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Research Article

EFFECTS OF HEAVY METALS (Cu AND Zn) ON ACCUMULATION OF BIOMASS IN *CHLAMYDOMONAS REINHARDTII* AND *ASTERARCYS QUADRICELLULARE*

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ABSTRACT

The present work aims to know the effects of heavy metals such as copper (Cu) and zinc (Zn) on growth and accumulation of biomass *i.e.* content of chlorophylls in *Chlamydomonas reinhardtii* (*C. reinhardtii*) and *Asterarcys quadricellulare* (*A. quadricellulare*). Both the species are fresh water group of green algae which belong to class chlorophyceae. Algal species were grown in tris-acetate phosphate (TAP) medium along with various concentrations (0.0, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/l) of copper and zinc individually under *in vitro* conditions. In order to assess the impact of heavy metals on both the algal species, *in vitro* grown log phase cells were used to estimate the chlorophyll a and b including total chlorophylls. Best growth condition and high total chlorophyll content was observed at TAP with 0.2 mg/l of copper in *A. quadricellulare*, when compared to *C. reinhardtii* which preferred 0.4 mg/l to generate more content. In zinc treatment, total chlorophyll content was more at 0.4 mg/l dose in *A. quadricellulare* and 1.6 mg/l dose in *C. reinhardtii* and earlier one possess more biomass. In conclusion, both the species exhibited differential dose resistance with the case of copper and zinc metals. Both being essential heavy metals, copper showed more toxicity when compared to zinc metal.

Keywords: Heavy metal, Copper, Zinc, *C. reinhardtii*, *A. quadricellulare*, Chlorophylls.

1. INTRODUCTION

Enhancement of various pollutants in environment due to natural as well anthropogenic reasons urges the society to study in depth about causative agents. Heavy metal stress is a major concern apart from other abiotic stresses such as drought, salinity, extreme temperature, light etc. Main sources of heavy metal pollution originate from both natural and anthropogenic reasons which include weathering, mining, smelting, chemical industries and agricultural waste [1] (fig. 1). Specifically through industries, heavy metals such as cadmium, lead, cobalt, chromium, copper, zinc, mercury etc., were infiltrated in to the environment rapidly [2]. This situation directly affects the ecosystem's growth and balance. Broadly, heavy metals are divided into essential and non-essential groups and accumulated in all the living organisms including animals, plants and microorganisms [3]. Initially, these heavy metals are accumulating in the soil and later moved to surface waters and finally enter into the living organisms. The rate of movement and entry is completely depends on the metal, its interaction with soil and water, its concentration and also the type of living organism. The effect of heavy metals on plants including primitive plant group such as algae is insufficient because of their complex influence [4].

Algae are a broad group of organisms and mostly autotrophic in nature. They are classified diversely by several researchers based on origin, habitat, pigments and phenotype. Algal species are directly used as food and also for production of food supplements, nutra-ceuticles, medicines and biofuels. Recently, certain algal species are using for water quality analysis [5]. Algae are available in the form of single or multicellular organisms and found mainly as aquatic species. Among aquatic species, both fresh and marine algae are available in nature in a large number. In fact, microalgae can grow in any kind of water and utilize the nutrients from all kinds of effluent water, thereby play a crucial role in wastewater remediation [6]. Under optimal conditions, they exhibited intense growth with sufficient biomass.

But in the natural environment, rapid growth of algae called as algal bloom can be seen where the nutrients such as phosphorous and nitrogen runoff from fertilizers and enter into the aquatic system. In this condition, algae generate toxins which in turn exhibit impact on surrounding environment. Apart from algal bloom toxins, excess chemical fertilizers and pesticides, industrial waste and lack of sufficient wastewater treatment methods enhances the biogenic compounds in ground water [7].

