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Original Research Paper

Botany

EFFECT OF COPPER AND ZINC ON THE PHOTOSYNTHETIC PIGMENTS OF OEDOGONIUM CAPILLARE

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ABSTRACT In this present communication, the variation in photosynthetic pigment content specially Chlorophyll (Chl a & Chl b) and carotenoids in Oedogonium capillare under two heavy metals viz. copper (Cu) and zinc (Zn) stress was studied. The filamentous green alga O. capillare was exposed to 10 mM Copper Sulphate and Zinc Acetate solution separately for 72 h. All the experimental sets were kept at room temperature under light. For a comparative pigment content analysis in control and metal treated O. capillare, a metal free experimental set was also maintained in same condition. Control and metal treated biomass was collected at 1, 24 and 72 h of experiment for pigment extraction and estimation. The pigments were extracted using chilled 80% acetone and their concentrations were determined by spectrophotometric method. It was observed that 85% and 81% Chl a disappeared after 72 h of reaction with 10 mM Cu and Zn respectively. Similarly, it was also confirmed that only 17% and 12% Chl b was left in Cu and Zn treated O. capillare respectively after 72 h of metal exposure. After 72 h of metal treatment, a sharp decrease in carotenoids content viz. 97% and 92% in Cu and Zn treated O. capillare respectively was also documented. The significant decrease of photosynthetic pigments in O. capillare confirmed that the selected metal dose is toxic to the selected green alga.

KEYWORDS : Algae, Chlorophyll, Carotenoids, Copper, Zinc

INTRODUCTION

Algae are found in almost all parts of the Earth, from oceans and rivers to even the most humid places on land. They show incredibly diverse shapes, sizes and colors; ranging from microscopic to macroscopic. They are photoautotrophic organisms and can be found among both prokaryotes and eukaryotes. Algae are categorized mainly into green algae (Chlorophyceae), red algae (Rhodophyceae), and brown algae (Phaeophyceae) including diatoms (Bacillariophyceae). Though Chlorophycean members are ubiquitous nature, they are commonly found in fresh water. In this research work also the studied green alga, *O. capillare* has been collected from fresh water reservoir of Badshahithaul, Uttarakhand, India.

The major pigments in green algae are chlorophyll a, b and carotenoids, through which they carry out photosynthesis that fulfill a key role in the ecosystem by which they provide oxygen and serve the role of producers in ecological food chain. Any kind of outside stress shows effect on photosynthetic pigment. Therefore, variation in pigment content is a very common stress response in algae. Like other toxic material, heavy metal is considered one of the major pollutants and easily bioaccumulated by biological systems [1]. Heavy metal shows toxicity in a dose dependent manner and researchers have proved various metal toxicity to algal system by pigment content specially chlorophyll and carotenoids analysis [2].

It was reported that chlorophyll content decreased in filamentous green alga, *Rhizoclonium riparium* during treatment with sublethal (1 mM) and lethal (9 mM) doses of silver nitrate [3]. However, in the same alga, increase and initial increase with subsequent decrease of carotenoids content was mentioned by researchers in sublethal and lethal doses of silver, respectively [3]. In our previous research work, initial increase and gradual decrease in total chlorophyll and carotenoids content was also observed in gold treated diatom *Nanofrustulum shiloi* [4]. A sharp increase in carotenoids content followed by a decrease was also observed by our team in gold treated cyanobacterium, *Anabaena sphaerica* and green alga, *Chlorococcum infusionum* [5]. Disappearance of pigment content was also reported in iron and silver treated *Pseudostaurosira trainorii* [6] and *Gedaniella* sp. [7] respectively. In our previous studies, it was observed that the chlorophyll a and b content in *O. capillare* decreased with reaction time due to iron stress [8]. No other report is available regarding the pigment content variation in *O. capillare* due to other metal stress.

Like other metals, the toxicity of Cu and Zn to algal system was also reported by researchers. The reduction of photosynthetic pigment content in *Chlorella vulgaris* due to Cu and Zn stress was reported [9]. Researchers also documented dropping of pigment concentration in *Chlorella pyrenoidosa* as Cu concentration increased [10]. In this present investigation, the variation in photosynthetic pigments content including chlorophyll a, b, and carotenoids in Cu/Zn treated green alga, *O. capillare* have been calculated first time at various metal exposure time.

