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Study onaccumulation of copper and cadmium metals in Gracilariaverrucossa in nutrition enriching condition and their effect on growth of the organism

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Abstract: *Pollution being the measure threat in this developing world, different methodologies are being invented or discovered every year. Algae, sea weeds, aquatic plants have been identified as potential resources for accumulating and bioconcentrating heavymetals. In this study a test is formulated how nutrient enrichment enhances the metaltolerance of floating macrophytes taking Gracilariaverrucossa as the test organism. Relative growth rates were measured inGracilariaverrucossaexposed to different cadmium and copper concentrations in laboratory conditions.Photosynthetic pigment levels were negatively correlated with metal exposures, and nutrientaddition attenuated chlorophyll decrease in response to metal exposures. MDA content and EC alsoshowed sharp increases at higher concentrations, indicating oxidative stress. Relative growth rates were observed with metal exposure, it is found that the additionof nutrientshelped the growth of the test organism, which often suppressed in the concentrated heavy metal exposure. This study indicates thatnutrient enrichment increases the tolerance of Gracilariaverrucossato metals, which is asuitable organismfor controlling low level of Cadmimum and copper in waste water.*

Keywords*: Gracillariaverrucosa, heavy metals, nutrient, toxic effect , biological activity*

I. Introduction

Water contamination withheavy metals has become a severe problem in industrialized areas. Thepresence of toxic heavy metals in ditch, lake and riverwater badly affects the aquatic life and well-being of people whodepend upon these sources for their daily waterrequirements.Consumption ofaquatic foodstuffs contaminated with toxic metalsmay cause serious health hazards through bio-magnification (Wenhua et al., 2007). A numberof methods are available to remove toxic metals fromwater, including ion exchange, reverse osmosis, electrolysis,precipitation and adsorption which are very expensive. The latter is byfar the most versatile and widely used. However, thesemethods have different efficiencies for differentmetals and can be prohibitively expensive, especiallyin large volumes, at low metal concentrations and inthe presence of biota and suspended solids. Thechoice of purification method depends on the compositionof the system, the pH of the water, redoxconditions, and the nature of the pollutants.It is well known that aquatic plants can accumulatemetals taken in from the environment and concentratethem in trophic chains with accumulative effect(Wenhua, H., Chen, X., Song, G., Wang, Q. and Chang, C.C. (2007)). Significant copperaccumulation has been observed in other aquaticplants such as Ceratophyllumdemersum, Hydrilla verticillataLemnatrisulca Vallisneria spiralis andLemna minor (Razinger et al.,2009).The use of aquatic macrophytes, such as waterhyacinth, duckweed, and water lettuce, in wastewatertreatment has attracted global attention in recent years(Mohan, B.S. and Hosetti, B.B. (1997), asthese plants can be grown on the surface of stabilizationponds, and may contribute to nutrient recoveryfrom wastewater. Duckweed is a floating aquaticmacrophyte belonging to the botanical familyLemnaceae, which can be found world-wide on theChemical Speciation and Bioavailability (2010), surface of nutrient-rich fresh and brackish waters.Mohan, B.S. and Hosetti, B.B. (1997) reported that an increase innitrate concentration resulted in a significant increasein cadmium accumulation in Ulva fasciata.According to Padhi et al., 2010, nutrient enrichmenteither attenuated (chromium and zinc) orsuppressed (nickel) root biomass decrease in responseto metal exposure in Salvinia herzogii. Althoughheavy metal accumulation has been studied in L.gibba (Demirezen et al., 2007), the effect of nutrient additionon this species' metal tolerance is unknown.The aim of this study was to determine whethernutrient enrichment enhances the metal tolerance offloating macrophytes, which would therefore enablethe growth of floating vegetation in constructedwetlands at metal concentrations that would otherwiseinhibit plant viability.