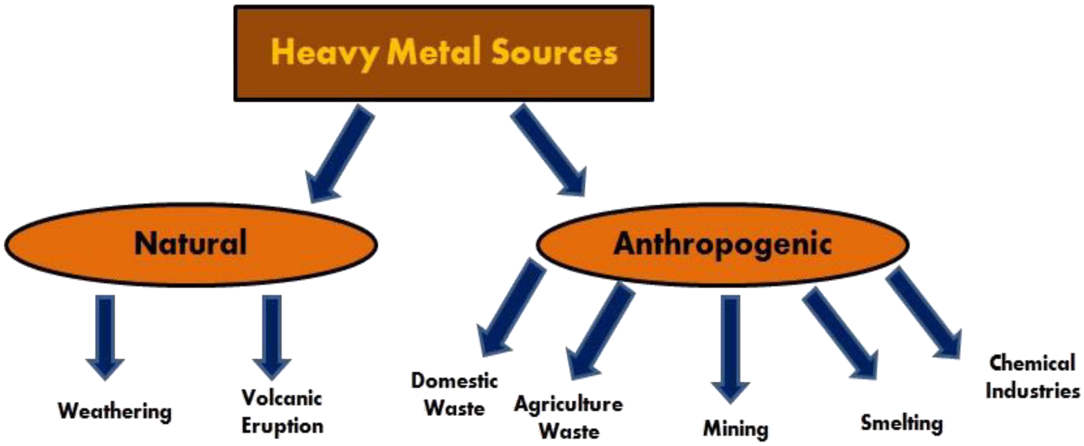

Fig. 1: Natural and anthropogenic sources of heavy metals

Algal growth in contaminated areas is generally distinguished by their resistance to the heavy metal content [8]. Though numbers of species of algae are available in nature, few are suitable for wastewater treatment. The algal species which showed the heavy metal resistance may be used for phytoremediation [9]. This in turn also leads to isolation of rare earth metals from metal rich areas. Recently, research is being focused on wastewater treatment using algae for biogenic compounds and also for certain medicinal compounds. However, few essential heavy metals are reported to be toxic at higher doses, for example zinc, manganese and copper are essential only at very low concentrations [10-11]. The effects of heavy metals on growth and development including physiological, biochemical and molecular properties have been studied by a number of workers [1, 12]. Several works proved the beneficial and lethal effects of heavy metals on various algal species [13-15]. The main aim of this study is to evaluate the effect of copper and zinc on the accumulation of photosynthetic pigments such as chlorophylls in the cells of *Chlamydomonas reinhardtii* and *Asterarcys quadricellulare*.

2. MATERIAL AND METHODS

In the current work, green algal species *i.e. C. reinhardtii* and *A. quadricellulare* MN179327 were collected from Acharya Nagarjuna University and University of Madras, India. Later, collected species were stored as conventional glycerol stocks in -80°C and some of the cultures were maintained in agar plates or agar tubes for regular experiments. These agar plates and tubes were sub cultured every 10 days to get fresh algal cultures for inoculation. Further algal medium preparation, inoculation and maintenance of *in vitro* cultures including extraction and estimation of pigments are the various stages in the present investigation. Before going to prepare various algal media, glassware were washed properly using appropriate detergent solution. Later, washed glassware were cleaned under running tap water, rinsed with distilled water and finally dried in a hot air oven. In the present experiment, tris-acetate phosphate (TAP) medium along with various doses (0, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/l) of Zinc chloride and Copper chloride used as heavy metal source to test the algal growth and development.

Media preparation, inoculation and culture growth were carried out under *in vitro* conditions. The pH of the algal medium was adjusted to 7.0 using 0.1 N HCl or 0.1 N NaOH solutions. Generally 250 ml conical flasks were used for 50 ml TAP medium but algal culture initiation was carried out in 30 ml serum vials with only 5.0 ml TAP medium. All the algal media prepared were autoclaved at 121°C and 15 lbs/in^2 for 20 min. A minimum ¾ empty space is compulsory in the flask or vial for better algal growth and development. Generally each alga will take a period of 2-3 days for optimal growth and this depends on the species in TAP medium. Hence, it is must to maintain gaseous phase in flasks or vials. After the completion of sterilization of the medium, inoculation was performed in laminar air flow (LAF) chamber. Before going to inoculation, all the required materials such as sterilized loops and needles, small wood sticks, algal media including algal plates and tubes were transferred to inside of the LAF chamber. After inoculation, all the cultures were incubated in an orbital shaker with 120 rpm at 25°C. Inoculated algal species were grown under normal light condition using white fluorescent tubes. After reaching the log phase both the algal cultures were removed and used for chlorophyll estimation. Further, extraction and estimation of chlorophyll a and b including total chlorophylls was carried out using Arnon's method [16]. Statistical works have been carried out using personal computer programs with obtained data.

