Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Research Paper

Bioremediation of Cadmium and Lead And It's Effect on Chlorophyll, Enzymatic and Non Enzymatic Antioxidants of Cyanobacteria

Microbiology

KEYWORDS : blue green algae, heavy metal, antioxidative enzymes, phyco-biliproteins

Aditi Nag Chaudhuri — Lady Brabourne College; P1/2 Suhrawardy Avenue, Kolkata 700017

Madhulika Gupta — Lady Brabourne College; P1/2 Suhrawardy Avenue, Kolkata 700017

ABSTRACT

Cyanobacterial biomass has emerged as an alternative for developing economic and ecofriendly waste treatment process. Various concentrations of Cadmium (Cd+2)and Lead (Pb +2) salts were prepared with a stock of 1gm/50ml. Effect of chlorophyll on heavy metals along with pH variation was studied. The cell morphology of metal treated Cyanobacteria was studied by Scanning Electron Microscope(SEM). It was observed that Cd+2 uptake increased from 10-100mg/l and that of Pb+2 upto 80mg/l. Effect of Cd +2 and Pb+2 on Cyanobacterial antioxidative enzymes such as superoxide dismutase, catalase and ascorbate peroxidase were studied. Superoxide dismutase (SOD) enzyme is first line of defense against antioxidative stress as it converts superoxide anion to peroxide which can be converted to water by catalase or peroxidase. On exposure to cadmium stress the phycobiliproteins, the non enzymatic antioxidants, seem to be most affected while in case of lead phycocyanin remained unaffected compared to allophycocyanin and phycoerythrin.

Introduction

Heavy metal pollution in the aquatic system has become a serious threat today and of great environmental concern as they are non-biodegradable and thus persistent. Metals are mobilized and carried into food web as a result of leaching from waste dumps, polluted soils and water. The metals increase in concentration at every level of food chain and are passed on to the next higher level a phenomenon called biomagnification. Heavy metals even at low concentration can cause toxicity to humans and other forms of life. The toxicity of metal ion is owing to their ability to bind with protein molecules and prevent replication of DNA and thus subsequent cell division.To avoid health hazards it is essential to remove these toxic heavy metals from waste water before it's disposal. Main sources of heavy metal contamination include urban industrial aerosols, solid wastes from animals, mining activities, industrial and agricultural chemicals. Heavy metals of concern include lead, chromium, mercury, uranium, nickel, zinc, selenium, arsenic, cadmium, silver and gold (Ahalya et al.,2003)

Techniques presently involved in metal removal include: reverse osmosis, electrodialysis, ultrafiltration, ion exchange, chemical precipitation etc. However all these methods have disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge etc. With increasing environmental awareness and legal constraints being imposed on discharge of effluents, a need for cost effective alternative techniques are essential. In this endeavor, blue green algal biomass has emerged as an option for developing economic and ecofriendly waste treatment process.

The cell wall of blue green algae(cyanobacteria) has many functional groups such as hydroxyl, phosphoryl, amino, carboxyl, sulphydryl which confer negative charge to cell surface.In water metal ions are present in cationic form, they are adsorbed to the cell surface. Functional groups are found associated with various cell wall components such as peptidoglycan, teichoic acid, polysaccharides and proteins.

The accumulation of heavy metals by algae involves two processes- an initial rapid (passive) uptake followed by a much slower (active) uptake. During the passive uptake, metal ions adsorb onto the cell surface within a short period of time and the process is metabolism independent. Active uptake is metabolism dependent, causing the transport of metal ions across the cell membrane into cytoplasm. In some instances, the transport of metal ions may also occur through passive diffusion owing to metal induced increase in permeability of the cell membrane(Gadd,1988).Many of the strains of cyanobacteria have an outer sheath made up of polysaccharides.Sheath of cyanobacteria is anionic in nature due to the presence of uronic acid and other charged groups.Due to it's anionic nature cyanobacterial sheath shows very high affinity towards metal ions and may offer a promising chelating agent for the removal of heavy metals from waste water(De Philippis et.al,2001). However presence of sheath around the cyanobacterial cell may slow down the diffusion rate of metal ions into the chelating matrix of cell wall.Recent studies reveal that the process of heavy metal biosorption involves mechanisms such as ion exchange, complexation, electrostatic attraction and microprecipitation(Volesky and Holan,1995). Ion exchange has been shown to be the most important mechanism for biosorption of metal ions by algal biomass(Grist et.al,1990). Once the metals are inside the cell, they may bind to intracellular components or precipitate(Gadd,1988). Biological macromolecules and enzymes with appropriate functional groups or metal co-factors are impacted by metal activity. Factors affecting metal sorption of algae are- concentration of heavy metal, pH, biomass concentration, temperature and other factors include nutrient level, growth rate, illumination, presence of other anions and cations in solution. The affinity of various algal species for binding of metal ions shows different hierarchies. In general metal ions with greater electronegativity and smaller ionic radii are preferentially sorbed by algal biomass.Therefore algal biomass may serve as an economically feasible and efficient alternative to the existing physicochemical methods of metal removal and recovery from wastewaters.

