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Effect of Cadmium on Chlorophyll Accumulation in *Asterarcys quadricellulare* and *Chlorococcum minutum*

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ABSTRACT
Algae generally habitat both marine and fresh water bodies and hold the capacity to grow even in non-potable water sources. Due to useful carbohydrate, lipid, protein and significant molecular substrate production, algae are being explored as a possible feedstock for bioproducts including fuel production. But environmental stress such as heavy metal pollution influences the growth and development of algal species. Even certain essential heavy metals exhibit the deleterious effects. In contrast, certain algae will used as heavy metal remediation process. Cadmium is one of the toxic heavy metal which affect the growth and development of several plant species including algae. In the present study, we focused on the effect of cadmium on chlorophyll accumulation in two algal species namely Asterarcys quadricellulare and Chlorococcum minutum. Tris-acetate-phosphate medium along with 0.4 mg/L cadmium chloride exhibited improvement in growth and total chlorophyll content in A. quadricellulare cultures when compared to control cultures. In contrast, enhancement of total chlorophyll content was noticed in TAP with 0.8mg/L cadmium chloride in C. minutum. Overall, differential behavior with respect to chlorophyll accumulation was observed in both the cultures when treated with cadmium chloride.
Keywords: *Heavy metal, Cadmium, Chlorophyll, Asterarcys quadricellulare, Chlorococcum minutum.*

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INTRODUCTION

Algae are a crucial staple for food, fodder, medicine, fertilizer and energy industries. Some of the known algae contain significant quantities of carbohydrates, proteins, vitamins, minerals and lipids [1]. Algae are divided into micro and macro groups which belong to both terrestrial and aquatic species based on the habitat. Among aquatic species, both marine and freshwater algal species are available in mass number [2]. In general, unicellular algae are also considered as microorganisms. Also, based on the pigment accumulation, algae are divided into blue-green, green, red and brown groups. Specifically, both green and blue-green algae are popular species for generation of bioproducts and biofuels due to their efficient photosynthetic pathway [3, 4]. Due to simple life cycle and short harvesting cycle algae considered as bioproduct resource. In algal species, the nutrient content may vary from species to species which in turn depends on the temperature, location, season etc. But certain countries are neglecting algal species and therefore they became orphans without use [5].

Generally, unicellular microalgae mainly possess simple structure and have chlorophyll as their primary photosynthetic pigment. Therefore, chlorophyll accumulation in unicellular species is a paramount concern and key for further growth and development of the algae [6]. Further, it is very important to focus on chlorophyll content which in turn dictates the overall yield. But pollutants such as heavy metals influence the growth and development of living organisms including algae. Heavy metals are generally classified as essential and non-essential based on their usage. Certain essential heavy metals when cross their critical level may cause metabolic toxicity and growth inhibition [7]. Moreover, non-essential heavy metals such as mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb) etc., are normally not used due to their noxious nature and they may become hazard to growth and development. In contrast, certain algal species are used for phytoremediation process to remove or isolate the heavy metals [8]. Knowledge on toxicity of various heavy metals is known since several decades and it is interesting that the molecular research on this area focused recently. Some of the earlier studies had focused on heavy metal effects on different algal species [9].

Cadmium is extremely toxic metal which cause inhibition of growth and development in plant species including algae. Cadmium commonly found in industrial places specifically in manufacturing of batteries, paints etc., which appears frequently in the food chain [10]. Cadmium stress generally lead to chlorosis which seriously affects the photosynthesis in turn yield. Reduction in ATPase activity of plasma membrane was noticed by cadmium treatment [11]. Cadmium stress also inhibits the nitrate reductase activity in turn reduced nitrogen fixation in plant groups [12]. Cadmium causes chloroplast alteration, inactivates CO_2 fixing enzymes and also influences the lipid peroxidation [13]. Involvement of cadmium in ROS formation and differential antioxidant responses was observed by Cheng et al. [14] in *Chlorella vulgaris*. Sanyal et al. [9] conducted biosorption and biomass accumulation studies using cadmium in both *Scenedesmus acutus* and *Chlorella pyrenoidosa* cultures. In the present investigation, the *in vitro* effects of cadmium on *Asterarcys quadricellulare* and *Chlorococcum minutum* growth, particularly chlorophyll accumulation was carried out. Both the species belong to green algae group with unicellular nature. For the first time, both the algal species were screened with cadmium under *in vitro* conditions.