II. Materials And Methods

2.1Gracilariaverrucosa (Hudson) Papenfus

Gracilariaverrucosa was collected from Kalijai area of Chilika Lake. Plants variable in length, about 15-35 cm in length attach to small stones and often worm tubes in bottom.(Padhi et al., 2010) Thallus erect, terete, highly branched, and attached by a small circular disc found with a single main axis, uniformly thin throughout branching lateral, occasional sub-dichotomous, opposite or sub-opposite in arrangement, frequently alternate branch tips attenuated, branches up to the third or even fourth order, ultimate branches 4-12 cm in length and 0.5 mm in thickness. Cortex of two to four layers of cells. Outermost radially elongated 10 µm x 7 µm. Some being modified to form enlarged cyst like cells produced on the surface as tubular hairs, inner cortical cell slightly larger and more or less oval. Medulla of 3-5 layers of cells around a large spherical or ovoid central cell about 300 µm across cells of medulla progressively smaller from the center outwards, oval 18 µm x 13µm – 14µmx 15µm.(Panigrahy, H.B, 2013). Transition in size from medulla to cortex is abrupt, Spermatangiain deep cavities. Carpogonial branching system with a supporting cell bearing a two celled carpogonial branch and two sterile branches of one or two cells each. Cystocarps sub-spherical elevated and scattered, 700 µm-900µm in height, 750-850 µm wide at the base and 900-1050 µm wide at the broadest part. Gonimoblast of densly aggregated radiating filaments. Carpospores in a short series, 10-13 µm in diameter sysmpodially developed at ends of gonimoblast filaments. Gonimonemata radiating. Tetrasporangia scattered 28-35µm long and 16-22 µm wide. (Swain, P. K., 2009)

2.2 Treatment condition:

The chemicalcomposition of the lake water was (mean ± standarddeviation): pH=6.5±0.2; conductivity = 92±8µScm^{-1};SO_4^{2-} =0.32±0.02 mg L^{-1}NH_4^+-N= 0.023±0.01 mg L^{-1};NO_3^--N= 0.022±0.001 mgL^{-1},NO_2^--N=0.001±0.001 mg L^{-1}.Before metal treatment, plants were acclimatized for5 days in laboratory conditions in lake water (23^{0}C and 14h photoperiod, 350 µmol m^2 s^{-1}). Plants werelater transferred to deionized water with nutrient anddifferent metal concentrations. Plants were treatedwith different concentrations of copper (0, 1, 2, 5,10 mg L^{-1}) and cadmium (0, 0.5, 1, 2, 4 mg L^{-1})and maintained in double deionized water in 500mLconical flasks under the aforementioned conditionsfor periods of 1, 3, 5 and 7 days. They were grown indeionized water added with the nutrients in concentrationsthat mimicked natural pond water. Maximumconcentrations of copper and cadmium were takenfrom Wenhua (2007). Plant growth rates in responseto metal exposures were compared with exposuresenriched with 5 mg L^{-1}P (KH_2PO_4), 5 mg L^{-1}NO_3^--N(KNO_3) and SO_4^{2-}(K_2SO_4). Flaskswithout metals grown alongside each set of experimentalgroups served as controls. The experimentslasted 7 days, when the plants exposed to the highestmetal concentrations developed chlorosis andnecrosis.After harvesting, plants were washed withdouble deionized water. Plants were placed on blotting paper and allowed to drain for 5 min beforeweighing. All treatments were carried out in triplicate.Gracilariaverrucossa relative growth rates were calculated ineach group according to Hunt's equation:

$R = \ln W_2 - \ln W_1 / T_2 - T_1$,

Where, R is the relative growth rate (gg^{-1}d^{-1}), $W_1$and W_2 are the initial and final fresh weights,respectively, and (T_2–T_1) is the experimentalperiod (Hunt, 1978).

2.3 Quantification of heavy metals:

After harvesting the plants were washed thoroughly withdouble deionized water, blotted and oven dried at 85^{0}C. Each sample was then digested with 10mLpure HNO_3, using a CEM-MARS 5 (CEMCorporation Mathews, NC, USA) microwave digestionsystem (maximum power: 1,200 W, power:100%, ramp: 20:00 min, pressure: 180 psi, temperature:210^{0}C and hold time: 10:00 min). After digestion,the volume of each sample was adjusted to25mL using double deionized water. Determinationsof Cd and Cu concentrations in all sampleswere carried out by inductively coupled plasmaoptical emission spectroscopy (Varian-Liberty II,ICP-OES) (Demirezen, 2007). Peach leaves (NIST,SRM-1547) and CRM 039-050 were used as referencematerial and also all analytical procedures wereperformed for reference materials. Samples wereanalyzed in triplicate.

