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An Interactive effect of six heavy metals on the growth characteristics of *Gracilaria blodgettii* Harvey and *Enteromorpha intestinalis* (Linn.)Link

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ABSTRACT

The heavy metal toxicity in *Gracilaria blodgettii* and *Enteromorpha intestinalis* showed reduction in their fresh weight, carbohydrate and protein concentration with that of control. There was a significant decrease in the relative growth and total chlorophyll content when concentrations of heavy metals were increased. At low metal concentration many of the biochemical characteristics of the algae were not affected suggesting that the metals have not gained access to sensitive sites of the cells, while higher metal concentrations resulted in enzyme poisoning. The toxicity of heavy metals on these two seaweeds are in the following order *Gracilaria blodgettii* Hg>Cd>Cr=Pb=Zn>Cu and *Enteromorpha intestinalis* Hg>Cd>Pb=Zn>Cr=Cu

Key words: *Gracilaria blodgettii*, *Enteromorpha intestinalis*,heavy metals, Cadium, Mercury, chromium, Zinc, Lead, Copper tolerance

INTRODUCTION

Seaweeds are marine macro algae mostly occurring on the inter tidal and sub tidal regions of the rocky surfaces as well as in the back water and brackish water conditions. Studies on the uptake and accumulation of heavy metals by algae have been investigated more as a fascinating basic study to understand the aspects of selective permeability and excluding the excessive amount taken into the cells. (Whiton, 1970; Rice *et al*., 1973; Sorentino, 1979.) Heavy metal uptake by algae from the points of view of possible removal of problematic toxicants from effluents, pollution control and safety of cultivated Algae which may remove them from the medium containing heavy metals inadvertently.

(Venkataraman and Becker, 1986). Thus uptake, accumulation and consequent toxicity in algae assume a practical significance. Effective concentration which reduced cells of a diatom, *Navicula obtusa* by 50% after 10 days of exposure to cadmium was determined at 1.34mg/L (Gowrinathan and Rao, 1995).

In the present study, the red seaweed *Gracilaria blodgettii* Harvey and green seaweed *Enteromorpha intestinalis* (Linn.)Link were collected from the Muttukadu back water near Chennai, were chosen for intensive investigation. The effect of heavy metals such as Copper, Cadmium, Chromium, Mercury, Lead, and Zinc on the fresh weight, pigment composition, total carbohydrate, total protein and protein profile of the seaweed were carried out.

MATERIAL AND METHODS

Algal specimens were inoculated in ESP1 basal medium (Richard,*et al*.,1987). The blue green algae, such as *Phormidium* sp., *Oscillatoria* sp., etc. were eliminated by treating them with 2000mg/L of

streptomycin for 30 mts under 30mts Em-2S-1 and then transferring back to antibiotic free medium (Rengasamy *et al*., 1987).

The contaminants of diatoms such as *Navicula* sp., etc. were eliminated by adding 3mg of Germanium dioxide (GeO2) to 1L of medium (Levin 1966, Markham and Hag Meier; 1982; Shyamala, 1981).

Growth study

The experiment was carried out for a period of 15 days on these two algal forms, at every 5 days of interval fresh weight of the seaweeds was recorded.

Effect of heavy metals

Cu - ($CuSO_4.5H_2O$)	-	1.0µg, 2.0µg, 5.0µg and 10µg
Cd - ($Cd\ Cl_2.H_2O$)	-	0.1µg, 0.5µg, 1µg
Cr - $(Cr_4CSO_4)_5(OH)_2$	-	1µg, 2µg, 5µg, 10µg
Hg - ($HgCl_2$)	-	0.005µg, 0.01µg, 0.02µg
Pb - ($PbCl_2$)	-	1µg, 2µg, 5µg
Zn - ($ZnSO_4.7H_20$)	-	1µg, 2µg, 5µg

The above concentrations of heavy metals were amended in the basal medium to investigate the growth characteristics of *E.intestinalis*. The concentration of chlorophyll a was estimated by Ramus method (1983).

