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ORIGINAL RESEARCH ARTICLE

Effect of Heavy Metal Copper on the Marine Cyanobacterium *Phormidium tenue* (Mengh.) Gomont

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ABSTRACT

The present study "Effect of heavy metal copper on the cyanobacterium ***"Phormidium tenue"*** was conducted in the laboratory to test the effect of growth and biochemical composition. Growth was measured in terms of chlorophyll 'a' content and it was decreased in all the concentrations (1, 2, 5, 10, 25, 50 and 100 ppm) of copper. The carbohydrate content was gradual decrease from lower to higher concentration. Protein content was decreased with increased concentration of copper 25 to 100 ppm. The amino acid and lipid contents were also decreased except at control, 1 and 2 ppm. Cyanobacteria to remove toxic metals from contaminated water could be advantageous, since they are ubiquitous and have colonized almost all parts of the world. They can be grown easily and have very simple growth requirements. An advantage of using living organisms over dead biomass is that they have fast growth rates and hence produce a regenerating supply of metal – removal material. There is much evidence that cyanobacteria could accumulate heavy metals in their tissues when grown in polluted waters. These cynobacteria must be identified from the laboratory as well as in the water polluted environment will solve metal toxicity problem in aquatic ecosystem.

Key words: Cynobacteria, copper, chlorophyll, protein, amino acid, lipid.

1. INTRODUTION

Aquatic ecosystems are particularly susceptible to accumulating contaminants. Due to their widespread industrial use, large quantities of metal compounds are discharged into freshwater ecosystems and the levels of these have increased substantially world-wide over the last century (Nriagu and Pacyna, 1988; Penuelas and Filella, 2002). Although metal compounds are originate from the bedrock, these chemicals largely enter the eco-environment through industrial and agricultural activities, and then transferred to the food chain (Schutzendubel and Polle, 2002), and can ultimately have significant toxic effects on organisms, causing ecological disturbances (Scoccianti *et al.*, 2008).

Cyanobacteria, also known as blue-green algae or blue-green bacteria obtain their energy through photosynthesis. The name "cyanobacteria" comes from the colour of the bacteria (Greek: κυανός (kyanós) = blue). Cyanobacteria are found in almost every conceivable environment, from oceans to fresh water to bare rock to soil. Most are found in fresh water, while others are marine, occur in damp soil, or even temporarily moistened rocks in deserts. Cyanobacteria are the only organisms that actively evolve oxygen as a by-product of oxygenic photosynthesis within the same cell or colony of cells where nitrogen fixation occurs. The presence of oxygen triggered biochemical and morphological adaptations in diazotrophic phototrophs aimed at limiting the inhibitory effects of oxygen on nitrogenase (Gallon, 1992; Bergman *et al.*, 1997; Berman-Frank *et al.*, 2003). Cyanobacteria get their name from the bluish pigment phycocyanin, which they use to capture light for photosynthesis. They also contain chlorophyll 'a' the same photosynthetic pigment present in higher plants.

Copper is an essential micronutrient for numerous physiological processes at low concentrations but a toxic metal at high concentrations (Gaetke and Chow, 2003). The progressive increase of Cu in

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aquatic ecosystems arises from various anthropogenic sources including copper mine drainage, copper-based pesticides, industrial and domestic wastes, and antifouling paints (Andrade *et al.,* 2004; Ma *et al.,* 2003).

Microalgae, specially cyanobacteria can sequester heavy metal ions by adsorption and by absorption, as do by other microorganisms, therefore, they used for metal removal as a chief greater performance at a lower cost than conventional wastewater treatment technologies, this is consistent with the recent trend for growing interest in biosorbent technology for removal of trace amounts of toxic metal from aqueous waste (Inthorn *et al.,* 2002). Converti *et al.* (2006) used *Spirulina platensis* biomass as adsorbent for copper removal from water solution while El-Sheekh *et al.* (2005) found that copper was removed by 12.5-81.8% from wastewater by using cyanobacterial cultures of *Nostoc muscorum* and *Anabaena subcylindrica* and they found that single culture in most cases was better than the mixed cultures in heavy metal removal.

