IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

THE EFFECT OF COPPER AND CADMIUM ON THE GROWTH RESPONSE FOR FRESHWATER ALGAE *CLADOPHORA CRISPATA* (L.)

*Komala E[1] and Vidyavati.[2]

*[1]Department of Botany, Govt. Degree College Husnabad Dist; Siddipet– 505467

[2]Phycology laboratory, Department of Botany Kakatiya University, Warangal – 506001, T.S.

The objective of this work was to evaluate the effect of exogenously applied cadmium on the physiological response of algae *Clodophora crispata*. The study of the investigation of Green alga *Clodophora crispata*., was treated by high concentrations of Cu and Cd added into the nutrition medium. Cd strongly inhibited growth – it had been 60% decreased, followed by Cu – about 40% decreased, while the action of Cu gave the impression to be the less harmful. Cd impaired cell morphology and development, and worsen the algal culture. It had been found a progressive decrease within the amount of chlorophyl, b, and carotenoids adore the duration of heavy metals action. Cd had the foremost pronounced negative effect on the pigment composition of Cu in *C. crispata*. Meanwhile, Cu-treated variants remained less full of the metal toxicity. These results were supported by the changes in malondialdehyde (MDA) content showing an enhanced lipid peroxidation under Cu and Cd pollution. Therefore, it may be suggested that Cu influenced in a very different way metabolic processes within the algal cells than Cd. possessed a decent adsorbing capacity for metal ions, especially for cadmium, which was absorbed during a much greater extent than copper. For that reason, we assumed that the strain may well be employed in the treatment of wastewater, polluted by Cu and Cd.

Key Words: *Clodophora crispata*. Cu and Cd metals, pigments, malondialdehyde.

Introduction:

Macroscopic green algae are a highly diversified group and, because of their ability of fast spreading, they are considered to be pioneering organisms (Van den Hoek *et al.* 1995). Despite the common occurrence of macroscopic algae communities, information on the ecology of filamentous algae in scientific literature is scarce and fragmentary. Freshwater macroalgae consist of filamentous forms (*Cladophora, Oedogonium and Spirogyra*). *Cladophora* belongs to the group of macroscopic green algae with over 183 species (Munir *et al.*

2019). The occurrence of this annual filamentous macroalga is mainly due to the increasing content of nutrients (nitrogen and phosphorus) in the water. This nutrient enrichment results from the intensive agriculture (the use of mineral fertilizers), usage of detergents containing phosphorus, increased human populations, wastewater treatment plants etc. (Parker and Maberly 2000; Mihranyan 2011). Seasonal blooms of this alga constitute a serious environmental problem. To increase our capabilities for prediction of both the spread and cosmopolitan nature of *Cladophora*, detailed and relevant information on the ecology of the species is imperative.

Heavy metals are a class of potentially harmful contaminants. When heavy metals get into the water through various means, the first victims are algae. When microalgae have a long-term state with heavy metals stress in water, it will produce a series of corresponding adaptive mechanisms to mitigate the hazards of heavy metals on algae. Microalgae can make their own heavy metal concentration be maintained within a certain range of levels by absorption, accumulation, transfer, discharge of the heavy metals. Thus it can minimize the effects by heavy metals, ensuring its own growth.

Metals are necessary components of all ecosystems and occur naturally in the earth's crust (Pinto *et al.*, 2003). They appear in a wide range of oxidative states and coordination numbers, influencing their chemical characteristics and thus their bioavailability and toxicity (Pinto *et al.*, 2003; Verbruggen *et al.*, 2009). Certain metals such as iron (Fe), copper (Cu) and zinc (Zn) are considered essential nutrients to plants and are needed for photosynthesis and as cofactors for many enzymes (e.g. Kovacik *et al.*, 2010; Shanmugam *et al.*, 2011). Plants take up essential elements from their surroundings, but they are also able to accumulate elements, which have no known biological function, such as heavy metals like cadmium (Cd), chromium (Cr) or lead (Pb) (Mendoza-Cozatl and Moreno-Sanchez, 2005; Peralta-Videa *et al.*, 2009). These nonessential metals are able to enter plant cells via metal transporters and carriers for the uptake of essential metals (Shanker *et al.*, 2005). Aquatic environments are particularly exposed to increasing amounts of industrial and agricultural wastes (Kovacik *et al.*, 2010). They may contain Cd, Cr and Pb which are toxic to most organisms at low concentration and have serious negative effects on plant growth, development and photosynthesis (Panda and Choudhury, 2005). Experimental amelioration of heavy metal effects by addition of antioxidants or essential ions provides insight into uptake and distribution mechanisms as well as on physiological and sub-structural targets of metals and increases our understanding on possibilities to limit damage to an aquatic ecosystem.