Ten mg of sample was disrupted in 5 ml of cold 90% acetone. After 24 hours Incubation in dark the homogenate was centrifµged at 12,000Xg for 10 minutes. The supernatant was quantified as 630nm and 663nm. The pallet was recovered and analyzed for the estimation of accessory pigments.

Chlorophyll a (µg /ml) = 11.43 E 663 - 0.64 E 630

E denotes extinction at the particular wave length. Accessory pigments such as phyroerythrin (PE), phyrocyanin (PC) and allophyrococyanin (APC) were estimated by Kursar *et al.,* (1983).

The pellets were re suspended in 5 ml of ice cold phosphate buffer at pH 6.8.(0.1M KH2PO4 and 0.1m K2HPO4. The aqueous supernatant by centrifµging at 4000Xg for 10 mins was read at 498.5, 614 and 651nm for PE, PC and APC.

PE = 158.8 E 498.5 - 40.0E 614 - 10.5 A 651

PC = 151.1 E 614 - 99.1 E 651

APC = 181.3 E 651 - 22.3 E 614

For *Enteromorpha intestinalis* chlorophyll a and chlorophyll b were calculated using the formula

Chlorophyll a (mg/g) = 12.7 a 663 - 2.69 A 645 X v / aX1000Xw

Chlorophyll b (mg/g) = 22.9 A 645 - 4.68 A 663 X v / a X 1000 X w

a = length of path light in the cells (1cm)

v = volume of the extract in ml

w = fresh weight of the sample in mg.

Estimation of total carbohydrate was estimated by phenol-sulphuric acid method (Dubois, et al., 1956).Ten microgram of sample was ground in 10ml of Sodium - phosphate buffer at 120ºc under 15 Lb /square inch pressure. After cooling centrifµged at 5000 × g for 10 minutes and supernatant was analyzed for total carbohydrate. 1ml sample added with 5% phenol and (5g phenol crystal in 100ml glass distilled water) 5ml of concentrated H2SO4(96% analytical reagent grade).The mixture was read at 490nm after ten minutes. Checked with the standard graph of glucose from 10 to 100 µg/ml to determine the total carbohydrate.

Estimation of protein was done by Bradford method (1976). Ten microgram of seaweeds was homogenized in 5ml of phosphate buffer at pH 7. The extract was centrifµged at 10000 × g for 15 minutes and supernatant was ollected. Protein was precipitated by adding equal volume of 10% ice cold TCA and centrifµged at 12,000 xg for 10 minutes. The pellet was collected, re dissolved in 2ml of 1N NaOH. To 0.1ml of the sample 5ml of protein reagent was added and mixed thoroµghly. The absorbance was read at 595nm against a reagent blank.

Analysis of polypeptides by sds - page (Sambrook *et. al*., 1989)

Sodium dodecyl Sulphate (SDS) – Polyacrylamide Gel electrophoresis was carried out by the modified method of Laemilli (1970). SDS-PAGE was run on the slab gel system.

Both seaweeds showed an optimum growth at 15th day. On 15th day the fresh weight of both the plants was increased to about one and half folds when compared to the initial inoculum.

RESULTS

The effect of six heavy metals at different concentrations on growth characteristics of the red seaweed *Gracilaria blodgettii* and the green seaweed *Enteromorpha intestinalis* revealed the following observations. Both seaweeds showed an optimum growth at 15th day. On 15th day the fresh weight of *G.blodgettii* and E.intestinalis was increased to about one and half folds and two and half folds, respectively when compared to the initial inoculum**.**

Effect of heavy metals on *G. blodgettii*

Effect of cu ($CuSO_4$ $5H_2O$)

The seaweed was able to tolerate up to 10.0µg concentration of Cu. Addition of Cu in the medium decreased the fresh weight of the seaweed. The seaweed grown in 5.0µg showed 168mg fresh weight which was about 65% less than control. The concentration of Chl a and accessory pigments such as PE, PC, and APC also decreased due to the addition of Cu. The amount of total carbohydrate and total protein was also decreased while the medium was amended with Cu.