Heavy metals enter algal cells either by means of active transport or by endocytosis through chelating proteins and affect various physiological and biochemical processes of algae.The toxicity primarily results from their binding to the sulphhydryl groups in proteins or disrupting protein structure or displacing essential elements. Metals can break the oxidative balance of the algae, inducing antioxidant enzymes such as superoxide dismutase(SOD), catalase(CAT) and ascorbate peroxidase(APX). The amount of oxidized proteins and lipids in the algal cells thus indicate the severity of the stress. Algal tolerance to heavy metal is highly dependent upon defense response against the probable oxidative damages. Production of binding factors and proteins, exclusion of metals from cells by ion-selective transporters and excretion or compartmentalization have been suggested with regard to reducing heavy metal toxicity.

Phycobiliproteins, the non enzymatic antioxidants, are the predominant accessory light harvesting pigments of cyanobacteria. They are reported to be used in the measurement of peroxy radical damage and have been shown to have antioxidizing activity.

Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Phycobiliproteins are a part of huge light harvesting antennae protein complexes called phycobilisomes.

Materials and Methods

Culture and Isolation:

To culture blue green algae(cyanobacteria) water samples were collected from Kolkata (E.M.ByPass area) and it's outskirts. The crude water sample collected was first observed under compound microscope to analyse various groups of algae. Water samples were taken from stagnant greenish coloured ponds. Media used for blue green algal growth were- Modified medium 18(sodium nitrate1.5g;magnesium sulphate380mg;dipotassium hydrogen phosphate120mg; calcium chloride110mg; sodium-chloride 70mg; ferrous sulphate10mg; sodium molbdate9mg; boric acid3mg; manganous sulphate2mg; zinc sulphate0.3mg; copper sulphate0.08mg; cobaltous chloride0.04mg; distilled water1000ml) and BG11 media (sodium nitrate1.5g; dipotassium hydrogen phosphate0.04g; magnesium sulphate0.075g; calcium chloride0.036g; citric acid0.006g; ferric ammonium citrate0.006g;EDTA0.001g;sodium carbonate0.02g;trace metal mix1ml;distilled water1000ml). In Modified medium 18 and BG11 the collected sample was centrifuged at 2,500rpm for10mins, supernatant discarded and pellet added to sterilized medium.The process was repeated a number of times in order to get a considerable amount of algal pellet.pH of both the media were maintained at 7.5 The media were incubated in a light regime of 12 hour light/ 12 hour darkness at room temperature and were kept undisturbed for 14-21 days.

As growth occurred the blue green algae were transferred to fresh broth and incubated in the same way. After sufficient growth occurred, a loopfull of inoculum was aseptically taken out, stained with Lactophenol cotton blue stain. A grease free slide was taken and adrop of lactophenol was added on the slide. Loopfull of culture was taken on the lactophenol and it was teased properly with a needle. The cotton blue stain was added to the sample and kept for 1min.Excess stain was removed by the mounting medium lactophenol. Cover slip was placed on it so that no air bubbles remain with the sample on the slide.Cover slip was sealed with wax to preserve the slide. Staining was performed with both the collected sample and cultured sample. Camera lucida drawing along with magnification was calculated under low and high power objective.(Desikachary,1959). Camera lucida is an instrument which when attached with a compound microscope , helps to draw the microscopic images of objects on paper.The three main parts of a camera lucida are :the attachment ring, the prism, and the mirror.It works on simple optical principle of reflecting beam of light through a prism and a plane mirror The microscopic image of the object is reflected by the prism on to the plane mirror and there from the image is reflected on to the plane paper. The observer moved the pencil on the lines of the image and a correct and faithful figure of the object on the paper was drawn.