The toxicity of heavy metals on microalgae there are two roles of effects of heavy metals on microalgae. On the one hand, some heavy metals are essential trace elements in algae growth, such as Fe, Mo, etc. But these essential trace elements also need to be controlled within a certain range of concentrations. On the other hand, some heavy metals will have toxic effects on algae (Liu *et., al.*, 2015).The toxic effects of heavy metals on the microalgae is mainly reflected in the way of hindering cell division, inhibiting the photosynthesis as well as the synthesis of organic compounds, reducing the activity of enzyme, etc.

Cu and Cd are among the most abundant environmental pollutants. Cu and Cd are nonessential but highly toxic metals to living organisms (Leborans and Novillo 1996): and copper, at excessive concentrations, is also harmful. The ability of green alga *Cladophora crispata*, to modulate polluted environments via its metabolic activity determined the purpose of the present work investigation of Cu^{2+} and Cd^{2+} influence on some physiological and biochemical parameters of *Cladophora crispata* cells.

Materials and methods

Algal Materials *Cladophara crispata* were collected from natural ponds and grown in a modified Chu No.10 medium18 in the laboratory under controlled conditions (25 ± 2^0C, 45 mmol m-2s-1 photon flux intensity, and 16h/8h light and dark cycle). The final pH of the solutions were adjusted to 5.0. One gram fresh weight of algae was inoculated into each flask containing various concentrations of Cu and Cd. Algae cultured in the nutrient medium without heavy metals served as controls. All experiments were performed in triplicate. Heavy metals (Cu and Cd) treatment was performed by adding into the nutrition medium (25, 50, 75 and 100 μM) of Cu^{2+} and Cd^{2+} (added as $CuSO_4$ and $CdCl_2$). Growth and physiological changes of the algal cultures were determined on the 3rd, 5th and 7th day from the start of the treatment.

Cladophara crispata growth rate was measured by the increase of dry weight. Pigment content was determined spectrophotometrically after methanol extraction and calculated according to (McKinney 1944). Malone dialdehyde (MDA) content was determined according to (Dhindsa *et al*.1981), using the extinction coefficient of 155 mM−1 cm−1. The content of Cu, and Cd in dry algal biomass was analysed by an atomic absorption spectrophotometer Perkin-Elmer. The experimental data were averaged of triplicate measurements. The significance of differences between control and each treatment was determined using Student t-test, $p \leq 0.05$

Toxicity Symtoms oxicity: Symtoms After the exposure of *C. crispata* to Cu and Cd, the algae were harvested at the end of each test duration (2, 4, 6 and 8 days). Toxicity symptoms of treated and control algae were observed under a compound transmission light microscope. Relative Growth Treated and control algae were gently blotted and weighed after each harvest on day 2, 4, and 8. Relative growth of control and treated algae were calculated as follows.

Results and Discussion:

Responses of algal cells to metal exposure are typically measured in terms of cell density, biomass, growth rate, and chlorophyll content or chlorophyll absorbance. The strongest toxic effect of heavy metal ions that occurred was found in the cultures of *C. crispata* polluted by cadmium (Fig. 1). Cu^{2+}-treatment caused lower levels of toxicity, followed by Cd^{2+} which appeared to be the least harmful. The highest reduction of algal dry weight was measured after 100 μM Cd was added to the medium – growth rate was more than 60% reduced as compared to control. A similar inhibition of growth was also observed after applying of 100 μM Cu – about 50% below the control. However, algal cells exposed to Cu (50 and 100 μM), did not significantly reduce their growth rate until 72nd hour of treatment, when the dry weight of biomass remained close to the control samples.