Effect of Zn ($ZnSO_4$ 7 H_2O)

The seaweed was able to grow up to 2.0µg ZnSO4 7H2O. The concentrations above 2.0µg were toxic to alga.The seaweed grown in 2.0µg concentration showed 162mg of fresh weight which was less than about 65% when compared to control. Concentration of pigments and accumulation of total carbohydrate and total protein were also decreased due to the addition of ZnSO4 7H2o in the basal medium. The amount of total carbohydrate and total protein recorded at 2.0µg ZnSO4 was decreased to about 50% and 25%, respectively when compared to control.

Effect of Cr (CrCl2):

The tolerance of seaweed to CrCl2 µg was up to 2.0µg. The tolerance of seaweed to CrCl2 was up to 2.0µg . Concentrations above 2.0µg were toxic to alga. The fresh weight observed at 2.0µg concentration was 155mg which was less than about 62% when compared to control. The concentration of PE showed a decrease of about 50% to that of control. The amount of total carbohydrate and total protein at 2.0µg was also decreased by about 60% and 75%, respectively when compared to control.

Effect of Pb ($PbCl_2$)

The seaweed was bleached in all concentrations of PbCl2 except 1.0µg and 2.0µg. The fresh weight showed 155mg at 2.0µg after 15 days was less than about 62% to that of control. The concentration of Chl a recorded at 2.0µg was 0.5µg /mg which was less than about 40% compared to control. Concentrations of accessory pigments such as PE, PC and APC were also decreased due to Pb amendment. The alga grown at 1.0µg PbCl2 showed the PE concentration of 2.3µg /mg fresh weight which was less than about 40% when compared to control. The accumulation of total carbohydrate and total protein also inhibited due to Pb amendment.The amount of total protein recorded at 2.oµg was 0.8µg /mg compared to 1.6µg /mg fresh weight in control.

Effect of Cd ($CdCl_2$ H_2O)

The seaweed survived up to 0.5µg concentration of CdCl2 H2O. Concentrations above 0.5µg were toxic to the organism. The seaweed grown in 0.5CdCl2 H2O showed a fresh weight of 146mg which was about 60%less than control. The concentration of Chl a and accessory pigments (PE, PC & APC) was also decreased due to the amendment of CdCl2 H2O. Among the accessory

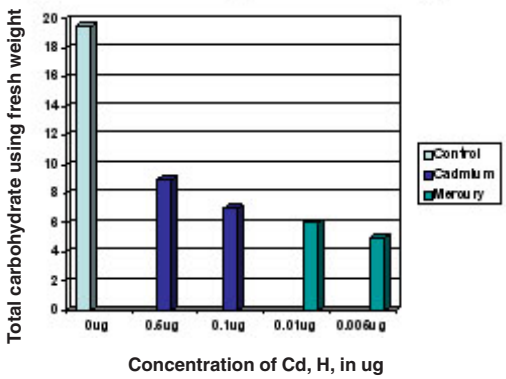

Fig. 1: Effect of Cd, Hg on total carbohydrte in G. blodgetti

pigments, the inhibition of synthesis of PC was higher than others. The seaweed grown in0.1µg showed the PC concentration of 0.13µg /mg fresh weight which was less than about eight folds compared to control. The amount of total protein and total carbohydrate recorded at 0.5µg was less than about 50% and 40% respectively to that of control.

Effect of Hg ($HgCl_2$)

The seaweed was able to survive in 0.005µg and 0.01µg of HgCl2 in the medium. Concentrations above 0.01µg were toxic to alga. The fresh weight recorded in 0.01µg was 130mg which was less than about 50% to that of control. Addition of HgCl2 decreased the fresh weight and