Effect of Heavy metals on Chlorophyll:

Stock solution of the following metals cadmium nitrate and lead nitrate were prepared with concentration of 1gm/50ml. Various concentration of respective metals were prepared- 5mg/l, 10 mg/l, 20 mg/l, 40 mg/l, 80 mg/l, 100 mg/l, 150 mg/l and 200 mg/l. A control set up was maintained without metal solution. Media, buffer and metal solution was added sequentially, then 2 loopfull of culture was added to each conical flask of 100ml. The conical flasks were incubated for 7 days maintaining 12 hour light/12 hour darkness. After incubation the chlorophyll was extracted in order to measure the absorbance of chlorophyll to see the effect of respective metal concentrations on chlorophyll. Extraction of chlorophyll was performed by methanol and absorbance was measured at 665nm.(Knudson et al 1977).The culture that had grown were taken in centrifuge tubes. These tubes were kept in deep freeze for half an hour. After incubation 2ml of methanol was added to each tube. Then the tubes were kept in deep freeze for next 48 hours. After the incubation the cultures were centrifuged and the supernatant was collected in screw capped tubes. Then again 1ml of methanol was added to each tube and were centrifuged at 5000rpm for 10mins.The supernatant were collected in the same test tubes.The absorbance was measured at 665nm. Formula to measure the amount of chlorophyll:0.0165 X O.D.at 665nm – 0.0083X O.D.at 649nm = amount of chlorophyll(mg/ml). Net O.D. at 649nm=0 since chlorophyll a (665nm) is present but chlorophyll b(649nm)is absent in blue green algae.

Staining of Algal cells after metal treatment:

Algal staining was performed with the algal cells taken from the control and the concentration of 5mg/l, 150mg/l and 200mg/l. The stained slides were observed under high and low power objective.

Treatment for AAS(Atomic Absorption Spectroscopy):

Different metal concentrations and control were collected, air dried and dry weight was measured. Then the cell masses were taken in different crucibles and 5ml of a mixture containing concentrated HNO_3 : HClO4 (40:3v/v) was added. The mixture was boiled to evaporate to dryness and allowed to cool. 5ml of 0.3% nitric acid was added to each crucible and collected separately. Then the solutions were measured by AAS.(Scott1978)

Analysis of the effect of metals on the cell morphology by SEM:

Metal treated blue green algal cells were suspended in 3% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2). The cells were kept in this solution for 24hr. After incubation the cells were gradually passed through alcohol grades with concentrations of 30% to 100%. The cells were stored in deep freeze suspended in 70% alcohol. Then SEM was performed with the samples.(Wilson et al.1996)

Protein estimation

Protein was estimated according to the method of Lowry taking crystalline bovine serum albumin as the standard (Lowry et al.1951)

Enzyme extraction from cyanobacteria

Cyanobacterial culture exposed to different concentrations of two metals, cadmium and lead as well as the control were transferred to sterilized centrifuge tubes. Chilled 0.5ml phosphate buffer (pH7.5)) was added to the culture pellets and subjected to sonication for a total of 3mins(6 times for 30secs for each time with a gap of 30secs between successive sonications). The crude lysate was clarified several times by centrifugation at 10,000g for 10mins.The supernatant served as extracellular enzyme source.The enzyme extracts thus prepared by sonication of cyanobacterial pellets obtained by centrifugation were subjected to protein estimation by Lowry method to estimate the protein content in the extract and antioxidative and non antioxidative enzyme assays.

Antioxidative enzyme assays

Catalase prevents excessive hydrogen peroxide,a powerful and potentially harmful oxidizing agent, build up and converts to water and molecular oxygen. It allows important cellular processes which produce hydrogen peroxide as a by product, to take place safely. It is a tetramer of 4 polypeptide chains which are at least 500 amino acids in length. Within this tetramer there are 4 porphyrin heme groups which allows it to react with hydrogen peroxide.It's optimum pH is at neutral level.It performs a very elegant reshuffling of toxic compounds.