2.3 Plant growth parameters

Plant biomass was measured on the basis of freshweight. Photosynthetic pigments of treated anduntreated plants (100 mg) were extracted in 80%chilled acetone in dark. After centrifugation at10,000 g for 10 min, absorbance of the supernatantwas taken at 450, 645 and 663 nm. The content ofchlorophylls and carotenoids were estimated by aspreviously described (Witham, 1971).

2.4Lipid peroxidation and electrical conductivity

Lipid peroxidation was determined by estimation ofthe malondialdehyde (MDA) content following theprotocol of Health and Packer (1968). MDA and ECcontent were not determined for the treatments158 Influence of nutrient addition on growth and accumulation of cadmium and copper in

Gracilariaverrucossaenriched with nutrients. Plant material (500 mg) washomogenized with 3mL of 0.5% thiobarbituric acidin 20% trichloroacetic acid. The homogenatewas incubated at 95^{0}C for 30 min and reactions werestopped on ice.

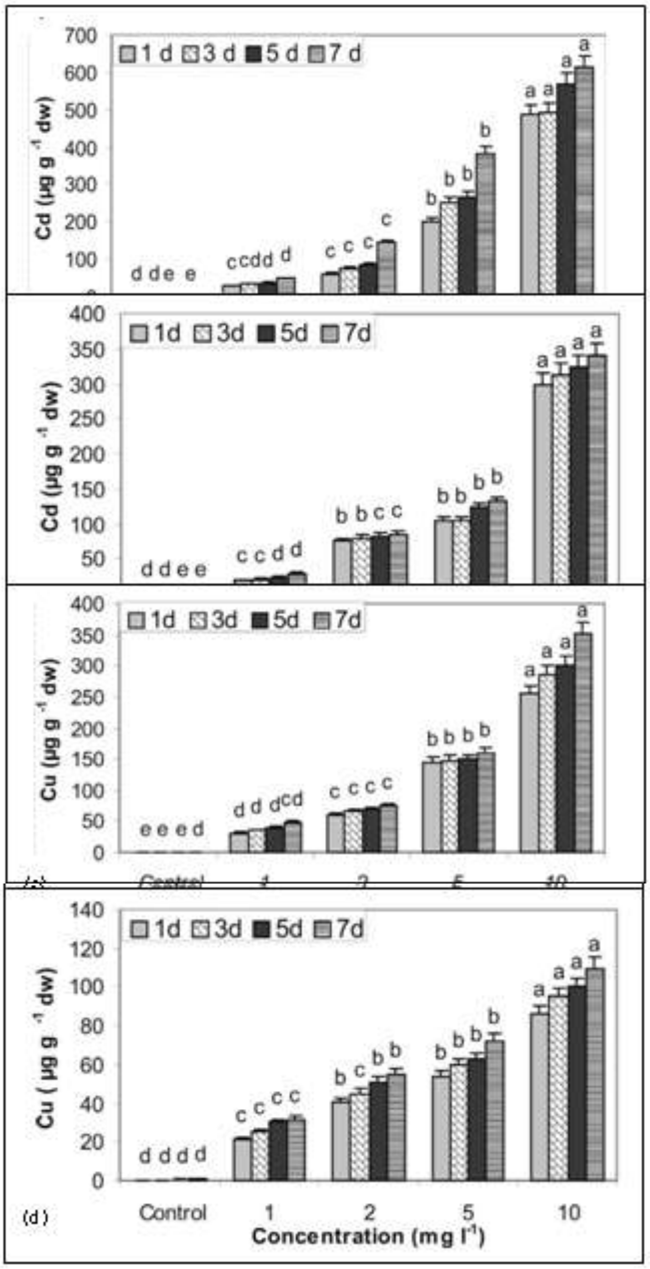

Figure.1 Accumulationof(a)Cd(b)Cdþnutrients(c)uand(d)CuþnutrientsbyGracilariaverrucossa exposedtodifferentconcentrations overvariousperiodsoftime.Allvaluesaremeansoftriplicates+SD.ANOVAsignificancewassetatP≤0:01.Differentlettersindicate significantly different values at a particular time point (DMRT, P ≤0:05).