MATERIAL AND METHODS

In the present investigation, both *Chlorococcum minutum* and *Asterarcys quadricellulare* were obtained from University of Madras, Chennai, India. All the algal cells were preserved as per the standard protocol using glycerol and kept at -80ºC. In addition, regular cultures were maintained in petriplates and test tubes and frequently they were used depends on the experiment. All the experiments were carried out under *in vitro* conditions.

Both general and contaminated glassware (Borosil, India) and vessels were decontaminated by autoclaving (Forged bail valve, RBI, Italy) at 15 lbs/in2 for 20 min. Further, all the glassware was washed with detergent cleanly and later tap water and finally oven dried (Kemi, K04.3, Ernakulam, India). The media which were used in the present study contain TAP nutrients along with selected heavy metal i.e. cadmium chloride. Specifically, TAP along with various concentrations of cadmium chloride i.e. 0, 0.2, 0.4, 0.8 and 1.6 mg/L were prepared as per the modified method of Raga Sudha et al. [8]. All the algal media were maintained pH 7.0 using a pH meter (Elico Limited, India) and were made to known volumes. Algal medium was dispensed in to conical flask and finally all the media were autoclaved (Inlab Equipment, Madras, India) at 15 lbs/ in^2 for 20 min. Comfortable space was maintained in the conical flask to enable algal growth, because optimal growth of algae will take around two to three days depending on the species in TAP medium [15].

After the completion of sterilization, media flasks were removed from the autoclave and were placed in a laminar air flow [(LAF) (Hi-Tech Products, India)]. Before going to inoculation, the LAF chamber was smeared with 100 percent ethanol and the chamber was sterilized by switching on the Ultra Violet (U.V.) lamp for around 20-30 min. Hands were cleaned with ethanol from frequently to minimize contaminations. Inoculation was carried out with sterilized loops. After inoculation, all the algal cultures were incubated in a culture room at 25°C with a relative humidity of 50-60% and 24 h photoperiod at a photo flux density of 30-40 μEm^2S^{-1} of white fluorescent tubes.

After inoculation a routine observation and collection of data i.e. biomass estimation was carried out using standard spectrophotometer (Shimadzu UV-1800). Chlorophyll estimation was carried out by collecting 100 µl of each algal sample as aliquot solution in to 1.0 ml vials along with 900 µl of 80% acetone and mixed well and later centrifuge at 5000 rpm up to 2-5 min. Collect supernatant solution then take O.D values at 663 nm (Chl- a) and 645 nm (Chl-b) initiated for chlorophyll experiments [16-17] and finally total chlorophyll estimation was carried out using standard procedure. A minimum of three replicates were involved in each experiment and mean values were used in excel program for various parameters.

RESULTS AND DISCUSSION

In the current work, algal species namely *Asterarcys quadricellulare* and *Chlorococcum minutum* were used. Both the species were treated with cadmium chloride under *in vitro* conditions. Specifically, an attempt has been made to standardize the optimum conditions to check the chlorophyll (biomass) content using different cadmium chloride doses along with TAP medium. Our study is under normal light and temperature culture room conditions to know the effects of cadmium chloride. Biomass analysis was done by visual observation of algal culture growth regularly and further estimating the chlorophyll content using spectrophotometer method. The results of the present experiments using the TAP medium alone as well TAP with cadmium chloride were documented below.

Effect of cadmium chloride on *Asterarcys quadricellulare* cultures

The composition of the algal culture medium is important factor in the successful establishment of any *in vitro* culture. TAP medium alone used as a control and also TAP along with various concentrations of cadmium chloride were screened. Cultures with 0.4 mg/L cadmium chloride exhibited improvement in growth and total chlorophyll contents when compared to control cultures (AQ2 in Fig. 1 and Fig. 2).