The goal of this study was to determine the effect of copper on the biomass, chlorophyll 'a', carbohydrate, protein, amino acid and lipid content of cyanobacterium *Phormidium tenue*.

2. MATERIALS AND METHOD

2.1 Description about *Phormidium tenue* (Mengh.) Gomont

Phormidium tenue, a marine algae collected from Nagapattinam coast, is a single thallus, pale blue-green, thin membranes, expaned; trichome straight or slightly bend, densely entangled, slightly constricted at the cross-walls, attenuated at the ends. 1-2 µ broad, pale blue green; sheath thin difficult, cell up to three times longer than broad, 2.5-5 µ long septa not granulated, cross walls not commonly visible end cell acute - calyptras absent.

2.2 Collection of Cyanobacteria

The test organism *Phormidium tenue*, a marine blue green alga was collected from Nagapattinam coastal area located on the south east coast of India (Latitude 10^0 49^0 N, Longitude 79^0 43^0 E) and transported to the laboratory for the isolation of the test organism. Pure culture of this species was carefully examined under microscope and identified with the help of standard works of Desikachary (1959).

2.3 Culture medium

The culture medium used in the present study was ASN-III-N^- medium. The composition used for preparing the medium was described by Waterbury (1976) and Rippka *et al* (1979).

2.4 Stock culture and sub-culturing

- Stock of the test organism was maintained in 250 ml conical flask containing 100 ml of culture medium under the following conditions.
- Light density 3000 lux provided from overhead cool while fluorescent tubes.
- Lighting cycle 12 hr. light followed by 12 hr. darkness, room temperature 22 ± 1^0C and salinity 35 ppt.

2.5 Isolation of the test organism

During the culturing of *Phormidium tenue* some other cyanobacteria like *Oscillatoria* occurred as contaminants. These were avoided by repeated sub culturing with of ASN-III-N^- medium since the test algae is a filamentous form.

2.6 Effect of heavy metal – copper ($CuSO_4$)

To test the effect of heavy metal on growth, *Phormidium tenue* was treated with different concentrations of copper which was used as copper sulphate ($CuSO_4\ 5H_2O$).

Molecular weight of copper sulphate - 249.68

Atomic weight of copper - 63.54

(Or)

63.54 mg of copper is present in 249.68 mg of copper sulphate

Therefore,

100 mg of copper = $\frac{249.68 \times 100}{63.54}$

=392.95 mg of $CuSO_4$

100 ppm (100 mg of copper)

=392.95 mg/Cu SO_4H_2O/1000 ml

2.7 Stock solution

Stock solution of the heavy metal of copper was prepared in sterile media (ASN-III-N^- free medium) from which different concentrations of Copper (ppm) were prepared *viz.,* 1, 2, 5, 10, 25, 50, 100 ppm and control respectively.

Exponentially growing *P. tenue* was inoculated into 100 ml of each test solution taken in 250 ml conical flasks. This experiment was conducted for 21 days in a culture room, illuminated with white fluorescent light (3,500 lux) by maintaining a 12-12 light/dark cycle at 22±1^0C. Duplicates were run in all the experiments.

2.8 Growth measurements

Growth rate was measured in terms of chlorophyll 'a' as biomass components at initial (0) day 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th}, 18^{th} and 21^{th} days after the inoculation. (McKinney's method, 1941).

All the growth analysis *viz*., carbohydrate, protein, amino acid and lipid content calculated at 21^{st} day after the inoculation. Estimation of carbohydrates followed by Dubois *et al.*, 1956, estimation of protein followed by Lowery *et al.*, 1951, estimation of amino acid followed by Jayaraman, 1981 and estimation of lipid followed by Sato and Murata, 1988.

2.9 Statistical Evaluation

The observation was taken in triplicate. The mean data of triplicate value was put in statistical analysis by taking their standard deviation.

