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TOXICOLOGICAL RESPONSE OF THE BLUEGREEN ALGA *OSCILLATORIA AGARDHII*, TO HEAVY METALS

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ABSTRACT

Problems statement: The disturbance of aquatic ecosystems provoked by heavy metals pollution from industrial and domestic sources has as consequence the loss of biological diversity, as well as increased bioaccumulation and magnification of toxicants in the food chain. The aim of this study was to evaluate the effect of some heavy metals on some physiological activities of *Oscillatoria agardhii* with special references to metal bioaccumulation. Approach: *Oscillatoria agardhii* was isolated from Jalmahal lake,Jaipur. A standard initial inoculum of the isolated algae was inoculated to culture flasks. The culture flasks were supplied with various concentrations of Cobalt and Zinc ranging from 1.5 ppm 2.5 ppm 3.5 ppm & 5 ppm At the end of the incubation period cultures were filtered and washed several times by distilled water for measurements the various experimental parameters. Results: The data show that the lower doses of the tested metals had stimulatory effect in biomass yield of *Oscillatoria agardhii*, whereas the higher doses were inhibitory depending on the type of the metal. On the other hand, bioaccumulation of cobalt and zinc by *Oscillatoria agardhii* cells were parallel to increasing the concentrations in the culture medium. Conclusion: The inhibitory and stimulatory effects of either of the used heavy metals depend on concentration. Different organisms, however, have different sensitivities to the same metal and the same organisms may be more or less damaged by different metals. The uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the water.

KEY WORDS: **Bioaccumulation, *Oscillatoria agardhii*, heavy metals, metabolism**

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INTRODUCTION

Many pollutants like pesticides, oil hydrocarbons, and heavy metals as well as thermal and radioactive pollution can get into aquatic environments after direct or indirect release from industries, agriculture and households (Fathi *et al*., 2008). As an important group of these various chemical substances, heavy metals may be deposited into all ecosystems (Mutlak *et al*.,1979).The disturbance of aquatic ecosystems provoked by heavy metals pollution from industrial and domestic sources, has as consequence the loss of biological diversity as well as increased bioaccumulation & magnification of toxicants in the food chain (Pena-Castro *et al*., 2004). Zinc is a major industrial pollutant of the terrestrial and aquatic environment (Foy.et.al 1978, Collins 1981). Zinc is also major contaminants in areas where oil is burned for heating purpose (lagerwerff, 1967). Zinc forms aerosol which disperse and participate out with dust (Freiberg et al.1971). It may be then absorbed by plants from such surface contamination or from water and subsequently transported to other organisms (Ernst, 1972). The aim of this study was to evaluate the effect of Zinc (heavy metals) on some physiological activities of *Oscillatoria agardhi* with special references to metal bioaccumulation.

MATERIALS AND METHODS

Organism and culture condition:

Oscillatoria agardhii was isolated from jalmahal lake, Jaipur. Isolation and purification was made by dilution and plating technique. grown in modified Zarrouk's medium (Zarrouk 1966) in distilled water at 26+2°C with a photoperiod 12h/ day.Three day old prepared inoculums of unialgal culture were added to the three sets of 500ml conical flask containing 250ml sterilized Zarrouk's medium. The flasks were covered perfectly by cotton wools and sealed with laboratory sealing film. All cultures were shaken twice daily to prevent cells from clumping.

Determination of Growth:

Growth was recorded through optical density with the help of a photochem colorimeter at 650 nm every 7^{th} day, over a period of one month.

Determination of dry weight:

A definite volume (50 mL) of algal suspension was filtered through weighted glass fiber (Schleicher and Schull, Germany). The cells, after being precipitated on the filter study, were washed twice with distilled water and dried overnight in an oven at 105°C. Data were given as µg mL-1 algal suspension.

Algal counting:

Cell number was determined using a Hematocytometer Chamber. Hemacytometer 0.1 mm deep, having improved Naubauer ruling was used. One drop of the algal suspension was pipetted on the slide, covered and left for two minutes for algal settling. The mean counts of three replicates were taken into consideration and the data were given as cell mL-1 algal suspension.

Sterile technique determinations of pigment content:

Basically blue green algae have chlorophyll-a as the light harvesting pigment. The quantity of the chlorophyll present in the known amount of algal sample was determined by procedure and equation, suggested by Parson and Strickland (1965).

Treatment:

A standard initial inoculum of the isolated algae was inoculated to culture flasks (500 mL each) that contained 200 mL of sterile nutrient medium (Zarrouks medium). The culture flasks were supplied with various concentrations of Zinc and cobalt ranging and control were used.