The samples were centrifuged at10,000 g for 10 min and absorbance was recorded at532 and 600 nm. The amount of MDA was calculatedby subtracting non-specific absorbance at 600 nmfrom absorbance at 532 nm.EC as a measure of ion leakage was determinedaccording to Devi and Prasad (1998). Cadmium andcopper exposed plants were washed with doubledeionized water and 500 mg of plant material wasthen transferred to 100mL of deionized water for24 h to facilitate maximum ion leakage. EC of thewater was then recorded.

III. Results

3.1 Accumulation of heavy metals and effect ongrowth of plants

Plants accumulated high amounts of cadmium in aconcentration-time dependent manner. The highestCd accumulation was seen at a dose of 4 mg L^{-1}.The percent of the total Cd accumulated (615 mgg^{-1}) on day 7 at 4 mg L^{-1} was 79.1% on day 1,80.6% on day 3, and 92.8% on day 5 (Figure 1a). Inthe groups enriched with nutrients, Cd accumulationat 4 mg $L^{-1}$was 87.9% on day 1, 90.8% on day 3,and 94.7% on day 5 of the total Cd accumulated(341.2 mgg^{-1}) on day 7 (Figure 1b). Maximum Cuaccumulation was observed at a dose of 10 mg L^{-1}.The percent of the total Cu accumulated (351.6 mgg^{-1}) on day 7 at 10 mg $L^{-1}$was 72.7% on day 1,81% on day 3, and 85.3% on day 5 (Figure 1c). In thegroups enriched with nutrients, Cu accumulation at10 mg $L^{-1}$was 78.1% on day 1, 86.3% on day 3,and 90.9% on day 5 of the total Cu accumulated(110 mgg^{-1}) on day 7 (Figure 1d).Relative growth ratesof Gracilariaverrucossa decreased in thepresence of cadmium in a concentration dependentmanner (Figure 2). However, the treatments enrichedwith nutrients did not show a similar correlation (R=0.980). Relative growth rates of Gracilariaverrucossadecreased with copper in a concentration dependentmanner (Figure 2), but the treatments enriched withnutrients did not show similar correlation(R=0.940). Therefore, nutrient addition attenuatedthe decline in relative growth rates in response tometal exposure (Figure 2).

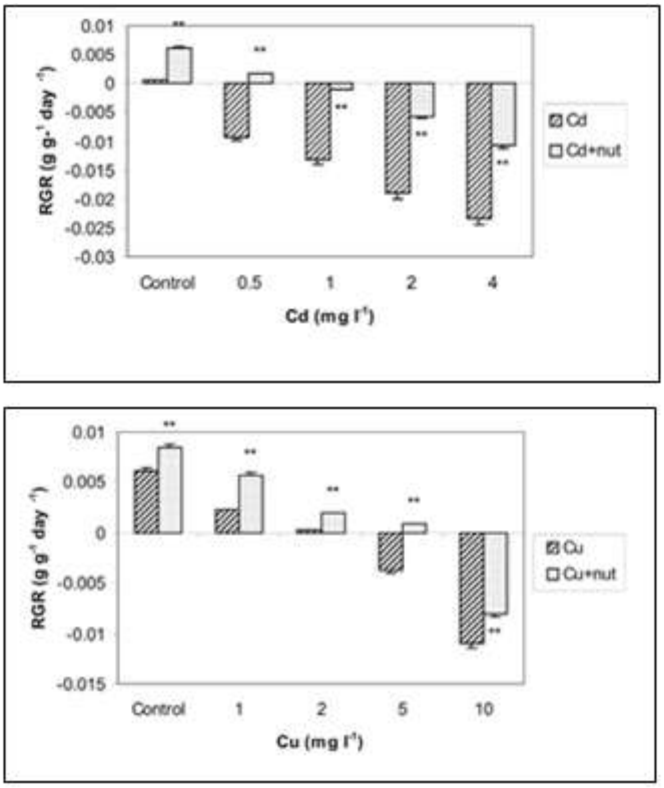

Figure2 RelativegrowthratesofCuandCdtreatedplants(after7day).Thebarsrepresentstandarddeviation.**(P＜0:01)indicate significant difference between the two treatment