Catalase activity was measured according to the method of(Brannan et al,1981)using 30% peroxide solution, phosphate

buffer (50mM;pH7) and sodium azide 0.1mM at 240nm taken after 15s,30s and 1min of enzyme addition.Sodium azide was added in blank only and not in experimental test tubes.

Cyanobacteria may be classified into two groups on the presence and absence of ascorbate peroxidase-i)one that scavenges hydrogen peroxide with the peroxidase using a photoreductant as the electron donor and ii) the other which scavenges hydrogen peroxide with catalase. In photosynthetic organisms, superoxide is formed by the univalent photoreduction of dioxygen in PSI on thylakoid membranes as photosynthesis is saturated by light(Miyake and Yokota,2000). Hydrogen peroxide which is formed from superoxide by the disproportionation catalyzed by superoxide dismutase is reduced to water by ascorbate peroxidase. Oxidised ascorbate is reduced to ascorbate by ferredoxin , monodehydroascorbate reductase and dehydroascorbate reductase(Asada,1999;Shimaoka etal.2000)

Ascorbate peroxide activity was determined by the method of(Diallinas et al.1997)using ascorbate (1.5mM), peroxide(1.5mM) and phosphate buffer (50mM;pH7) at 240nm taken after 15s,30s and 1min of enzyme addition.

Superoxide dismutase takes two molecules of superoxide, strips the extra electron off of one and places on other.So, one ends up with an electron less forming normal oxygen and the other one with an extra electron which picks up two hydrogen ions to form hydrogen peroxide. Superoxide dismutases are metalloenzymes that are the first arsenal in defence mechanism against oxidative stress followed by an array of antioxidative systems. Multiple isoforms of SOD are possessed by cyanobacteria.

Superoxide dismutase activity was measured by the method of (Marklund and Marklund,1974) using DTPA (1mM), Catalase (40ng), phosphate buffer (50Mm;8.5) and pyrogallol (2.6mM in 10mM HCl) at 420nm for 2mins.

Non- enzyme antioxidant protein assay

Phycobiliproteins are water soluble proteins present in blue green algae and certain other algae that capture light energy, which is then passed on to chlorophylls during photosynthesis. The phycobiliproteins are made up of two subunits (α and α) having a protein backbone to which 1-2 linear tetrapyrrole chromophores are covalently bound. It comprises of phycocyanin, allophycocyanin and phycoerythrin. Phycocyanin is a light blue colour pigment absorbing orange and red light, particularly near 620nm. Allophycocyanin absorbs and emits red light at 650 and 660nm repectively. It emits and absorbs at longer wavelengths than phycocyanin and phycoerythrin.Phycoerythrins are the phycobiliproteins that bind highest number of phycobilins. Absorption peaks in the visible light spectrum are measured at 495 and 545/566nm, depending on the chromophores bound and the considered organism.

Estimation of Phycobiliproteins was performed by following the method of (Jensen and Bennet) using phosphate buffer(0.05M;pH7.5) and distilled water.The absorbance was taken at 562nm,615nm and 652nm.The Phycobiliproteins were calculated from the following equations:

Phycocyanin(mg/ml) = $(A_{615}$-.474 X $A_{652})/5.34$

Allophycocyanin(mg/ml) = $(A_{652}$-.208 X$A_{615})/5.06$

Phycoerythrin(mg/ml) = $(A_{562}$-{2.41 X (phycocyanin).849 X (allophycocyanin)/9.62})

Results and Discussion

After the mentioned period of incubation for culture of blue green algae, thick brownish, in some cases greenish net like filamentous growth was observed in M18 media. BG11 showed profuse growth. Genera observed were *Microcystis, Nostoc, Anabaena, Oscillatoria* and *Gloeotrichia* by Lactophenol Cotton blue staining under low and high power objectives. Irregular compact or loose aggregated colonies were observed under the microscope (depending on the media type). Cells in the colonies were embedded in mucilage and gelatinous sheath.