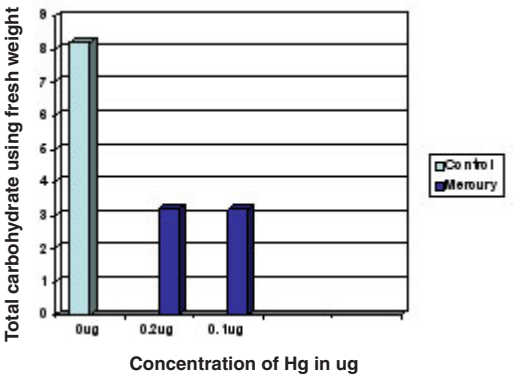

Fig. 2: Effect of Hg on total carbohydrate in E. ingestinalis

also the biochemical characteristics investigated. Concentration of Chl a recorded at 0.01µg was 0.05µg/mg fresh weight, which was less than about sixteen folds compared to control. Among the accessory pigments, PE was least affected due to the amendment of Hg in basal medium. Concentration of PE recorded at 0.01µg was 1.29µg /mg against 3.73µg /mg in control. The accumulation of total carbohydrate and total protein was also inhibited while the medium was amended with Hg. The amount of total carbohydrate and total protein was decreased at 0.1µg to about 35% and 40%, respectively when compared to control.

Effect of heavy metals on e.intestinalis
Effectof Cu (CuSO4 5H2O)

The fresh weight of the green seaweed was decreased to about 70% while the medium amended with 10µg CuSO4 5H2O. The seaweed inoculated in the basal medium amended with above 10µg $CuSO_4$ $5H_2O$ died within 3 days. The concentration of Chl a and Chl b also decreased due to Cu amendment. At 10µg CuSO4 5H2O the concentration of pigments, Chl a 6.75µg /mg and Chl b 0.35µg /mg) was less than about 50% and 25% respectively to that of control. The amount of total carbohydrate and total protein recorded at 10µg $CuSO_4$ $5H_2O$ was also decreased to about 30% and 35% respectively than control.

Effect of Cr (CrCl2)

The green seaweed E.intestinalis was able to tolerate 10µg concentration of CrCl2. Addition of Chromium inhibited the growth of alga. The algal

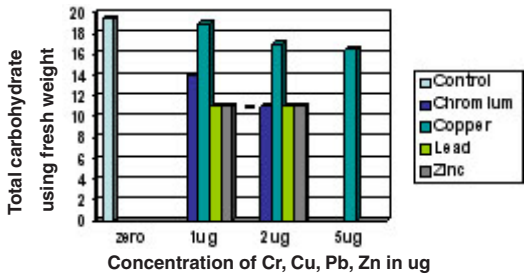

Fig. 3:Effect of Cr, Cu, Pb, Zn on total carbohydrate ratio in G. blodgetti

fresh weight 211mg recorded at 10µg was less than about 65% to that of control. Addition of Chromium inhibited the synthesis of pigment composition also. The concentration of Chl a and Chl b recorded at 10µg was 0.56µg /mg and 0.51µg /mg respectively, whereas 1.26µg /mg(Chl a) and 0.38µg /mg(Chl b) in control. The accumulation of total carbohydrate and total protein recorded at 10µg was about each 35% less than control.

Effect of Pb($PbCl_2$)

The seaweed was able to survive up to 10µg concentration of $PbCl_2$. Concentrations above 10µg were toxic to the alga. Amendment of Pb inhibited the growth of seaweed. The algal fresh weight recorded at 10µg was 174mg which was less than about 55% compared to control. Concentrations of Chl a and Chl b recorded at 10µg was decreased to about 75% and 60%, respectively when compared to control. The accumulation of total

carbohydrate and total protein recorded at 10µg was less than about 40% each to that of control.

Effect of Zn ($ZnSO_4$ $5H_2O$)

The seaweed was able to grow up to 10µg concentration of $ZnSO_4$ $5H_2O$. The algal fresh weight recorded at 10µg was 182mg which was less than about 55% compared to control. Concentrations of Chl a and Chl b recorded at 10µg was 0.53µg / mgChl a and 0.34µg /mg Chl b fresh weight against 1.26µg /mg Chl a and 0.78µg /mg Chl bin control. The accumulation of total carbohydrate and total protein recorded at 10µg was less than about 30% than control.

Effect of Cd ($CdCl_2$)

The green seaweed was able to grow up to 10µg concentration of $CdCl_2$. The algal fresh weight recorded at 10µg was 196mg which was less than about 60% compared to control. Addition of CdCl2 in the medium decreased the concentration of photosynthetic pigment. Concentrations of Chl a and Chl b recorded at 10µg was 0.42µg /mg Chl a and 0.26µg /mg Chl b fresh weight against 1.26µg /mg Chl a and 0.78µg /mg Chl bin control.