Metal uptake:

For the analysis of metal contents, the cultures were centrifuged to harvest the algal mass (50

mL). The algal pellet was washed with 2 mM EDFA for 10 min. to remove surface-bound metal. After centrifugation the pellet was digested 5 mL mixture containing HNO3 (70%), H2O2 (30%) and deionized water in 1:1:3 ratio (Bates *et al*., 1982). After digestion the samples were analyzed for metal content with a Perkin-Elmer atomic absorption spectrophotometer.

Calculation of bioaccumulation factor*:*

The bioaccumulation factor defined by (Brooks and Rumsby, 1965) is the ratio of concentration of an element in dry plant biomass and in the water.

RESULTS AND DISCUSSION

It is well known that algal cells exposed to heavy metals may suffer serious morphological and biochemical alterations (Rocchetta *et al*., 2006). The results of this investigation show that the inhibitory and stimulatory effects of either of the used heavy metals depend on concentration.

The bioassay results as illustrated in Fig. 1 showed clear differences in pigments content (Chlorophyll a) of algal cells between control and treated ones when algae were exposed to different concentrations of the tested metals. The pigments content gradually increased in the culture supplemented by concentration 1.5ppm, 2.5 ppm, 3.5ppm during exposure periods, whereas other concentration 5 cause a clear reduction in the pigments content of *Oscillatoria agardhii.* The same effect was observed with respect to growth rate as another indicator of algal growth as shown in Fig. 2. The growth rates decreased in respect of increasing metals concentration more than 5 ppm. Generally results shows that the low dose (1.5, 2.5, 3.5 ppm) of the tested metals had stimulatory effect in biomass yield of *Oscillatoria agardhii*, whereas the higher doses were inhibitory depending on the type of the metal. These findings are in agreement with several previously published data (El-Sheekh *et al*., 2003; Osman *et al*., 2004; Fathi *et al*., 2005; Muwafq and Bernd, 2006; Romera *et al*., 2007; Cecilia *et al*., 2007; Deng *et al*.,2007).

The inhibitory effect of the zinc on the studied growth parameters at higher doses depended on their concentration in the culture medium. Fisher and Jones (1981), who reported that low Zn2levels enhanced the total chlorophyll in *Asterionella japonica*. Prassad and Prassad (1987) found that heavy metals inhibit the enzymes that are responsible for the chlorophyll (che) synthesis. De Filippis and Pallaghy (1994) reported that toxicity of Zn results from their binding to SH groups and disruption of enzyme structure (Omar, 2002). On the other hand, Zn does not directly accelerate the formation of reactive oxygen species due to its redox inertness and it, therefore, exerted comparatively less stress on the test organism (Tripathi and Gaur, 2006). The same effect was observed with respect to dry weight as another indicator of algal growth as shown in Fig. 2. The dry weight decreased in respect of increasing metals concentration more than 5 ppm. Generally results shows that the low dose (1.5, 2.5, 3.5 ppm) of the tested metals had stimulatory effect in biomass yield of *Oscillatoria agardhii*, whereas higher concentration shows reduction in the dry weight which is indicator of the biomass presence. Which is further use to estimate another parameters.

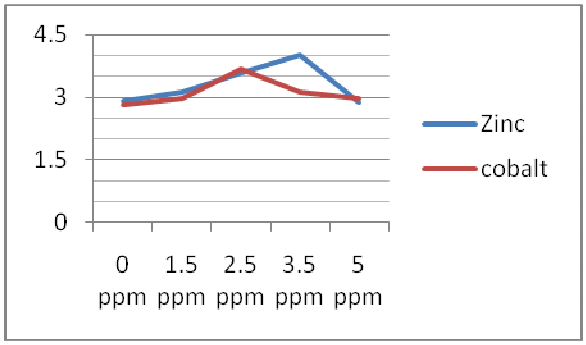

Figure 1

Effect of various concentrations of cobalt, and zinc on pigments content (Chl a mg/l) of Oscillatoria agardhii after 7 days incubation period. Vertical bars indicate chlorophyll-a whereas horizontal line indicate heavy metal concentration

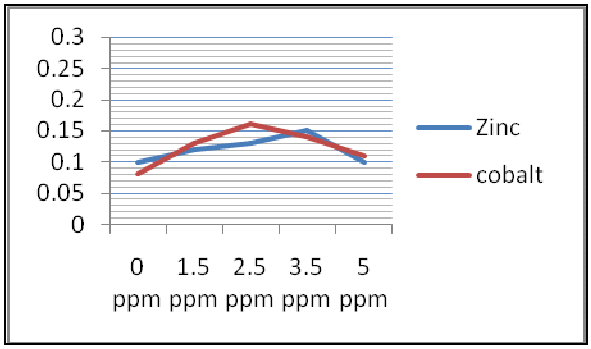

Figure 2

Effect of various concentrations of cobalt and zinc on growth rate of Oscillatoria agardhii after 7 days incubation period.