Effect of cadmium and lead on chlorophyll

A steady decrease in chlorophyll content was observed in case of all the two metals- lead and cadmium with increasing concentrations of respective metals. In case of cadmium there is a steady increase in the metal uptake from 10mg/l to 100mg/l. From 150mg/l metal concentration cadmium uptake steadily decreased. In case of lead, metal uptake upto 80mg/l was observed but with a decrease in metal uptake by cyanobacterial cells from 100mg/l metal concentration(Fig1)

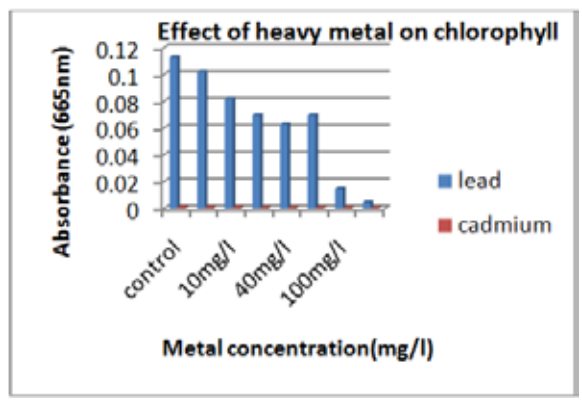

Fig1:Effect of Cd^{+2} AND PB^{+2}ON CHLOROPHYLL

In case of cadmium and lead steady decrease in chlorophyll a content may be due to two reasons- the central Mg atom of chlorophyll may get replaced by the respective metal cadmium and lead. The metals may arrest the electron flow in PSII thus hampering oxygenic photosynthesis. Cadmium caused strong inhibition on activities of PSII and the whole electron transport chain whereas PSI was the least affected. Thus the decrease in the absorbance of chlorophyll with the increase in metal concentrations may be due to the alteration in the whole chain and photosystem II catalyzed electron transport activities and alter the energy transfer preferentially in concentration dependent manner.

Uptake of heavy metals by Cyanobacteria

In case of cadmium there is a steady increase in the metal uptake from 10mg/l to 100mg/l. From 150mg/l metal concentration cadmium uptake steadily decreased. In case of lead, metal uptake upto 80mg/l was observed but with a decrease in metal uptake by blue green algal cells from 100mg/l metal concentration.(Fig2)

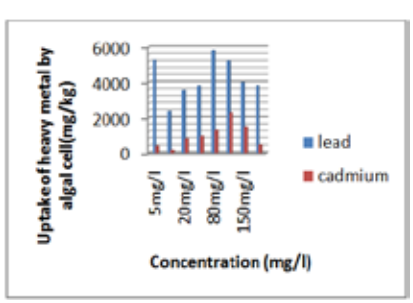

Fig2:Uptake of Cd^{+2} AND PB^{+2} BY CYANOBACTERIAL CELL

Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Lead uptake increases with increasing metal ion concentration upto a certain point. This is the pectin like substances present in capsule are thickened due to cross linking of polysaccharide chains by bonds between metal ion and the carboxylates, ring oxygens and bridging oxygen of polygalacturonate junction zones on adjacent chains. Then this metal uptake capability decreases with further increases in metal ion. The decreased viscosities seen at high (100-200mg/l) concentrations are influenced by removal of capsule from solution during formation of of condensed precipitates. Whereas increased metal uptake at 5mg/l concentration may be due to polymer chain association in early stages of cation induced gelatin, with resulting changes in radii of polymer gyration or formation of loose flocks with large amounts of trapped solvents.

At low ion concentration of cadmium the cyanobacterial cell showed the increased level of metal uptake due to the initial stages of polymer chain association in the sheath resulting in formation of loose flocs with large amount of trapped metal. But high concentration of cadmium causes removal of capsule from solution due to the precipitation of capsule by excess divalent ions of cadmium which finally results in decrease in metal uptake.

Effect of heavy metals on morphology of cyanobacteria observed under SEM

In case of SEM analysis it was observed (Fig.3) that the cyanobacterial filaments were intact in case of control set up with profuse quantity of thick gelatinous/mucilaginous sheath. At 5mg/l concentration of metals (Cd+2 and Pb+2) the number of filaments damaged were less and thick gelatinous sheath was observed but in case of highest metal concentration of 200mg/l, the filaments were rough, scattered, ruptured and shrinkage of gelatinous sheath was observed.