Studying the effect of cadmium on the algae several researchers found such a severe growth inhibition, as we did (Carr *et.al.*, 1998 Mohammed and Markert 2006)), etc. In addition to the decrease of growth, high cadmium concentrations disturb photosynthesis and reduce cell size (Leborans and Novillo 1996). The excess of Cd, in turn, causes stunted growth, chlorosis, inhibits photosynthesis, mineral nutrition and water balance

(Maldonado *et.al.* 2011). Cadmium and lead are considered to affect growth by different mechanisms than Cu they can form stable complexes with some structural proteins and enzymes, and thus negatively influence cellular metabolism. High Cu concentration could affect the normal growth and development of the plant cells by the ability of copper to displace other metal ions from physiologically important centers. Cu causes a decrease of chlorophyll a, photosynthetic O_2 evolution, cell division rates, as well as growing numbers of deformed and broken cells (Rijstenbil *et.al.*, 1994a).

In proliferating algal cultures, the growth of protozoa is obviously strongly inhibited by algae. However, we observed that the increased levels of heavy metals (Cu and Cd) caused a rapid development of unicellular eukaryotic organisms, especially in Cd and Cu treated variants. 100 µM Cd forced *C. crispata* to form rounded auto spores with slow development, as well as multiple agglutinates in the medium. Moreover, nearly 30% of the biomass belonged to protozoa. When Cd ions were added to the medium, about 20% of the cells were rounded in shape, with impaired morphology. There were very few single cells mostly agglutinates of 2–4 cells, and almost 10% of the biomass consisted of protozoan organisms (data not shown). Furthermore, depending on the type of heavy metal treatment, different morphological changes were observed in *C. crispata* cells. For example, when treated with divalent ions, such as Cd^{2+} and Cu^{2+}, the unicellular forms were predominantly observed, while Cr^{6+} determined the appearance of irregularly shaped prisms and grouped cells.

Table 1. **Effect of Cu and Cd (mg g–1 DW) in on the growth response of Fresh Water Algae *C. crispate.***

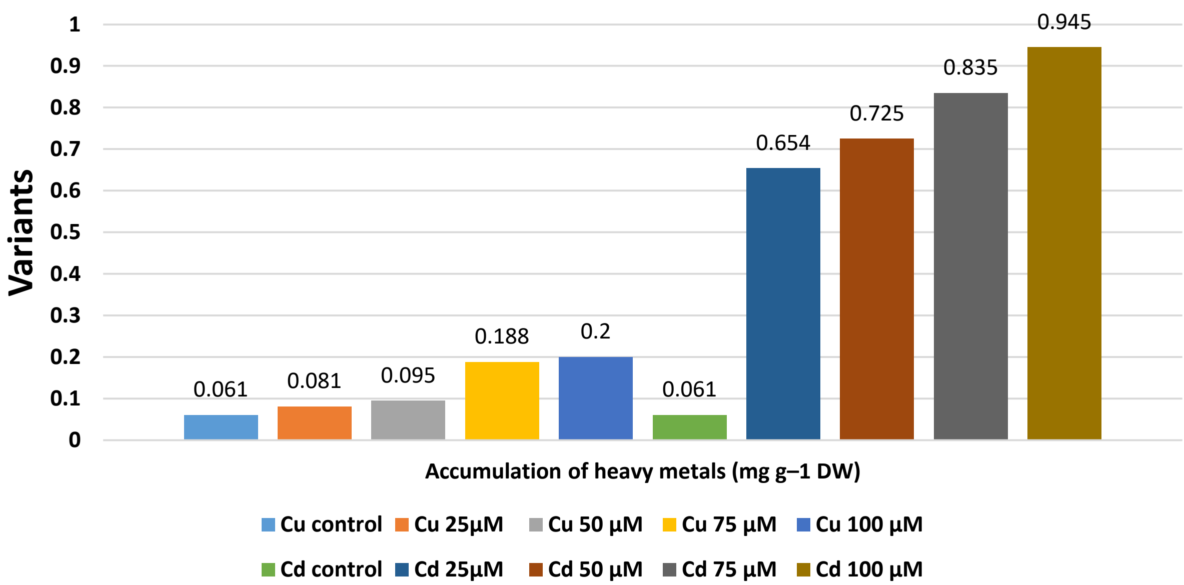
Heavy metals	Variants	Accumulation of heavy metals (mg g–1 DW)
Cu	control	0.061 ± 0.02
	25µM	0.081 ± 0.05
	50 µM	0.095 ± 0.04
	75 µM	0.188 ± 0.04
	100 µM	0.200± 0.05
Cd	control	0.061 ± 0.02
	25µM	0.654 ± 0.43
	50 µM	0.725 ± 0.32
	75 µM	0.835 ± 0.45
	100 µM	0.945 ± 0.46