Algae are known to be able to accumulate heavy metals. They are able to eliminate metal ions from aquatic solutions in a short time by biosorption in uncomplicated systems, without any problems of toxicity. It is an important biochemical function of algae in the shaping of proper ecological relationships and interactions between organisms in the aquatic environment (Wilde and Benemann, 1993; Sandau *et al*., 1996; Bajguz, 2000). The data of Fig. 3 performed that accumulation of cobalt and zinc by *Oscillatoria agardhii* cells was parallel to increasing the concentrations in the culture medium. Also, it was observed there is no significant difference observed between each of cobalt and Zinc. It is known that the uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the water. The data of Fig. 4 shows that the bioaccumulation factors (the ratio of concentration of an element in dry biomass and in the surrounding medium) of the tested metals were parallel also to increasing the concentrations in the culture medium. However, the bioaccumulation factor of cobalt is slightly higher than that of zinc in all treatments. Metal accumulation by *Oscillatoria agardhii* were shown to be Co2+>Zn2+.

The ability of microalgae to accumulate metals from aqueous solution is well-documented (Fathi and Falkner, 1997; Fathi *et al*., 2000; Giusti, 2001) as well as the possibility of using microbial biomass to remove metals from effluents (Macaskie, 1991; Hamdy, 2000). Algae take metals up both passively and actively. Some, such as Pb and Sr, may be passively adsorbed by charged polysaccharides in cell wall and intracellular

matrix (El-Sheekh *et al*., 2003; Osman *et al*., 2004; Fathi *et al*., 2000; 2005). Other metals (e.g., Zn, Cd) are taken up actively against large intracellular concentration gradients. On the other hand, Barbara and Michael (1994) reported that the phenomenon of metal accumulation by microbial cells is quite complex, two principal mechanisms to adsorption on to the surface of the cell and a slower, active uptake into the cytoplasm. As passive biosorption mainly depends on binding to functional surface ligands the cell wall structure is most important for rapid metal ion uptake. Hamdy (2000) reported that metal uptake dependent on the type of biosorbant, with different accumulation affinities towards the tested elements and the amount of metal uptake increased steeply by increasing the weight of the biomass. Fathi *et al*. (2005) reported that the uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the water.

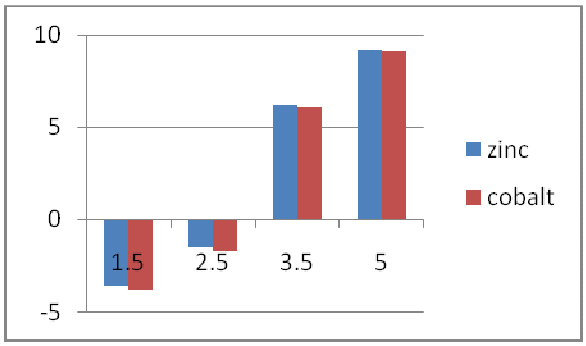

Figure 3

Bioaccumulation of cobalt, copper and zinc (□g mg̃1 dry mass) by Oscillatoria agardhii after seven days growth period

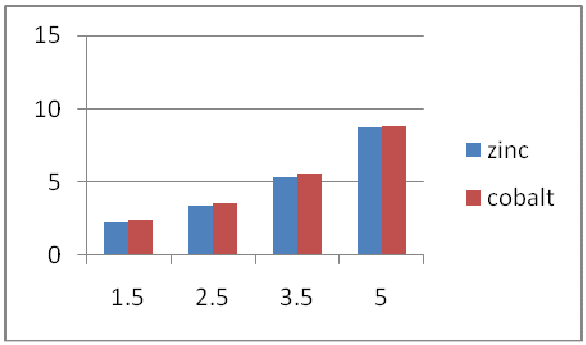

Figure 4.

Bioaccumulation factor of cobalt, copper and zinc by Oscillatoria agardhii after seven days growth period

CONCLUSION

The inhibitory and stimulatory effects of either of the used heavy metals depend on concentration.Different organisms, however, have different sensitivities to the same metal and the same organisms may be more or less damaged by different metals. The uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the water. Different organisms, however, have different sensitivities to the same metal and the same organisms may be more or less damaged by different metals.

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