

## DIFFERENT CONCENTRATION OF ZINC TOLERANCE IN *CHLORELLA VULGARIS* & THEIR EFFECT ON GROWTH AND BIOPIGMENT

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### **ABSTRACT**

**Objective:** This study, it has been concluded that accumulation of Zn uptake can be increased by step wise sub culturing *Chlorella vulgaris* could be tolerant at 3.5 ppm ZnSO<sub>4</sub>.H<sub>2</sub>O and a zinc tolerant strain with maximum tolerance concentration (MTC) 10 ppm ZnSO<sub>4</sub>.H<sub>2</sub>O was obtained by stepwise transfer to higher concentrations. The adaptation was irreversible even after three generation in metal free medium.

**Methods:** *Chlorella vulgaris* grown at 2.5 ppm, 3.5 ppm ZnSO<sub>4</sub>.H<sub>2</sub>O, 5 ppm and a stepwise transfer to higher concentrations strain with maximum tolerance concentration (MTC) 10 ppm ZnSO<sub>4</sub>.H<sub>2</sub>O.

**Results:** The tolerant strain grew with a shorter lag period of 4 days as against 6 days in the case of the wild strain. The tolerant strain had higher MTC than that of the wild strain. The zinc tolerant strain of *Chlorella vulgaris* was obtained by transferring a 10 days old wild strain from minimal medium containing known concentration of ZnSO<sub>4</sub>.H<sub>2</sub>O.

**Conclusion:** Ultra structural comparisons revealed no structural change in the tolerant strain exposure to zinc, whereas in the wild strain a thick extracellular matrix was observed. At same doses also estimated various biochemical activities including chlorophylls (chl-a, chl-b & total chl) and carotenoid in these experiment.

**Keywords:** *Chlorella vulgaris*, Zinc tolerance, Minimal media.

### **INTRODUCTION**

Rapid industrialization and urbanization in the last century have led to the problems of environmental pollution and ecological damage. An indiscriminate and excessive uses of synthetic fertilizers and chemicals have damaged the ecosystems considerably. Heavy metal contamination of water and soil may create major environmental and human health problems. Among the heavy metals, lead particularly has become a cosmopolitan environmental pollutant (Sharma & Dubey, 2005). Bioremediation typically provides an efficient and economical way to reduce environmental toxins using indigenous or introduced microbes that naturally degrade contaminants. The major advantage of bioremediation is that it is a natural process and can be used at much lower cost than many other treatment technologies.

Industrialization has led to increased emission of pollutants into ecosystems (Diagomanolin *et al.*, 2004). Metal pollutants can easily enter the food chain if heavy metal-contaminated soils are used for production of food crops. Farm productivity has decreased in toxic metal polluted areas (Gosavi *et al.*, 2004). Accumulation of toxic metals e.g. Hg, Cu, Cd, Cr and Zn in humans has several consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and wide range of other illnesses (Thiele, 1995). Elevated levels of such metal ions are generally toxic and cause major damage to cell (Inouhe *et al.*, 1996). Those microbes, which confer resistance by accumulating high amounts of heavy metals either intracellularly or extracellularly or by a combination of both mechanisms, can be harnessed to develop bioaccumulators for treating heavy metal-contaminated wastewater (Stauber JL *et.al* 1987). Hence, insight into the mechanisms of heavy metal resistance becomes essential for evaluating biotechnological utility of a metal-resistant strain. Here we present zinc tolerance mechanism of the *Chlorella Vulgaris*, which has already been reported by us to be an excellent biosorbent for copper, zinc, and cobalt (Twiss MR. 1990).