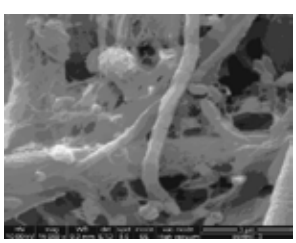

Fig3a. Control

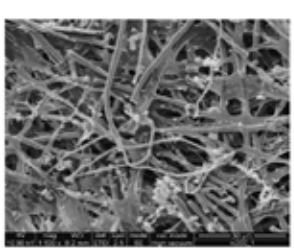

Fig3b. 5mg/l (Cd+2)

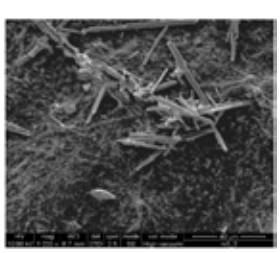

Fig3c. 200mg/l(Cd+2)

Effect of heavy metals on antioxi dative enzymes of Cyanobacteria

Superoxide dismutase(SOD) is the first line of defense against antioxidative stress as it converts superoxide anion to peroxide which can be converted to water by catalase or peroxidase. From the above graph (Fig.6) it can be seen that specific activity of the enzyme SOD gradually increased with increasing concentration of cadmium where at 200mg/l, the enzyme has

maximum specific activity which suggest that SOD effectively provide protection against oxidative damage induced by cadmium stress in cyanobacteria.This is also because the specific activity of the enzyme is increasing significantly and not being decreased as in case of lead where SOD specific activity has increased till 75mg/l concentration of lead. In case of catalase (Fig4) it can be clearly demonstrated from the graph that cadmium had more toxic effect on the specific activity of catalase compared to lead. When the cyanobacterium was cultured in presence of 10mg/l of cadmium specific activity of catalase has been seen to remain same as that of the control sample. Moreover the specific activity of catalase remains same in maximum and minimum concentration of cadmium while maximum specific activity of the enzyme catalase was at 75mg/l in case of lead. It could be said that catalase enzyme could not provide much protection to cyanobacteria against oxidative damage elicited by cadmium. From the graph it could be inferred that the specific activity of ascorbate peroxidase (Fig 5) increased from 10mg/l of cadmium but a fall in the specific activity at 75mg/l which then increased abruptly from 100mg/l to 200mg/l of cadmium compared to lead which attained maximum activity at 75mg/l concentration. Thus ascorbate peroxidase seemed to provide significant protection in response to to oxidative stress generated by cadmium compared to lead in cyanobacteria. In case of lead maximum specific activity was observed at 75mg/l concentration for all the three antioxidative enzymes.Thus increased intracellular concentration of oxy radicals triggered the antioxidative enzymes in the cyanobacterial cells. Thus a rise in the antioxidative enzymes suggested that there was an excess of toxic oxygen species that had to be scavenged for the protection of the cyanobacterial cells.