Studying the changes in *C. crispata* pigment composition, we found a strong decrease in the amount of chlorophyll a in all heavy metal polluted variants. Both, Cd and Cu severely reduced chlorophyll a content the lowest level was measured at 100 Cd µM – 88% decrease as compared to the control. Cu, in fact, caused a significantly lower reduction in chlorophyll a (nearly 45%). Changes in the amounts of chlorophyll b in the biomass followed an equal trend to that one of chlorophyll a. The decrease of chlorophyll b content was most pronounced in Cu and Cd treated suspensions 84% at 100 µM Cd and 87% at 100 µM Cu (Fig. 3).

When analysed the amount of carotenoids, we observed a similar tendency to decline in all experimental variants (Table-I). Their content progressively decreased with time of exposure and reached the lowest values on the 7th day of treatment. Moreover, the decrease of carotenoids was relatively independent of the heavy metals concentrations that were used 50 or 100 μM. The only exception was 50 μM Cd, wherein carotenoids responded by a relatively lower decrease at the beginning of the period they were 32% decreased. Thus, there was no reason to believe that carotenoids possessed any protective role against heavy metal stress developed in *C. crispata*.

Copper and Cadmium damage cell membranes by binding to the sulfhydryl groups of the membrane proteins, resulting in peroxidation of the lipids. Changes of malondialdehyde amounts are often used as a routine method to determine the degree of lipid peroxidation in the plant cells, and increased MDA content have been frequently registered as a result of heavy metal toxicity. In the course of the experimental work, we have also found severely elevated levels of MDA in Cu- and Cd polluted *C. crispata* cultures. The highest values were recorded at 50 μM and 100 μM Cd in the medium (2 2.5) times higher than the control, respectively). 100 μM Cd caused 70% increase of MDA, while Cu-treated variants remained close to the control. The only exception was at 50 μM Cd, where MDA was even reduced – it was 30% below the control value.

Fig I. Effect of Cu and Cd (mg g–1 DW) in on the growth response of Fresh Water Algae *C. crispate.*



On the other hand, it should be taken into account that the rate of heavy metals absorption by plants is highly pH-dependent (Pena-castro *et.al.*, 2004). Some of the appropriate culture media could affect the removal rate for some bivalent metal ions, and respectively, the efficiency of microalgal wastewater treatment. For practical microalgal application in bioremediation of heavy-metal polluted areas, a further study is necessary to ascertain the influence of higher pH values on the efficiencies in the heavy metals absorption/ adsorption. In the current experiments, the pH value was maintained around 7.0. *C. crispata*, grown under these conditions, in fact, cannot be considered as a strain that strongly accumulates heavy metals, unlike some other microalgae. This conclusion follows from the results of our previous studies (Chaneva, *et.al.*, 2005), in which we have found that some cyanobacteria and green algae accumulated heavy metals in their biomass at much higher concentrations than *C. crispata* did.

References:

Chaneva, J.G. Uzunova, S. Furnadzhieva (2005): Cadmium Toxicity in Coincubation System Pisum sativum/Plectonema boryanum. *J Environ Prot Ecol*, 6 (2), 271.

Dhindsa, R. S. Plumb-dhindsa, P.Thorpe T. A. (1981): Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase. *J Exp Bot*, 32, 93 (1981).