METHODS

The studied algal sample *O. capillare,* is a fresh water green alga and was collected from Badshahithaul, Uttarakhand, India which was already mentioned in our previous publication, Upasna et al., 2024 [8]. Before identification and isolation, a thorough cleaning of collected sample is always required. The steps of sample cleaning, identification and isolation of *O. capillare* was also mentioned in Upasna et al., 2024 [8]. As Bold basal medium (BBM), composition given by Nichols & Bold 1965 [11] is the best growth medium for fresh water green algae, the healthy biomass of filamentous *O. capillare* was maintained in this medium for further experiment.

The variation in pigment content specially Chlorophyll (Chl a & Chl b) and carotenoids in *O. capillare* during two heavy metals viz. Cu and Zn stress was analysed. The healthy biomass of *O. capillare* (400 mg FW) was exposed to 10 mM 400 mL Copper Sulphate ($CuSO_4$, MW:159.609, CDH) solution for 72 h. Similarly, another experimental set was also prepared separately using Zinc Chloride ($ZnCl_2$, MW:136.286, CDH) instead of $CuSO_4$ where the healthy biomass of *O. capillare* (400 mg FW) was exposed to 10 mM 400 mL $ZnCl_2$ solution for 72 h. All the experimental set was kept at room

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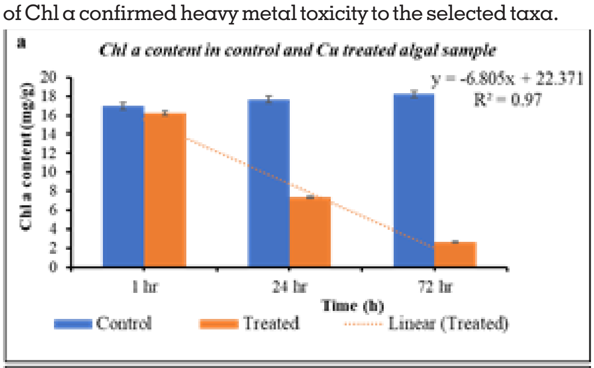
temperature under light. A control set of *O. capillare* without Cu/Zn stress was also maintained in the same condition throughout the experiment.

For determination of pigment content in metal treated experimental taxa, biomass was collected at various time of metal exposure. The metal treated biomass of *O. capillare* collected at 1, 24 and 72 h of metal treatment. Biomass from control set was also collected at the same experimental time to compare the pigment content in both control and metal treated *O. capillare*. For pigment extraction from control and Cu/Zn treated *O. capillare* the method of Arnon 1949 [12] was followed. A pre-weighed biomass was crushed with pre-chilled 80% acetone with help of mortar and pestle. Then the properly crushed sample was centrifuged at 10,000 rpm for 5 min. The extraction process continued until the residue became colorless. The supernatant was then collected for spectrophotometric analysis. The absorbance was noted at 663, 645 and 480 nm for Chl a, Chl b and carotenoids respectively using UV-Vis spectrophotometer (2375 spectrophotometer, Electronics India, Haryana, India). The measurement was performed against acetone as blank. The measurements were performed in triplicates. The chlorophyll (Chl a and Chl b) and carotenoid content were estimated following the formula given by Arnon 1949 [12] and Carreto 1977 [13] respectively. The pigment content in control and metal treated *O. capillare* was expressed as mg g^{-1}.

RESULTS

The variation of Chl a content in control and Cu/Zn treated *O. capillare* has been represented in Fig 1. Chl a content in control *O. capillare* was estimated as 16.99, 17.66 and 18.23 mg/g at 1, 24 and 72 h of experiment respectively. So, it can be said that amount of Chl a was increasing in control biomass of *O. capillare* with time which indicated experimental taxa was in healthy condition. However, in 10 mM Cu stress, the Chl a content started decreasing after 1 h of reaction. The estimated value of Chl a in Cu treated *O. capillare* was 16.25, 7.38 and 2.64 mg/g after 1, 24 and 72 h of metal exposure respectively. Similarly, a drastic decrease of Chl a content in Zn treated *O. capillare* was also observed. The amount of Chl a was documented as 6.45, 4.24 and 3.35 mg/g at 1, 24 and 72 h of reaction with 10 mM Zn respectively. It was observed that 85% and 81% Chl a disappeared after 72 h or reaction with 10 mM Cu and Zn respectively. The disappearance of a large amount of Chl a confirmed heavy metal toxicity to the selected taxa.