3. RESULTS

The Effect of different concentrations of copper on the growth and biochemical constituents such as carbohydrate, protein, amino acid and lipid of *Phormidium tenue* are shown in (**Figure 1 to 5**). Growth was measured in terms of chlorophyll 'a' in all the concentrations (*viz.* 1, 2, 5, 10, 25, 50 and 100 ppm and control). Growth was gradually decreased from the 3rd day onwards up to 21st day but there was not attained log phase. Different concentrations of copper (1, 2, 5, 10, 25, 50, and 100 ppm) were exhibited similar responses but in control the growth was increased up to 15th day and thereafter growth was decreased. Maximum growth was observed in control and decreased growth at concentrations on the 18th day, whereas maximum reduction of growth was decreased except in control (**Fig 1**). Proximate composition (carbohydrate, protein, amino acid and lipid) of *P. tenue* at the different concentration of copper is shown in figure 2, 3, 4 and 5. There was a gradual reduction in the level of carbohydrate content in control, 1, 2, 5, 10, 25, 50 and 100 ppm except in control on 21st day. Maximum carbohydrate content was observed in the control, 1 and 2 ppm treated cultures (0.318, 0.312 and 0.314 µg/ml) and minimum was observed in the 100 ppm on 21st day (0.095 µg/ml) (**Fig 2**).

There was a gradual reduction in the level of protein content in 2, 5, 10, 25, 50, 100 ppm on 21st day. Maximum protein contents (0.29 µg/ml) was observed in the control and the minimum was observed in 100 ppm on 21st day (0.06 µg/ml) (**Fig 3**). There was a gradual reduction in the level of amino acid content in 1, 2, 5, 10, 25, 50, 100 ppm except control on 21st day. Maximum amino acid content was observed in the control, 1 and 2 ppm (0.262, 0.261 and 0.260 µg/ml) and minimum was observed in 100 ppm on 21st day (0.098 µg/ml) (**Fig 4**). There was a gradual decrease in the level of lipid content in 2, 5, 10, 25, 50, 100 ppm, than control on 21st day. Maximum lipids were observed in the control, 1, 2 and 5 ppm concentrations of copper treated cultures, which were 112, 112, 110 and 109 µg/ml and minimum was observed in 100 ppm (76 µg/ml). Generally the amount of lipid was not affected by concentrations of heavy metals (**Fig 5**).

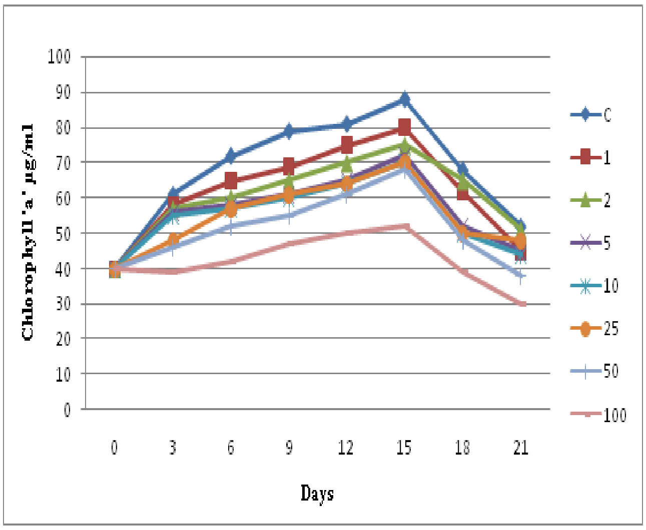

Fig 1: Effect of different concentrations of copper on chlorophyll 'a' content of *P. tenue*