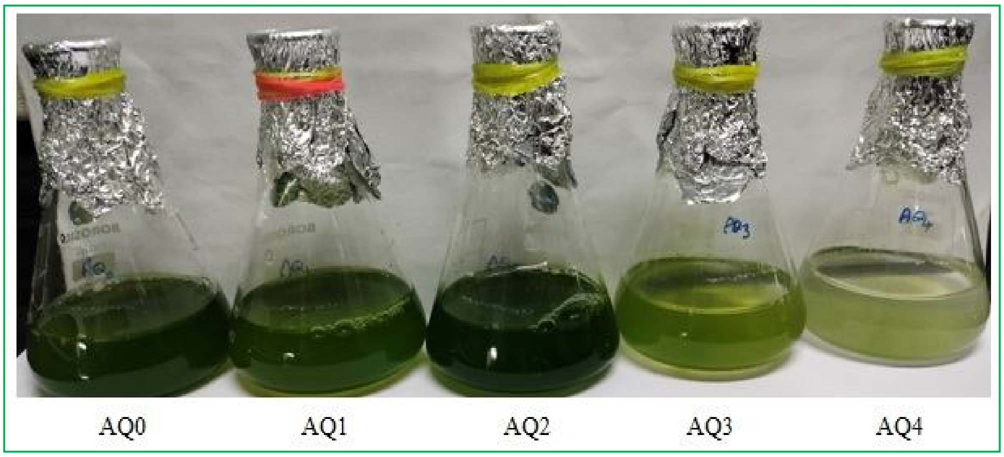

Figure-1: *Asterarcys quadricellulare* (AQ) cultures with TAP along with cadmium chloride

Cadmium effects in algal species in various species were explained in detail by Trevors et al. [18]. In contrast, cultures with more than 0.4 mg/L concentration reduced the growth and development of alga (Fig. 1). Likewise, Mo et al. [19] proved the cadmium toxicity in three algal species. Also, higher concentration of cadmium chloride reduced the total chlorophyll accumulation (Fig. 2). Visual observation of the cultures was also confirmed the same pattern (Figs. 1 and 2).

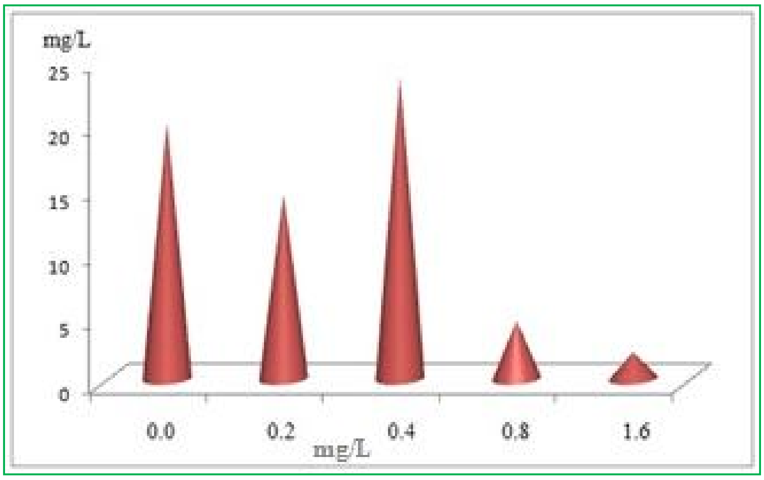

Figure-2: Total chlorophyll contents in *A. quadricellulare* in TAP with/without cadmium chloride

In the present work with this alga, cadmium chloride cultures are exhibiting enhanced chlorophylls up to 0.4 mg/L concentration along with TAP medium and after that cell may go for stress condition due to high concentration. In contrast, Valdez et al. [20] used immobilized *Chlorella* species for cadmium removal.

Effect of cadmium chloride on *Chlorococcum minutum* cultures

TAP medium alone along with cadmium chloride showed the variation in growth pattern of *Chlorococcum minutum* cultures. In *C. minutum* cultures, augmented growth and total chlorophyll content were noticed in TAP along with 0.8 mg/L (CM3) cadmium chloride (Fig. 3 and Fig. 4). Lower concentration of cadmium chloride (0.2 mg/L) exhibited less total chlorophyll accumulation when compared to untreated control cultures. Samadani et al. [7] proved the impact of cadmium accumulation in model alga, *Chlamydomonas reinhardtii* in detail.