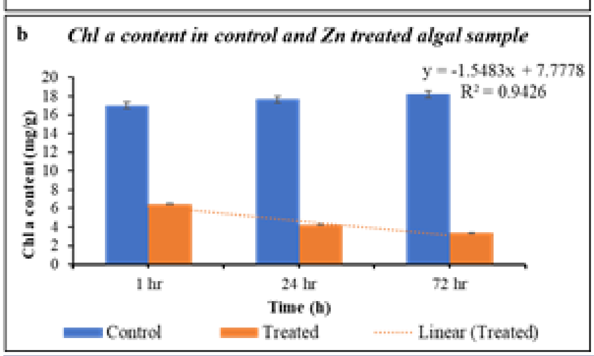


Fig 1: Showing Variation In Chlorophyll A Content In Control And Cu/zn Treated *O. Capillare*

A comparative graphical representation of Chl b content between control and Cu/Zn treated *O. capillare* has been shown in Fig 2. The amount of Chl b in control biomass of *O. capillare* was calculated as 27.54, 28.22 and 29.01 mg/g at 1, 24 and72 h of experiment respectively. Chl b content also showed increasing trend in control sample like Chl a which is very common phenomenon in healthy growing algal biomass. However, a distinct decrease of Chl b content was observed in both Cu and Zn treated *O. capillare* which confirmed the selected metal doses are lethal for *O. capillare*. The value of Chl b in Cu treated *O. capillare* was determined as 19.18, 8.3 and 4.67 mg/g at 1, 24 and 72 h of reaction respectively. The value of Chl b in Zn treated *O. capillare* was calculated as 6.4, 5.51, 3.39 mg/g at 1, 4, 72 h of reaction respectively.

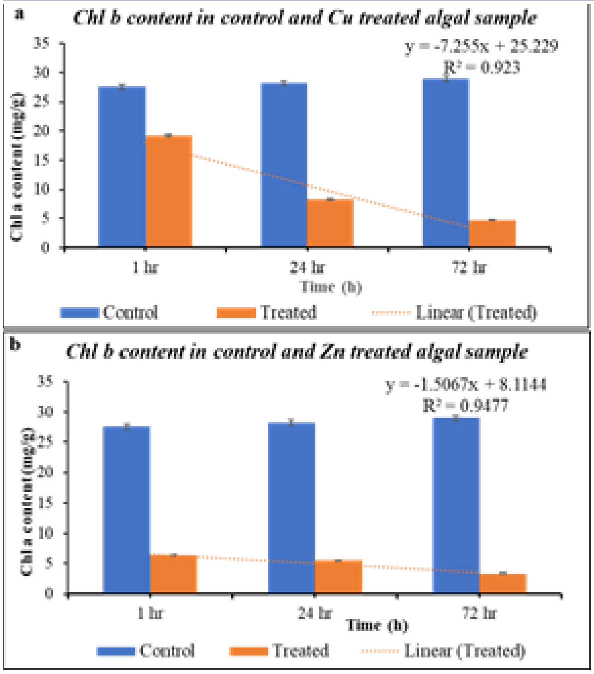
Likewise, a sharp decrease in carotenoids content in Cu and Zn treated *O. capillare* was also confirmed (Fig 3). The average carotenoid content in control *O. capillare* was measured as 1.3 mg/g. The 30% decrease of carotenoid content was observed only after 1 h of reaction with 10 mM Cu solution. However, after 72 h of reaction with Cu solution almost complete amount of carotenoids (97%) disappeared. The calculated amount of carotenoids was 0.9, 0.3 and 0.033 mg/ g at 1, 24 and 72 h of reaction with Cu respectively. Similarly, 92% of carotenoids content disappeared due to treatment with 10 mM Zn solution for 72 h. The values of carotenoids were estimated as 0.45, 0.36 and 0.095 at 1, 24 and 72 h of reaction with Zn respectively.