### **MATERIALS AND METHODS**

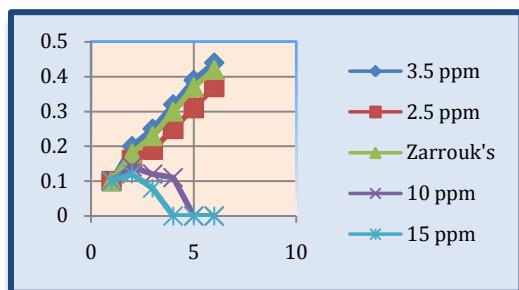
The wastewater alga *Chlorella vulgaris* was collected from industrial wastewater at Jalmahal and nearby area. Algae were thoroughly washed up by tap water to remove any epiphytic algae attached to it. *Chlorella vulgaris* isolation was carried out by identification of algal species by their characterization with the help of standard

monographs (Desikachary, 1959) books and papers published in different scientific journals. The media preparation and the culturing methods were carried out following standard methods of (Allen-Arnon method, 1955) to culture of *Chlorella vulgaris*. Growth was recorded through optical density with the help of a photochem colorimeter at 650 nm every 7th day, over a period of one month. 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<sup>-1</sup> algal suspension. Cell number was determined using a Hemacytometer 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<sup>-1</sup> algal suspension. 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). 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. *Chlorella vulgaris* grown at 2.5 ppm, 3.5 ppm ZnSO<sub>4</sub>.H<sub>2</sub>O, 5 ppm and a stepwise transfer to higher concentrations strain with maximum tolerance concentration (MTC) 10 ppm ZnSO<sub>4</sub>.H<sub>2</sub>O. The adaptation was irreversible even after three generation in metal free medium. The tolerant strain grew with a shorter lag period of 4 days as against 6 days in the case of the wild strain. The tolerant strain had higher MTC than that of the wild strain.

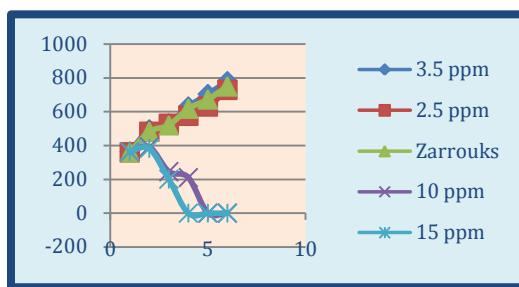
### **RESULTS AND DISCUSSION**

A large array of natural products of economic potential may be produced from fresh water algae. This also represent an attractive source of natural pigment such as chlorophyll, carotenoids and phycobiliprotein because of their very wide range of uses in food, feed, cosmetics and pharmaceutical area (Dhar and Kaushik, 2001). However, further development of this field is impeded by the high cost of the production. There is an urgent need for other strains and genera selection for obtaining high yield of biomass and special products. Biomass production

in turn was depended upon several factors. The growth of any unicellular alga is a manifest of an increase in size followed by the division of the cells, which is said to be influenced by the nutritive medium, light quality and photoperiods accompanied by temperature range.