The accumulation of total carbohydrate and total protein recorded at 10µg was less than about 55% than control.

Effect of Hg ($HgCl_2$)

The seaweed inoculated in the medium amended with above 0.2µg HgCl2 died within Two days. The concentration of Chl a and Chl b also decreased due to the amendment. At 0.2µg HgCl2 the alga showed 0.32µg/mg Chl a and 0.09µg /mg Chl b which were less than about 30% and 20%, respectively to that of control. The amount of total carbohydrate and total protein was decreased at 0.1µg to about 35% and 40%, respectively when compared to control. The fresh weight recorded in 0.01µg was 130mg which was less than about 50% to that of control. Addition of HgCl2 decreased the fresh weight and also the biochemical characteristics investigated. Concentration of Chl a recorded at 0.01µg was 0.05µg/mg fresh weight, which was less than about sixteen folds compared to control. Among the accessory pigments, PE was least affected due to the amendment of Hg in basal medium. Concentration of PE recorded at 0.01µg was 1.29µg/mg against 3.73µg/mg in control. The accumulation of total carbohydrate and total protein was also inhibited while the medium was amended with Hg. The amount of total carbohydrate and total protein was decreased at 0.1µg to about 35% and 40%, respectively when compared to control.

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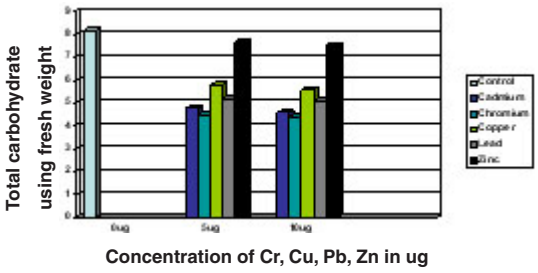

Fig. 4: Effect Cd, Cr, Cu, Pb, Zn or total carbohydrate ratio in E. intestinalis

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Effect of Cr ($CrCl_2$)

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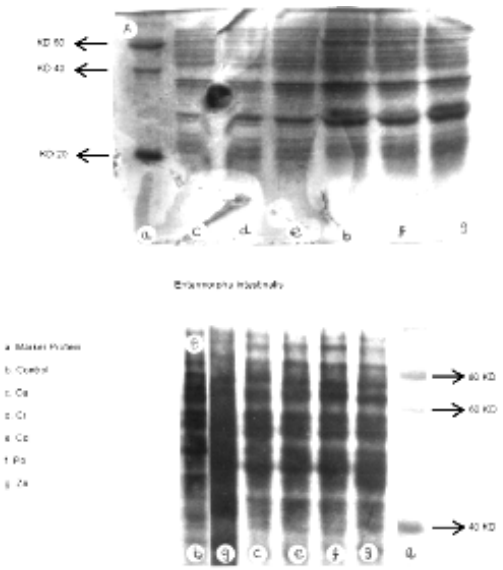

Fig. 5: Effect of metals on the protein profile of seaweeds

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Effect of heavy metals on the protein profile of *G.blodgettii* and E.intestinalis

Electrophoretic study on the protein samples of seaweeds revealed the significancance on the removal of salts to elucidate discrete bands on SDS PAGE. All the samples exhibited similar electrophoretic mobility of protein profile. Bands exhibited apparent molecular weight between 40 and 60 KD. Both high low molecular weight polypeptides were uniformly separated. The number of bands observed in *Gracilaria blodgettii* 20 was less than *Enteromorpha intestinalis* 25. However, the density of the bands observed in control was high when compared to low density bands appeared in the sample grown in heavy metals. There is no addition or deletion of bands seen in seaweeds due to heavy metals amendment in the basal medium.

DISCUSSION

Studies made on a comparative investigation on the red seaweed G. blodgettii Harvey and green seaweed *E. intestinalis* (Linn.) Link in relation to six heavy metals such as Cu, Cr,

Cd, Pb, Zn and Hg under laboratory conditions elucidate certain interesting findings.