3. RESULTS AND DISCUSSION

In the present study, an attempt has been made to know the effects of copper and zinc along with TAP medium on growth of algal cultures under *in vitro* conditions in turn estimated the biomass. Total data was collected with log phase cultures and all the results i.e. contents of chlorophylls obtained were documented below.

The present results as illustrated, exhibited clear differences in pigment content (chlorophyll a and b and total chlorophylls) of algal cells between treated and untreated control algae which were exposed to various doses of copper and zinc metals. The chlorophyll content gradually increased up to certain level of the metal concentration, whereas higher concentrations cause a clear reduction in the chlorophyll contents in both *C. reinhardtii* and *A. quadricellulare.* In copper treatment, improved total chlorophyll content was observed at TAP with 0.2 mg/l of copper in *A. quadricellulare*, when compared to *C. reinhardtii*. In contrast, *C. reinhardtii* generated more total chlorophyll content in TAP with 0.4 mg/l, but overall it is less when compared to *A. quadricellulare* (fig. 2 A, B and 3 A, B).

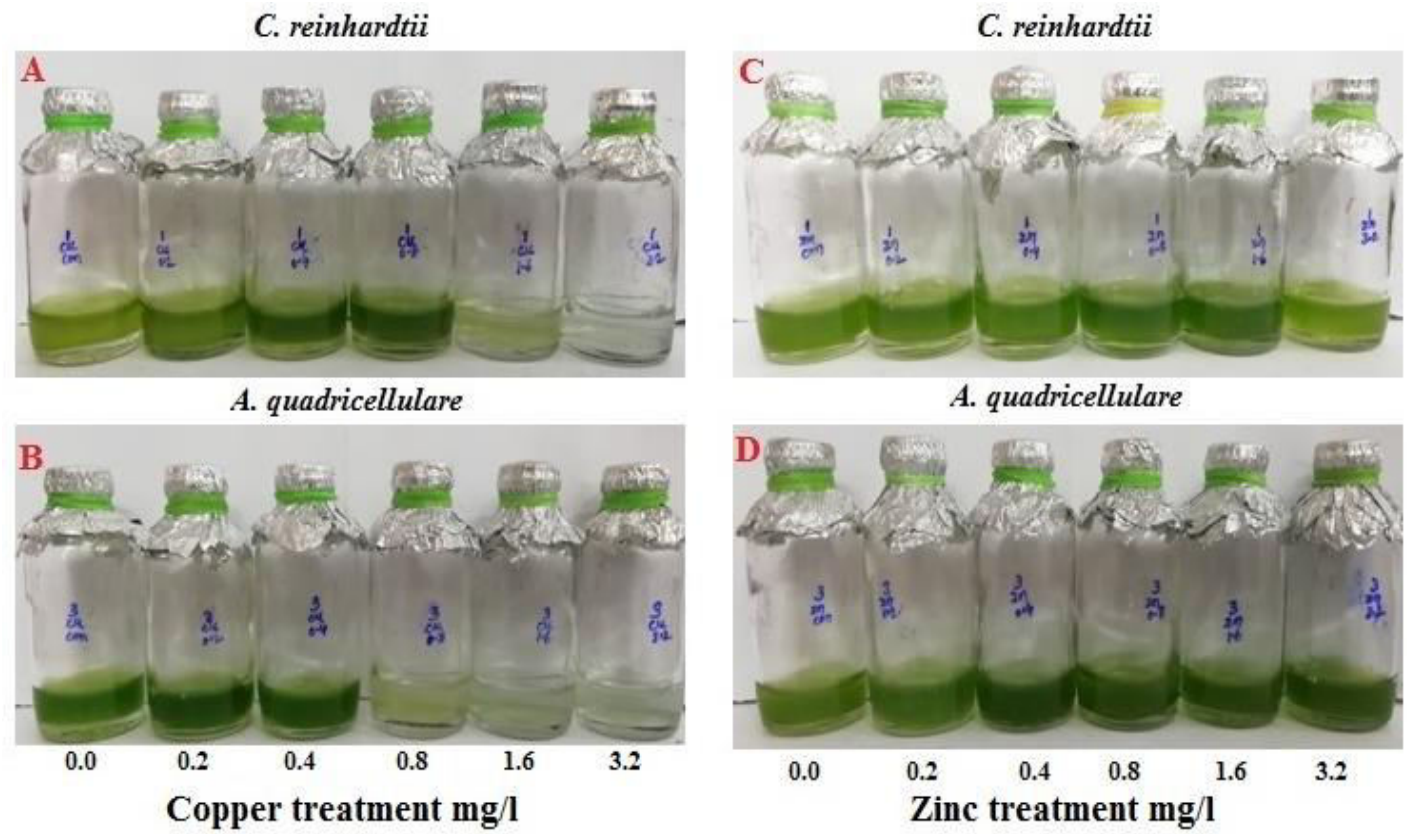

Fig. 2: Effects of Cu and Zn on both the algal species

In zinc treatment, we observed high growth rate and chlorophyll content at 1.6 mg/l in *C. reinhardtii* and 0.4 mg/l in *A. quadricellulare* compared to untreated control. Later, decreased growth and chlorophyll content in both the species at higher metal concentrations was noticed (fig. 2 C, D and 3 C, D). Decline in cell growth in both the species was noticed at the concentration of 3.2 mg/l of copper but with