DISCUSSION

Cu and Zn, both heavy metals are essential for algal growth but simultaneously toxic to them if their concentration exceed certain level. Cu plays important role in oxidation of O2 as well as one major component of plastocyanin which is required to complete electron transport chain in photosynthesis [14]. Similarly, Zn is also a vital component of different enzymes viz. superoxide dismutase, carbonic anhydrase, carboxypeptidase and an essential mineral nutrient for algal growth. Zn deficiency cause distinct reduction in electron transport [15]. However, the concentration of Cu and Zn above certain level is not beneficial for living organisms. High dose of heavy metal always shows detrimental effect on biotic factors of ecosystem. Heavy metals are non-biodegradable pollutant and generally present in industrial waste, pesticides, herbicides and fertilizer. These metal contaminants are continuously coming to the aquatic environment through anthropogenic activities. Therefore, these hazardous materials damage photosynthetic system of algae as well as other plants. In this study also, gradual disappearance of pigment in green alga, *O. capillare* due to Cu/Zn stress was confirmed.

Effect of Cu/Zn on algal growth is dose dependent and species specific. Two green microalgae - *Chlorella sorokiniana* and *Scenedesmus acuminatus* responded differently to Zn stress. *Chlorella sorokiniana* was less affected than *Scenedesmus acuminatus*. However, high metal concentration always reduces algal growth and pigment concentration with exposure time [16]. It was reported that high Cu concentration in culture medium caused significant changes in algal chlorophyll and carotenoids content in *Chlorella pyrenoidosa* [17] and *Odontella mobiliensis* [18]. In this study also, we observed disappearance of Chl a, chl b and carotenoids in filamentous green alga, *O. capillare* during treatment with both Cu and Zn heavy metals.

Fig 2: showing variation in Chlorophyll b content in control and Cu/Zn treated *O. capillare*



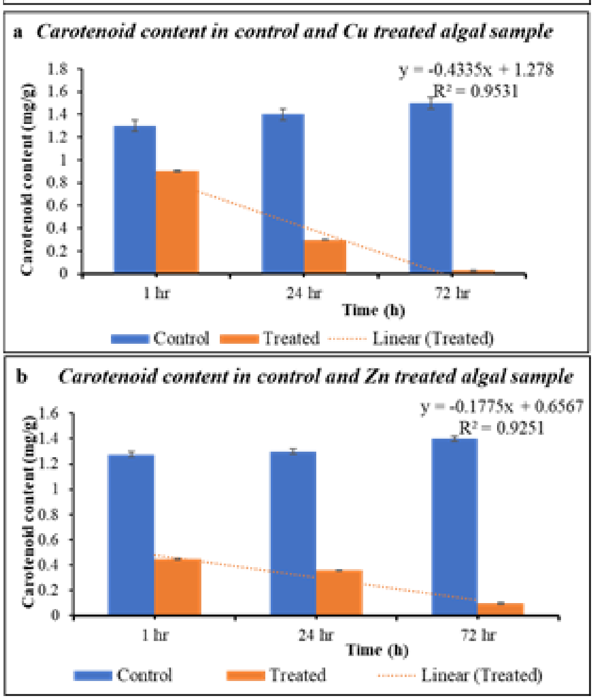


Fig 3: showing variation in Carotenoids content in control and Cu/Zn treated *O. capillare*

CONCLUSION:

The pigment content specially Chl a, Chl b and carotenoids gradually decrease in filamentous green alga, *O. capillare* due to Cu and Zn stress. Therefore, it can be concluded that *O. capillare* is sensitive to high dose of Cu/Zn and variation in photosynthetic pigment content is their common heavy metal stress response. It was observed that Cu/Zn stress also reduced the growth of *O. capillare*. Therefore, it can be hypothesized that the detrimental effect of heavy metals on algae could reduce the number of producers in aquatic ecosystem. Simultaneously, green algae could be utilized as an aquatic heavy metal pollution indicator.

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