Gracilaria blodgettii is well known phycocolloid-agar yielding seaweed. The agar is widely used as food, medicinal preparations and industrial and laboratory preparations.The genus Enteromorpha is also an abundant seaweed which bis rich in their protein content.

Enteromorpha is also occurring in the sea polluted with organic waters / sewage

Macroalgae are major primary producers in the marine environment and play an important role in food chains. Since marine pollution is most serious in coastal waters adjacent to major pollutant sources, macroalgae from marine environment are particularly suitable for pollution studies.Additionally, they have the ability to accumulate high levels of various metals in their cell walls (Burdin and Bird, 1994; Salgado *et al.,* 2005).

Heavy metals are environmental pollutants that have the potential to induce severe stress-reactions in organisms on land as well as in the sea. The effects of short term sublethal concentrations of copper (Cu^{2+}) and cadmium (Cd^{2+}) on the reactive oxygen metabolism of the marine red macroalga *Gracilaria tenuistipitata.* Additions of either 0.2 ppm Cu^{2+} or 1 ppm Cd^{2+} caused decreased growth (~60%), increased oxidation of lipids and increased oxidative damage to proteins as shown by increased content of protein carbonyl groups. Together this strongly sµggests an induction of oxidative stress. Cu^{2+} caused more oxidative damage than Cd^{2+}. As a response to the increased oxidative stress, addition of Cu^{2+} induced the activities of catalase, ascorbate peroxidase, and superoxide dismutase. In contrast, Cd^{2+} only caused increased catalase activity. Ten-fold lower concentrations of the metals did not cause an increase in enzyme activity. Both heavy metals also increased the content of the antioxidants -carotene and lutein. The results show that Cd^{2+} and, to a larger extent, Cu^{2+} induce oxidative stress in short-term experiments and the seaweed responds by increasing the activity of the reactive oxygen metabolism. Tropical green macroalga *Ulva reticulata* (Daleng Shen and Madeline Wu 1994)

Contamination of algae with heavy metals is one of the major problems in algae cultivation which may affect its safety. This fact attains paramount importance since it has been repeatedly reported that the metal concentration in algae may reach levels which may be toxic to the consumer.

Though some of the metals like copper which are required in micro quantities for various metabolic processes of plant growth however, lethal at high concentrations. (Venkataraman *et al*., 1992).

The biological variables such as concentration of photosynthetic pigments, accumulation of total carbohydrate etc. characteristic feature of heavy metal is that they interact bind themselves to biological macromolecules. Seaweeds are known to concentrate metals from seawater by ion exchange mechanism. Approximately 50% of metals were absorbed onto the cell surface. (Gowrinathan and Rao, 1992).

In the present study the green alga *Enteromorpha intestinalis* was able to tolerate higher concentrations of heavy metals than *Gracilaria blodgettii*. Among the six metals studied mercury was more toxic than others (Velayutham,et al., 1994; Rao, 1995). The tolerance of *G.blodgettii* and E.intestinalis on mercury was 0.01µg, indicating that both algae could not survive above the level in mercury polluted water. Mercury not only affected the growth of algae but also inhibited the concentration of photosynthetic pigments and accumulation of polysaccharides and polypetides.

High concentration of Zinc inhibited the growth of various algae. The plants which grow in seawater accumulate less Zinc than river water (Venkataraman, et al,. 1986). Concentration of pigments decreased due the addition of Zinc to the medium. The inhibition on the accumulation of total protein in G. blodgetti washigher than *E. intestinalis* in Zn amended medium.

Cadmium toxicity in some diatoms proved the effective concentration reduced cell number by 50% after exposure of Cadmium (Rao and Gowrinathan, 1994).

Cadmium accumulation was proved toxic in 0.2 µg concentration in red alga Porphyra umbilicalis (Mclean *et al*, .1977). The present study revealed that both seaweeds showed different sensitivity towards Cd amendment. *E. intestinalis* could tolerate higher concentration (10µg) of Cd than G. blodgettii (0.5µg). The concentration of pigment composition and the accumulation of total carbohydrate and total protein also decreased due to Cd amendment in the basal medium.