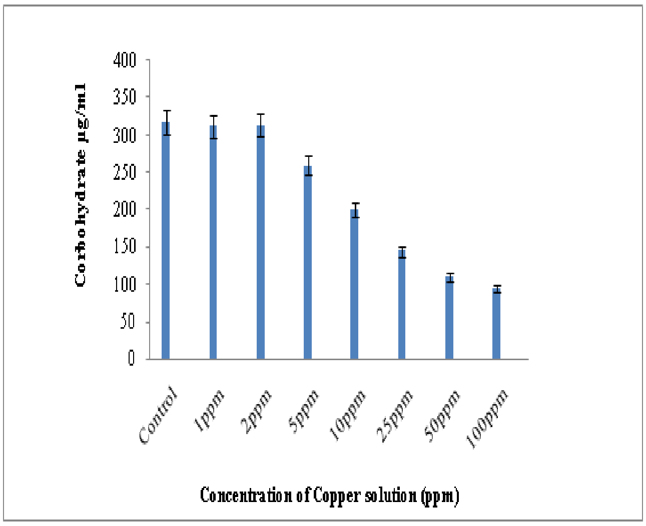

Fig 2: Effect of different concentrations of copper on carbohydrate content of *P. tenue*

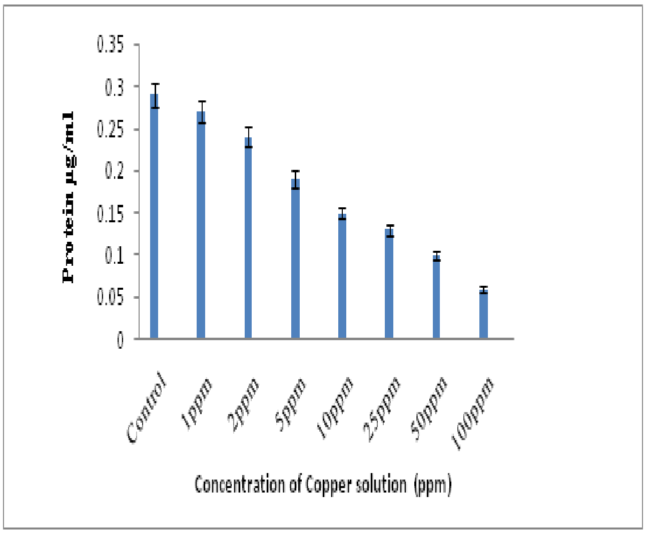

Fig 3: Effect of different concentrations of copper on protein content of *P. tenue*

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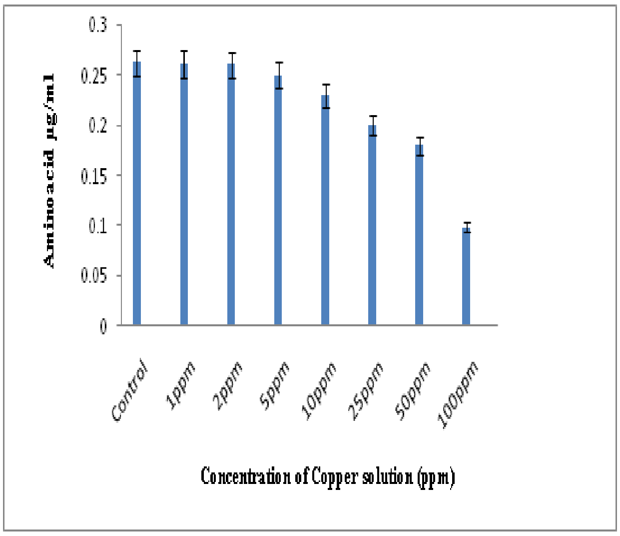

Fig 4: Effect of different concentrations of copper on amino acid content of *P. tenue*

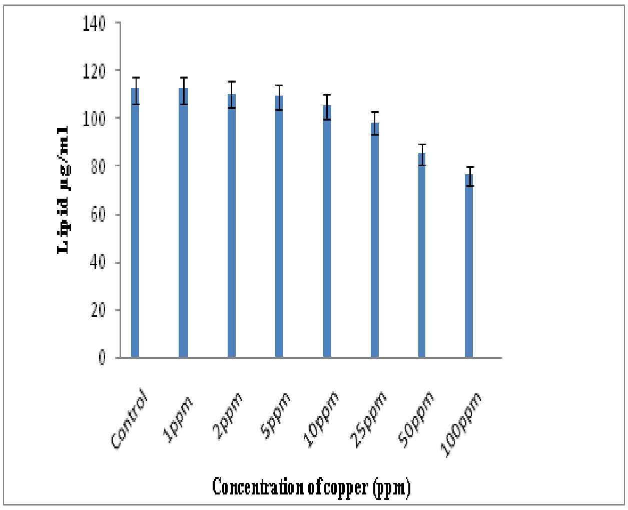

Fig 5: Effect of different concentrations of copper on lipid content of *P. tenue*