zinc treatment, both exhibited resistance at 3.2 mg/l concentration. Similar inhibition was noticed in *Chlorella vulgaris* in the earlier works [17]. In contrast, Kondzior and Butarewicz [18] proved that higher concentration of both zinc and copper damages the growth and reduce the chlorophyll content. Though it is essential heavy metal, once if it crosses its critical level then it shows adverse effects in most of the occasions i.e. alterations in morphological and biochemical pathways [19]. The current results exhibited that the low dose of the tested metals had stimulatory effects in biomass yield in both algal species, whereas the higher doses were inhibitory depending on the type of the metal. Moreover, reduction of chlorophyll content is a common symptom of heavy metal toxicity [20]. The results also showed that the inhibitory and stimulatory effect of heavy metals is completely depending on their concentrations. The adsorption efficiency completely depends on the type of metal ions, number of charges and the affinity of the binding site for each metal including type of algal species [21].

In several reports, it was proved that the inhibitory effect of stress become more with an increase in metal dose and suggested that the reduction in the growth rate is due to inhibition of synthesis of chlorophyll in turn photosynthesis. There is damage of cell wall at higher doses of heavy metals which can cause uncontrolled exchange of vital ions and can be liable for the inhibition of growth. Though variation in resistance, Wan Maznah et al [22] showed that both *Chlorella sps.* and *Chlamydomonas sps.* can be good for use in the biosorption of copper and zinc.