The heavy metal Cu is essential for various metabolic processes in trace amount with particular reference to plastocyanin (Gledhill, *et al*,. 1977). Tolerance of the present green alga was more than red alga while the medium amended with Cu. This abservation was similar to the earlier findings of Venkataraman, *et al*, (1992).

The total chlorophyll content of *C. fracta significantly* decreased when the exposure time and Pb or Cd concentration were increased. Pb and Cd at high concentrations destroyed chloroplasts of *C. fracta,* as shown in the toxicity symptom study. It is well known that Cd can cause disorganization of chloroplasts leading to a reduction of the photosynthetic pigments33. Both Cd and Pb were reported to inhibit chlorophyll biosynthesis, leading to the lowered chlorophyll contents 34. Sen and Mondal 35 reported that the decline in chlorophyll content might be caused by a reduction in the synthesis of chlorophyll, possibly by increasing chlorophyllase activity, by disorderness of chloroplast membrane and by inactivation of electron transport in photosystem I. (Chantana Lamaia, Maleeya Kruatrachuea , 2005). (Chantana Lamaia, Maleeya Kruatrachuea , 2005) Lead is a common pollutant in water systems and also affects the organisms.

Photosynthesis and cell division of various algae are inhibited by lead (Rivkin, 1979). In the present study, *E. intestinalis* was able to tolerate a high concentration of lead than other metals in contrast to Cu in G. blodgettii. The concentration of pigment and the accumulation of total carbohydrate and total protein were also decreased due to lead amendment. The effect of Cr was different to various organisms and also with various concentrations (Rao and Gowrinnathan, 1994). The present study showed that the tolerance of *G. blodgettii* and *E. intestinalis* to Cr was 2.0µg and 10µg respectively. Growth characteristics such as pigments, total carbohydrate and total protein also decreased due to Cr amendment. The electrophorogram of the present study on *G.blodgettii* and *E. intestinalis* grown from six heavy metals had shown high and low molecular weight proteins ranging from 40KD to 80KD. In *Phaeodactylum tricornutum* the soluble proteins of 22-24KD in size were detected in iron-limited but not in iron deplete cultures (Roche, *et al*, 1993). Nearly 20 to 25 bands were found in a green alga *Dunaliella tertiolecta* (Richard, *et al.,* 1998).

The present work provides informations regarding the protein profiles of *G. blodgettii* and *E. intestinalis*. Considerable interest is yet to be generated regarding the protein nature of the red algae and green algae. Further, the nature, type and various identification of polypeptides of the samples have to be investigated. Effect of heavy on the protein profiles of *G.blodgettii* and E. intestinalis revealed that there was no addition or deletion of protein, however the density was low. This indicated that heavy metals affected the synthesis of protein.

Several activities can contribute to heavy metal pollution in the marine environment, for example, seafloor and bedrock dredging, shipping activities, industrial and urban effluents, mining, agricultural fertilizer use, and burning of fossil fuels (Machiwa, 1992; UNEP, 1997; Lio-netto et al., 2003). Natural weathering of rocks is yet another source (Pyle and Mather, 2003).

Accumulations of copper and zinc and their effects on growth and maximum quantum yield of the brown macroalga *Padina gymnospora* was reduced as the concentration increased. (Mamboya, F.A *et al*,.2007)