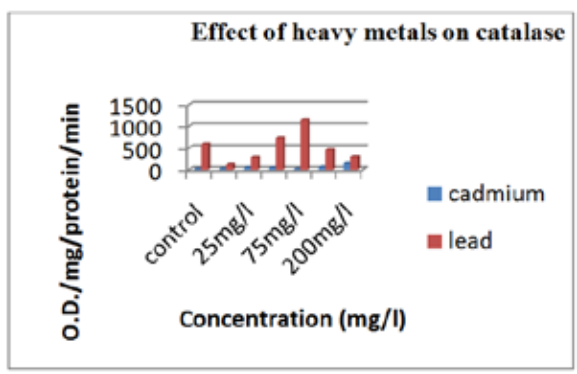

Fig4:Effect of Cd^{+2} and Pb^{+2} on Catalase

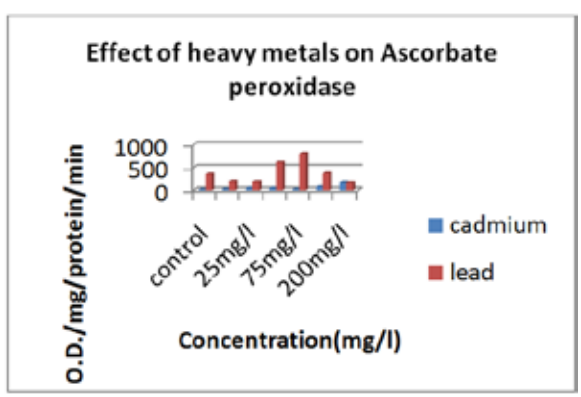

Fig5:Effect of Cd^{+2} and Pb^{+2} on Ascorbate peroxidase

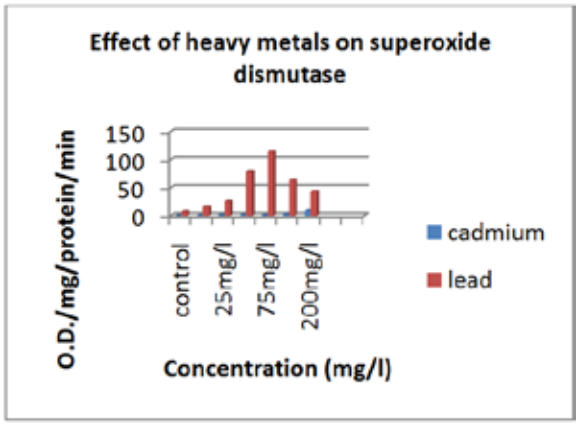

Fig6: Effect of Cd^{+2} and Pb^{+2} on Superoxide dismutase

Effect of heavy metals on Non- enzyme antioxidative proteins of Cyanobacteria

On exposure to cadmium stress all the three types of phycobiliproteins have declined especially allophycocyanin and phycocyanin (Fig. 8)which indicated that cadmium posed a more detrimental effect on these non enzymatic antioxidants. At 10, 25, 50, 75mg/l of cadmium concentration allophycocyanin and phycocyanin levels were lower than that of the control sample but resumes it's normal level at 100mg/l while phycoerythrin had shown a dramatic increase in it's level at 10mg/l with drastic decline in it's level when exposed to immediate higher concentrations of cadmium. In case of lead (Fig. 7)phycocyanin acted as a better antioxidant in oxidative stress than allophycocyanin and phycoerythrin. It has been reported that (Suroz and Palinska,2004) that cellular components of the thylakoid membranes of cyanobacteria deteriorates by increased metal concentration(cadmium and lead) causing a loss of photosynthetic activity and decrease in thylakoid surface in metal treated cells and decrease of proteinaceous lamellae affecting phycobilin content.

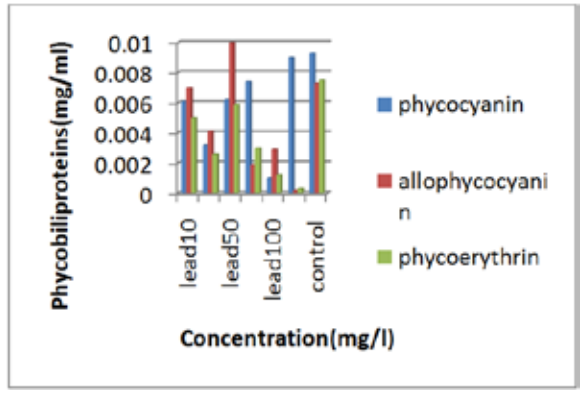

Fig7:Effect of Pb^{+2} on Phycobilins

Acknowledgement

We express our sincere gratitude to Wetland Management Institute, Saltlake, and Bose Institute (Rajabazar), Kolkata for their support.

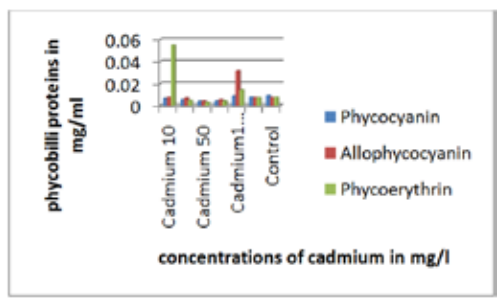

Fig8:Effect of Cd^{+2} on Phycobilins

Therefore , the algal species can be used for remediation of heavy metals like cadmium and lead upto a certain concentration at a very low cost and can be utilised in waste water treatment in specific industrial area .