G.F. Leborans, A. Novillo (1996): Toxicity and Bioaccumulation of Cadmium in *Olisthodiscus luteus.* Water Resources, 30 (1), 57

Kovacik J, Klejdus B, Hedbavny J, Backor M (2010). Effect of copper and salicylic acid on phenolic metabolites and free amino acids in *Scenedesmus quadricauda* (Chlorophyceae). *Plant Sci; 178*(3):307–11.

Leborans, G. F Novillo A. (1996) Toxicity and Bioaccumulation of Cadmium in *Olisthodiscus luteus.* Water Resources, 30 (1), 57.

Liu DM, Wang ZW, Zhu Q (2015). Drinking water toxicity study of the environmental contaminant—Bromate [J]. *Regulatory Toxicology and Pharmacology*, 73(3): 802-810

Maldonado, J. Sole A., Puyen Z., Esteve I. (2011): Selection of Bioindicators to Detect Lead Pollution in Ebro Delta Microbial Mats, Using High-resolution Microscopic Techniques. Aquat Toxicol, 104, 135 (2011).

Mckinney G. (1944): Absorption of Light by Chlorophyll Solution. J Biol Chem, 140 (2), 315 H. Carr, P, Carino F.A., Yang, M. S. Wong M. H. (1998): Characterization of Cadmium-binding Capacity of Chlorella vulgaris. Bull Environ *Contam Toxicol*, 60, 433

Mendoza-Cozatl DG, Moreno-Sanchez R. (2005) Cd2+ transport and storage in the chloroplast of *Euglena gracilis*. *Biochim Biophys Acta*; 1706(1–2):88–97

Mihranyan A (2011) Cellulose from Cladophorales green algae: from environmental problem to high-tech composite materials. *J Appl Polym Sci* 119:2449–2460.

Mohammed, M, Markert B (2006): Toxicity of Heavy Metals on *Scenedesmus quadricauda* (Turp.) de Brébisson in Batch Cultures. ESPR – *Environ Sci & Pollut Res*, 13 (2), 98 (

Munir M, Qureshi R, Bibi M, Khan AM (2019) Pharmaceutical aptitude of Cladophora: a comprehensive review. Algal Res 39:101476.

Panda SK, Choudhury S (2005). Chromium stress in plants. Braz J Plant Physiol;17(1):95–102.

Parker JE, Maberly SC (2000) Biological response to Lake Remediation by phosphate stripping: control of *Cladophora. Freshw Biol* 44:303– 309.

Pena-castro J., F. Martinez-jeronimo, F. Esparza-garcia, R. Canizares-villanueva (2004): Heavy Metals Removal by the Microalga *Scenedesmus Incrassatulus* in Continuous Cultures. Bioresour Technol, 94, 219.

Peralta-Videa JR, Lopez ML, Narayan M, Saupe G, Gardea-Torresdey J (2009).The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. Int J Biochem Cell Biol ;41 (8–9):1665–77

Pinto E, Sigaud-Kutner TCS, Leitao MAS, Okamoto OK, Morse D, Colepicolo P.(2003) Heavy metal-induced oxidative stress in algae. *J Phycol; 39*(6):1008–18

Rijstenbil, J. W. Derksen J., Gerringa, L. Poortvliet T., Sandee A., Vandeberg M., van Drie, J. Wijnholds (1994a): Oxidative Stress Induced by Copper: Defense and Damage in the Marine Planktonic Diatom Ditylum brightwellii Grown in Continuos Culture with High and Low Zinc Levels. Mar Biol, 119, 583 (1994a).

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005). Chromium toxicity in plants. *Environ Int;* 31(5):739–53.

Shanmugam V, Lo JC, Wu CL, Wang SL, Lai CC, Connolly EL, et al. (2011) Differential expression and regulation of iron-regulated metal transporters in Arabidopsis halleri and Arabidopsis thaliana - the role in zinc tolerance. *New Phytol*; 190 (1):125–37.

Van den Hoek C (1982). A taxonomic revision of the American species of *Cladophora (*Chlorophyceae) in the North Atlantic Ocean and their geographic distribution. Royal Netherlands Academy of Arts & Sciences, Amsterdam, pp 1–236.

Verbruggen N, Hermans C, Schat H (2009). Mechanisms to cope with arsenic or cadmium excess in plants. *Curr Opin Plant Biol*; 12(3):364–72.