**Graph 1: Effect of various Zn concentrations on Optical Density of *Chlorella Vulgaris*.**

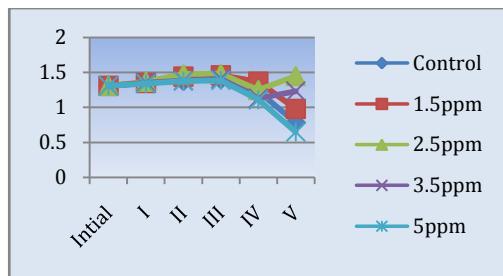


**Graph 2: Effect of various Zn concentrations on Cell Count of *Chlorella Vulgaris*.**

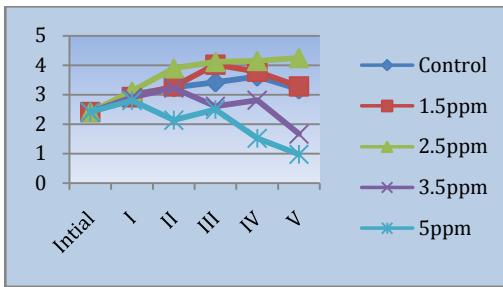
Carbonate has been suggested to be essential for the growth and metabolic activities of the algae (Huang and Lin, 1981 and Schlosser, 1982). Further, carbon provided skeleton for the assimilation of ammoniac nitrogen. Nitrogen, being essential requirement for protein synthesis seemed to be most needed by the algae. Zarrouk's medium with 3.5 ppm Zn conc, was the best suited for the growth of *Chlorella Vulgaris* had nitrate as NaNO<sub>3</sub> (1.5 gm/l) growth with respect to cell count and optical density was supported with graph 1 and 2. The excessive amount of nitrogen seemed to be undesirable for the alga under investigation. Minute quantities of nitrate in remaining solution could not support the growth of the algae. Agius and Jaccarini (1962), Singh and Srivastava (1991) and Bhatia (1996) also supported the above findings.

Phosphate as a source of phosphorus was additional for the substantial growth of the algae. In Zarrouk's media, K<sub>2</sub>HPO<sub>4</sub> and super phosphate has been the source of phosphorous. In green algae, sodium seemed to have influenced the growth and photosynthesis (Belkin and Padan, 1983). It was in the form of NaCl, NaNO<sub>3</sub> and NaHCO<sub>3</sub> in Zarrouk's media. However, it was inadequate amount in the rest of the media employed. MgSO<sub>4</sub>, a constituent of chlorophyll, appeared to be an absolute requirement, Rodhe (1948) and Round (1966) reported that Mg not only contributed to brilliant blue green coloration, rather accelerate the growth of the algae as well.

Besides chemical composition, pH also instructed the growth the algae. Media having alkaline pH showed better results, whereas it was retarded in media with acidic pH. Kratz and Myer (1955) and Sardeshpande and Goyel (1981) shared similar observations. Zarrouk's (10.2) media was employed throughout the period of this study. Cell counts were also found in favours of the above result, which showed an increase of about 1.7 times the initial number. Chlorophyll-a quantity also support similar growth patterns. Comparatively slower but steady growth was observed throughout the experiment. Finally on V week, total chlorophyll-content was 1.47 times the initial amount (Graph 3 &4).

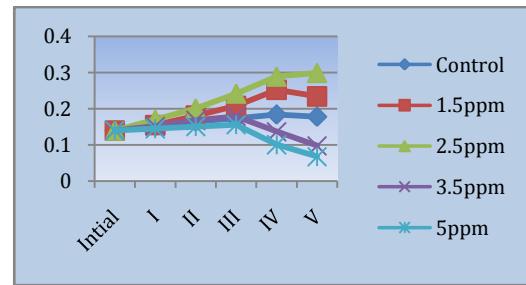


**Graph 3: Effect of different Zn concentrations on Chlorophyll-a (%) of *Chlorella Vulgaris*.**



**Graph 4: Effect of different Zn concentrations on Chlorophyll-b (%) of *Chlorella Vulgaris*.**

Cultures were brilliant blue green and trichomes were quite healthy upto III week. Thereafter some unhealthy and slightly yellowish trichomes were noted. Finally, cultures turned yellowish green in colour and trichomes were not as healthy as noted earlier. The most significant feature was shriveled and unhealthy pigment complex intermingled with swollen intercellular septa.



**Graph 5: Effect of different Zn concentrations on Carotenoid (%) of *Chlorella Vulgaris*.**

After a period of 1 month, culture contained 1.31 % chlorophyll-a, 0.214 % carotenoid (shown graph: 5). the adaptation was irreversible even after three generation in metal free medium. The media concentration was reached upto 10 ppm after 3 months by repeated sub culturing on metal free media. Thus the result showed higher uptake of heavy metal which can work as detoxifier.

## CONCLUSION

After this study, it has been concluded that accumulation of Zn uptake can be increased by step wise sub culturing. 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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