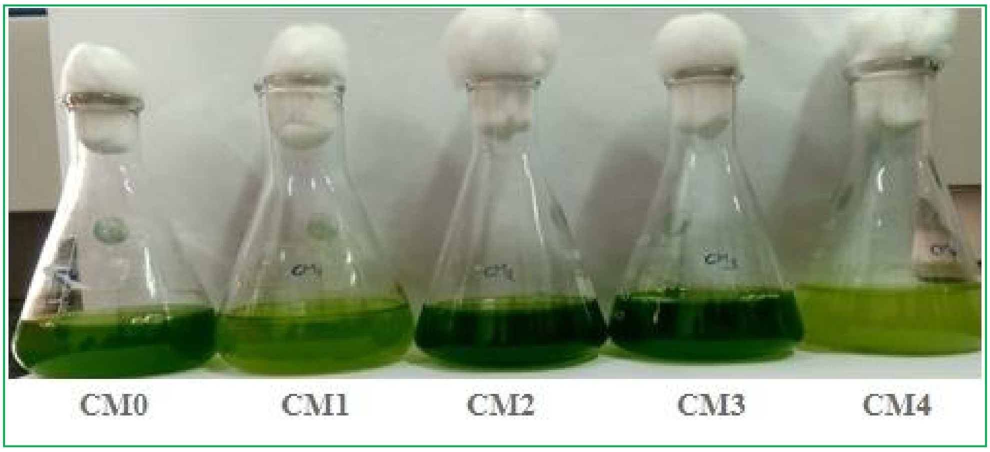

Figure-3: *Chlorococcum minutum* (CM) cultures with TAP along with cadmium chloride

Total chlorophyll contents were slightly high in TAP along with 0.4 mg/L cadmium chloride medium. High concentration of cadmium chloride (1.6 mg/L) reduced the growth and total chlorophyll accumulation in this alga (Figs. 3 and 4). Leon-Vaz et al. [6] also studied the biochemical pathways in cadmium treated *Chlorella sorokiniana* cultures. Similarly, number of research works was carried out with heavy metals using algal samples [8, 14].

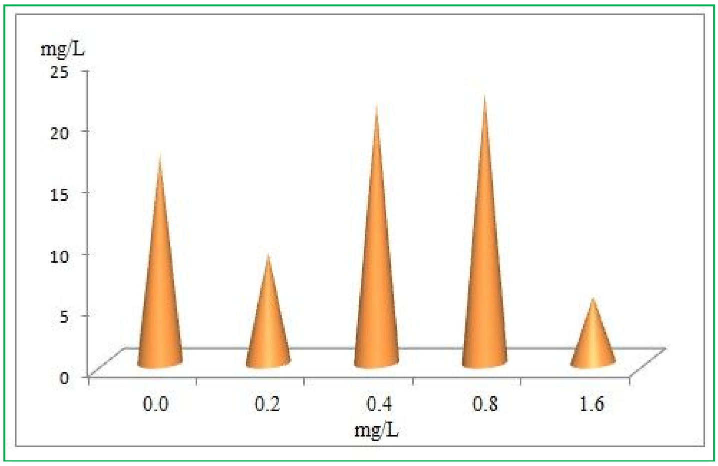

Figure-4: Total chlorophyll contents in *C. minutum* in TAP with/without cadmium chloride

CONCLUSIONS

In the present investigation, cadmium chloride cultures are exhibiting enhanced chlorophyll contents up to 0.4 mg/L concentration along with TAP medium in *A. quadricellulare* cultures and after that cell may go for stress condition due to high concentration. Moreover, in *C. minutum* cultures, TAP along with cadmium chloride cultures are giving slightly more total chlorophyll contents compared to TAP medium alone. On the whole, current work may be useful in future algal biomass research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest for this study.

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