REFERENCE

Allison, D.et al., Eds. (2000). Community Structure and Co-operation in | Bioflims. Socity for General Micro-biology Symposia. Cambridge University Press, Cambridge, UK. | | Barret, P. et al. (1996). The control of diatom and cyanobacterial blooms in reservoirs using barley straw. | | Biggs, B. J. F. (1996). Patterns in benthic algae of streams. In Algal Ecology, (Stevenson, R. J. Bothwell, M. and Lowe, R.L., Eds), pp.31-56. Academic Press, New York, USA. | | Booth, K. et al. (1987). Studies on the occurrence and elemental composition of bacteria in freshwater phytoplankton. Scanning Microscopy 1: 2033-2042. | | Bender,J., J.P. Gould, Y. Vatcharapijarn,J.S. Young, and P. Phillips, 1994, | Removal of Zinc and Manganese from Contaminated Water with Cyanobacteria | Mats, WaterEnviron. Research, 66, 5, p.679-683. | | Clegg, M. et al. (2003). The effect of photon irradiance on the behavioural ecology and potential niche separation of freshwater phytoplanktonic flagellates. Journal of phycology 39: 650-662 | | Fulton, R. and H.W. Paerl(1987). Toxic and inhibitory effects of the blue-green alga | Microcystis aeruginosa in herbivorous zooplankton. Journal of Plankton Research 9:837-855 | | Gardea-Torresdey, J.L., I. Cano-Agulera, K.J. Tiemann, and R. Webb, 1996c, | CopperAdsorption by Inactivated Cells of Mucor rouxii: Effect of Esterification | of CarboxylGroups, J. of Hazardous Materials, 48, p.171-180. | | Harper, D. (1992) Eutrophication of Freshwaters: principles, problems and restoration. Chapman and Hall, London, UK. | | H.K.Alluri,S.R.Ronda,J. Singh. Biosorption: An Ecofriendly Alternative for Heavy Metal Removal. African Journal of Biotechnology. Vol. 6(25), p.p. 2924-2913, 28th December,2007. | | Heavy Metals, Water Treatment Solutions. LENNTECH. www.lenntech.com. | | Lee, R. (1997) Phycology. Cambridge University Press, Cambridge, UK. | | M.K.Shukla,R.D.Tripathi,N. Sharma, S.Dwivedi, S.Mishra,R.Singh. Responses of Cyanobacterium Anabaena doliolum during Nick Stress. Journal ofEnvironmental Biology. September 2009, 30(5) 871-876 (2009). | | Minu Kesheri, Richa and Rajeshwar P.Sinha*, Anioxidants as Natural Arsenal against Multiple stresses in Cyanobacteria,Laboratoryof Photobiology and Molecular Microbiology, Centre of Advanced Study in Botany, Benaras Hindu University, Varanasi-221005, India volume 2/issue2/April-June 2011. | | Nayana Brahmbhatt, Rinku v. Patel and RT Jasrai, Comparison of antioxidant enzymes activity of Spirogyra and Oscillatoria sp.under heavy metal stress. | International Journal of Green and HerbalChemistry,IJGHC;2012-2013Vol2,No.1,1-4 | | Mehta, S.K, Gaur,J.P. Use of Algae | for Removing Heavy Metal Ions | From Wastewater: Progress and | Prospects. Source: Critical Reviews in | Biotechnology, Posted on Sunday,23rd | Oct 2005,03:01 CTD. | | Odum, E. (1985). Trends expected | in stressed ecosystems. | BioScience 35:419-422. | | P.K.Pandey,S.Choubey,Y.Verma | M.Pandey. Biosorptive Removal of | Ni from Wastewater and | Industrial Effluent. | International Journal of | Environmental Research and Public | Health.ISSN | 166-7827,2007.www.ijerph.org. | Park, C. (1980) Ecology | and EnvironmentalManagement, | Dawson Westview Press. | | Silver, S. and G. Ji, 1994, Newer Systems for Bacterial Resistances to Toxic | Heavy Metals, Environmental Health Perspectives, 102(3), p.107-113. | | Wang W.X.., Dei RCH (2001) Effects of major nutrient additions on metal uptake | inPhytoplankton. Environ Pollut 111:233-239.