IV. Discussion

In the present study, high accumulations of Cd andCu were observed in Gracilariaverrucossaover 7 days. Nutrientenrichment resulted in decreased accumulation of Cdand Cu in growing plants over the time. Growth ratesof Gracilariaverrucossa decreased with increasing cadmium andcopper concentrations. However, nutrient enrichmentled to increased growth rates at Cd and Cu concentrationsthat impaired growth in the non-enrichedgroups. Cd had a stronger effect on growth rates then Cu. LowconcentrationsofCuactuallyincreased plant growth rates, while higher concentrations had negativeeffects.

V. Conclusions

We have investigated the toxic effects of cadmiumand copper on Gracilariaverrucossa, and showed that nutrientenrichment increased the tolerance of Gracilariaverrucossa tometal contamination. This effect has important implicationsin the use of constructed wetlands for industrialwastewater treatment. Many metallurgicindustrial processes produce wastewater containinghigh concentrations of metal ions. Increased tolerancemay be useful for wastewater treatment by allowingmacrophyte growth at metal concentrations thatwould otherwise impair their development. Nutrientaddition will thus aid metal removal by increasingmacrophyte production, leading to a higher metaluptake by the macrophyte biomass, and also byenhancing overall biological activity, reaching ahigher metal retention in the detrital fractions.

References

[1]. Appenroth, K.J., Luther, A., Jetschke, G. and Gabrys, H. (2008) Modification of chromate toxicity by sulfate in duckweeds (Lemnaceae). Aquat. Toxicol., 89, 167 – 171.

[2]. Demirezen,D., Aksoy,A.andUruç,K.(2007) Effectofpopulation density on growth, biomass and nickel accumulation capacity of Lemnagibba (Lemnaceae). Chemosphere, 66, 553 – 567.

[3]. Demirezen Yamaz, D. (2007) Effects of salinity and nickel accumulation capacity of Lemnagibba (Lemnaceae). J. Hazard Mater., 147, 74 – 77.

[4]. Mohan, B.S. and Hosetti, B.B. (1997) Potentialphytotoxicity of lead and cadmium to Lemna minor grown in sewage stabi-lization ponds. Environ. Pollut., 98, 233– 238.

[5]. Padhi, S. B., Behera, G., Behura, S., Swain, P., Behera, S., Panigrahy, H., Panigrahy, M., Beja, S., Mishra A., Das N., Baidya S., Pradhan S. and Das P. (2010). Utilization of nitrate and ammonium by algal biomass available in prawn cultivation sites in Chilika Lake, Orissa. J. Bot. Res. **Vol. I.1**, pp.1-6

[6]. Padhi, S. B., Swain, P. K., Behera, S. K., Nayak, L., Panigrahy, H. and Samantray, S. M. (2008).Feasibility study for the cultivation of Kappaphycusalvarezii(Doty) Doty ex. P. Silva in the coastal areaof Ganjam district (Orissa). Indian Hydrobiology, 11(2), 297-301.

[7]. Panigrahy, H. B. (2013). "Studies on the effect of environmental factors on nitrogen uptake & assimilation in the red seaweed gracilariaverrucosa(hudson) &kappaphycusalvarezii(doty) doty ex. P. Silva." Doctoral thesis, Berhampur University. Berhampur.
Razinger, J., Drinovec, L. and Zrimec, A. (2009) Real-time visualization of oxidative stress in a floating macrophyte Lemna minor L. exposed to cadmium, copper, menadione, and AAPH. Environ. Toxicol., doi: 10.1002ytox.20518.

[8]. Swain, P. K. (2009). "Occurrence, seasonal productivity, growth dynamics, multiplication and conservation strategies of seaweed flora near Kalijaiarea of Chilika Lake under field and laboratory cultivation". Doctoral thesis, Berhampur University. Berhampur.

[9]. Wenhua, H., Chen, X., Song, G., Wang, Q. and Chang, C.C. (2007) Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (Lemna minor). Plant Physiol. Biochem., 45, 62 – 69.