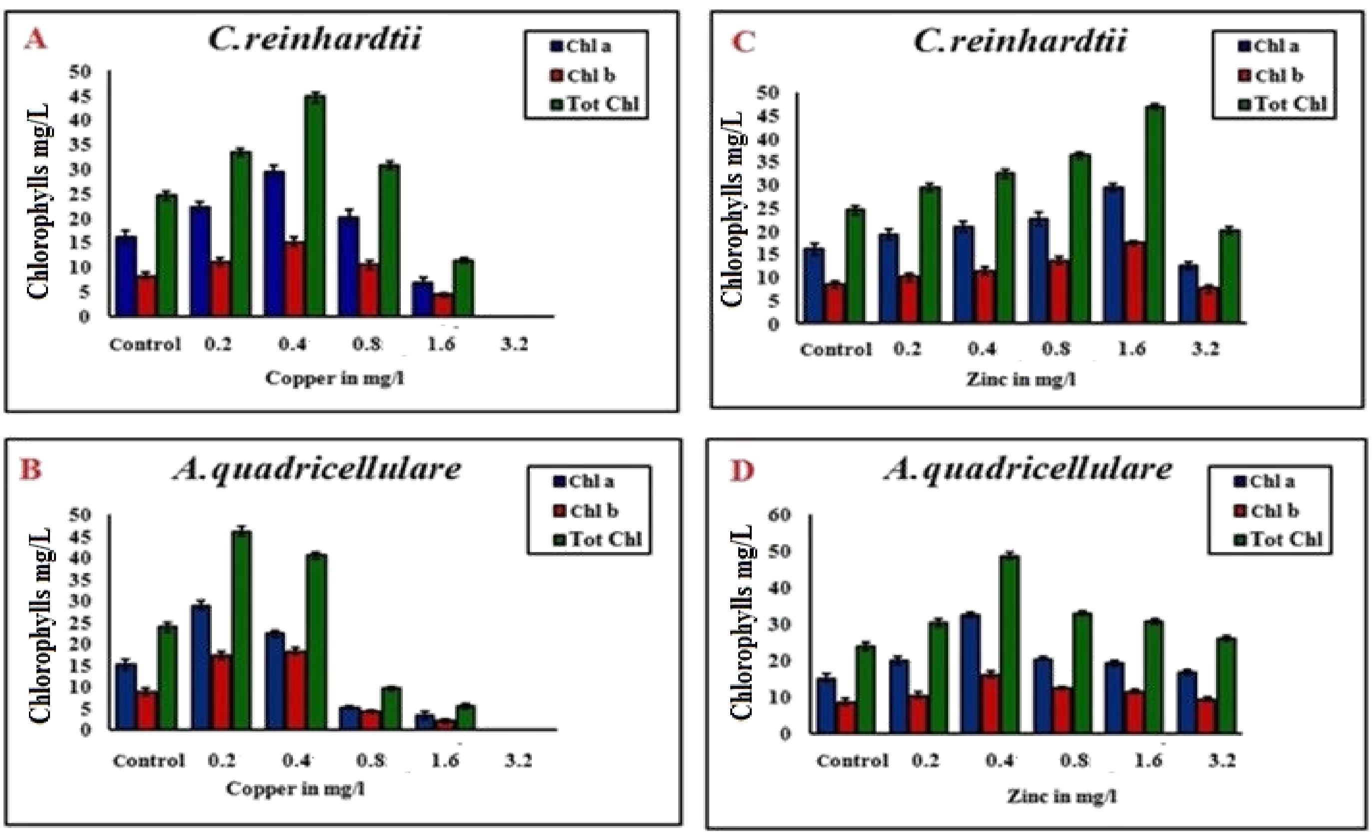

Fig. 3: Effect of copper and zinc on chlorophyll contents in *C. reinhardtii* and *A. quadricellulare*

4. CONCLUSION

Different algal species have different sensitivities to metals and therefore the same organisms could also be more or less damaged or promoted. Both copper and zinc are an essential micronutrient at lower concentration. In our work, the results showed that optimal concentrations of copper and zinc increased the growth and chlorophyll content in both species. But increased metal concentrations lead to decreased growth and chlorophyll content in both *C. reinhardtii* and *A. quadricellulare*. In conclusion, the inhibitory and stimulatory effects of both copper and zinc completely depend on their concentration and algal species.

5. ACKNOWLEDGEMENTS

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Conflicts of interest

The authors declare no conflict of interest

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