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REFERENCES

1. Bradford, M.M., A rapid and sensitive method for the quantification of microgram method for the quantification of microgram quantities of protein utilizing the principle dye binding. *Anal. Biochem.* **72**: 248-254 (1976).
2. Chantana Lamai, Maleeya Kruatra Chue,Prayad Pokethefiyook,F. Suchart Upatham,and Varasaya Soonthornsarathool, Toxicity and Accumulation of Lead and Cadmium in the filamentous Green Alga Cladophora fracta (O.F.Muller ex Vahl)Kutzing:A laboratory Study. Dubois M., Gilles.K.A., Hamilton.T.K., Rebers.P.A and Smith.F.,1956. Colorimetric method for determination Of sµgars and related substances. *Anal. Chem*. **28**: 350-356 (2004).
3. Govindarajan.S and Rao.V.N.R., Transfer of copper and zinc throµgh a marine food chain. *Acta Botinica Indica* (1992).
4. Gowrinathan.K.P. and Rao.V.N.R., Cadmium toxicity in *Nitzschia obtusa* Wm.Sm. A pennate diatom. *Seaweed Res. Utiln*. **17**: 165-175 (1995).
5. Kesava Rao C.H. and Induslkar.V.K., Chromium, Lead and Cadmium contents of certain seaweeds from Sawrastra Coast. *Phykos* **26**: 1-7 (1987).
6. Kursar T.A.,Vaudeer Meer J. and Alberte R.S.,1983. Light harvesting system of the red algae *Gracilaria tikvahiae. Pl. Physiol*. **73**: 353-360.
7. Laemmli V.D., Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**: 680-685 (1970).
8. Lay, P.L., M.P. Isaure, J.E. Sarry, L. Kuhn, B. Fayard, J.L. Le Bail, O. Bastien, J. Garin, C. Roby and J. Bourguignon., Meta-bolic, proteomic and biophysical analyses of *Arabidopsis thaliana* cells exposed to a caesium stress. *Influence of potassium supply. Biochimie.* **88**: 1533"1547 ((2006)).
9. Lewin J., Silicon metabolism in diatoms. Germanium dioxide a specific inhibitor of diatom growth. *J. Phycol.,* **6**: 1-12 (1966).
10. Lewis, S., M.E. Donkin, and M.H. Depledge Hsp70 expression in *Enteromorpha intestinalis* (Chlorophyta) exposed to environmental stressors. *Aquat. Toxicol.* **51**: 277–291 (2001).
11. Mackineey.G., Absorption of light by chlorophyll solutions.*J. Biol. Chem*. **140**: 315-322 (1941).
12. Mamboya, F.A., H.B. Pratap, M. Mtolera, and M. Björk., Accumulations of copper and zinc and their effects on growth and maximum quantum yield of the brown macroalga *Padina gymnospora*. *Western Indian Ocean J. Mar. Sci* (2007).
13. Mamboya, F.A., T.J. Lyimo, T. Landberg, and M. Björk., Influence of combined changes in salinity and ambient copper concentrations on growth and copper accumulation in the tropical green macroalga *Ulva reticulata* (2007).
14. Markham W. and Hagmeier. E., Observations on the effects of Geranium dioxide on the growth of macroalgae and diatoms. *Phycologia*, **21**:125-130 (1982).
15. Ramus J., A physiological test to the theory of complementary chromatic adaptation in brown, green and red seaweeds. *J. Phycol*. 173-178 (1983).
16. Rao V.N.R., Adaptation of algae to heavy metal toxicity. *Seaweed Res. Utiln*. **17**: 111-116 (1995).
17. Rengasamy R., Prema M., Govindarajan S. and Ilanchelian K., Effect of antibiotics on the growth of *Hypnea valentiae* (1987).
18. Rice H.V., Leighrty D.P. and Mc Lead G.C., The effect of some trace metals on marine phytoplankton. *CRC Crit. Rev. Micro bio*l. **3**: 27-49 (1973).
19. Richard C.S. and Zeikus J.A., UTEX - The culture collection of algae at the University Texas at Austin. *J. Phycol*. **23**: 36-42 (1987).
20. Sambrook J., Fritsch E.F., Maniatis T., Molecular cloning, A laboratory Manuel, 2nd Cd - Vol.I cold spring Harbor, Newyork. 18.52 (1989).
21. Sorentino C., The effects of heavy metals on phytoplankton: *A review Phykos* **18**:

149-161(1979).

22. Venkataraman L.V. and Becker E.W., In Biotechnology and Utilization of algae. The Indian Experience Pub. Department of Science and Technology, New Delhi, India. 4 (1986).

23. Velayutham P.G., Jegatheesan G. and Jeyachandran P., Total mercury content of seaweeds collected from tuticorin coast, Fisheries college & Research Institute. 20-23 (1994).