4. DISCUSSION

In recent years, heavy metal pollution has become one of the most serious environmental pollution. Presence of heavy metal even in traces is toxic and detrimental to both flora and fauna. The use of biological materials in general, and microalgae including cyanobacteria in particular, has received considerable attention during recent decades for the removal of heavy metals as the environment friendly alternative technology (Romera *et al.*, 2006).

In the present study, the growth of *Phormidium tenue***,** was found to be influenced by the heavy metal copper at the different concentration viz 1, 2, 5, 10, 25, 50 and 100 ppm. The blue green algae used as biomonitors because of the unique feature of bioaccumulation of heavy metal in their internal compartments by tens of thousand times through various physio-ecological process. The effect of different concentrations of copper on the cynobacterium *Phormidium tenue* revealed that the effect of chlorophyll- a, carbohydrates, protein, amino acids and lipid decreased with increased concentrations of copper from 1 ppm to 100 ppm. Similar observations were reported by Khalil (1997) in *P. fragile* exposed to mercury. Heavy metals stress caused reduction in growth, photosynthetic pigments (Al-Hejuje, 2008).

The growth enhancement was noticed from 3rd day to 15th day and after they reached to decline phase. Similar findings were reported in *Anabaena inaequalis* treated with nickel (Stratton and Corke, 1979). Singh and Rai (1989) reported inhibition of pigments in *Chlorella vulgaris* exposed to 20 µg/ml of chromium. Singh and Rai *et al.* (1989) suggested that the metals may cause disruption of thylakoid membrane resulting in the decrease of chlorophyll pigments.

Generally the presence of media containing the nitrogen source resulted in an increase in the protein content of the cells and a decrease in the levels of carbohydrate, protein and amino acids although biomass productivity was not affected significantly. Copper showed maximum inhibition of the total carbohydrate content of test algae up to 25 ppm and other higher concentrations showed reduction in carbohydrate content. Most studies indicated that acclimation or adaptation to higher metal concentrations is accompanied by the potential for increased tolerance by adjusting physiological or biochemical mechanisms (Devars *et al.*, 1998; Soldo *et al*, 2000). Earlier studies showed that metal efflux was an important mechanism to regulate the intracellular metal content in bacteria (Hassler *et al.*, 2005; Nies, 2003). Furthermore, the internal metals were found to be partitioned in different subcellular compartments, which may affect the tolerance capability (Wang and Rainbow, 2005; Wang and Wang, 2008).

The protein content was decreased with increased concentration of copper. Singh and Rai (1989) observed reduction in protein content of *Chlorella vulgaris* exposed to chromium. This reduction was suggested to be due to the blocking of sulfohydrexyl groups by heavy metal ions. Effect of copper on the amino acid content of the selected cynobacteria strain was decreased at the increased concentration. Similar findings were reported by Mishra and Nanda (1997) who showed that free amino acid content decreased significantly with the increasing concentration of the mercury contaminated soil. The lipid content was decreased at higher concentration but increased production of lipid content was noticed at the lowest concentration. Similar increase has also been reported in algae under, salinity and pollution stress (Saxena, 1998).

From the present study, it is clear that the heavy metal copper influence the blue green alga *Phormidium tenue* by reducing its growth and biochemical compositions. Further research on this organism with different heavy metal is needed to consider and use this organism as an indicator of the heavy metal pollution.

5. CONCLUSION

The present investigation concluded that, the cynobacteria posse's high metal adsorption capacity and very high multiplication rate. Such characters have encouraged the application of this microbial biomass in the laboratory as detoxification of effluents. These cynobacteria must be identified from the laboratory as well as in the water polluted environment will solve metal toxicity problem in aquatic ecosystem.

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