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Metal Effects on Carotenoid Content of Cyanobacteria

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Abstract: Cyanobacteria exhibit an extraordinary resistance to many environmental factors including metal pollution. The present study was conducted to explore the possibility of using cyanobacteria for bioremediation of Co^{2+} and Zn^{2+} as essential nutrient elements for the growth of cyanobacteria which detoxify these metals. *Anabaena oryzae* and *Tolypothrix tenuis* cells collected from paddy fields expressed different degrees of tolerance to metal(s) stress due to cobalt, zinc, copper and mercury. The tolerance of these species under different concentrations (1, 10 and 100 ppm) of heavy metals was determined. Observations were made on every 2nd day for the period of 12 days. Among the organisms studied, *T. tenuis* was more sensitive to the metals than *A. oryzae*. Copper at 1 ppm on the 8th day gave maximum carotenoid content (6.652 μg mL^{-1}) in *A. oryzae*. The mercury treated cells showed lethality at (1, 10 and 100 ppm). There was gradual increase of carotenoid content after 12 days, especially at (1 and 10 ppm) of Co^{2+} and Zn^{2+} in *A. oryzae*. This indicated the possibility of application of this species for detoxification of effluents.

Key words: Heavy-metal pollution, carotenoid, detoxification, cyanobacteria

INTRODUCTION

Carotenoids are naturally occurring pigments in cyanobacteria responsible for various shades of blue, pink and red. They serve as accessory pigments for light harvesting and help in prevention of photo-oxidative damage (Bryant, 1994).

Carotenoids absorb the photons and transfer the energy to chlorophyll (°CAR: CHL→CAR: CHL°) thus assisting in the harvesting of light in the range of 450 to 570 nm (Cogdell and Gardiner, 1993). Cobalt is one of the essential trace elements for the growth of cyanobacteria. The concentration of 5×10^{-6} to 10^{-5} mol L^{-1} of Co^{2+} exerted maximum stimulatory effect on *Chlorella pyrenoidosa* cells at the exponential growth phase in terms of fresh weight (150-160% increase), dry weight (50-60% increase), chlorophyll a and b (45-65%) increase, total carotenoids (55-65% increase), water soluble proteins (19-20% increase) and monosaccharide content (55-65% increase) (Czerpak *et al*., 1994). In *Chlamydomonas reinhardtii* there was reduction in growth at 10 ppm of Co^{2+} without change in the morphology of cells or pH. At 20 ppm Co^{2+}, growth was considerably reduced compared to the control and the colour of the organism became paler and the cells clumped (Lustigman *et al*., 1995). The chlorophyll fluorescence analysis could be a useful physiological tool to assess early stages of change in photosynthetic performance of algae in response to heavy metal pollution (Lu *et al*., 2000). Growth of *Synechocystis* PCC 6803 cells in 10 mol L^{-1} of Co^{2+} was found to stimulate the PSII electron transport rates (Tiwari and Mohanty, 1993). Cobalt permitted more vigorous synthesis of RNA than of protein in *Escherichia coli* (Blundell and Wild, 1969). They provided evidence that Co^{2+} specifically affects protein-synthesizing machinery.

Zinc is required for many biological functions, which include both structural and catalytic roles for a number of enzymes (Lipscomb and Strater, 1996). In photosynthetic organisms, Zn^{2+} is also needed for electron transport and photophosphorylation and for carbonic anhydrase activity involved in CO_2 supply to RuBisCO. It has been proposed that Zn^{2+} may limit the primary biomass production in oceans and therefore influence the global carbon cycle (Buitenhuis *et al*., 2003). Zinc is major industrial pollutant of terrestrial and aquatic environments (Foy *et al*., 1978; Collins *et al*., 1981). The presence of high levels of Zn^{+2}, Cd^{+2}, Cr^{+2} has been well documented in sediments, suspended particles, water column and organisms (Lima *et al*., 1986; Filho *et al*., 1997). Relative low levels of Zn^{2+} 30 μg L^{-1} have been shown to inhibit CO_2 fixation in marine phytoplanktons (Davies and Sleep, 1979). The photosynthetic activity is inhibited in spinach chloroplast by Zn^{2+} concentrations higher than 1.45 μg L^{-1} (Hampp *et al*., 1976). Moreover, Zn^{2+} concentrations higher than 130 μg L^{-1} were required to inhibit photosynthetic electron transport in plant chloroplasts (Tripathy and Mohanty, 1980).

Copper is an essential micronutrient required for photoautotrophic growth of cyanobacteria as it is component of plastocyanin in the photosynthetic electron transport chain. Nielsen and Wium-Andersen (1971) have observed pronounced inhibition of photosynthesis rather than of growth in *Nitzschia palea*, when treated with Cu^{2+}. The higher concentration of Cu^{2+} caused loss of photosynthetic pigments in *Chlorella* sp. (Gross *et al*., 1970). Similarly, other workers also have observed adverse effect of Cu^{2+} resulting in disruption of chloroplast membranes, degradation of photosynthetic pigment and cell proteins (Cedeno-Maldonado and Swader, 1972, 1974). In cyanobacteria, Cu^{2+} was found to cause increase in cellular bodies containing sulfoquinovosyl diglyceride to which Cu^{2+} was bound (Wolk, 1973). Copper uptake has been described by the Free Ion Activity Model (FIAM), which relates uptake to the chemical activity of the free metal ion (Cu^{2+}). Transport into the cell occurs via complexation to a ligand on the cell surface that takes Cu^{2+} across the plasma membrane, followed by complexation to a stronger internal ligand within the cell itself (Campbell and Laudenbach, 1995).

Mercury compounds have been used worldwide as active ingredients in pesticides and fungicides. The environmental significance of Hg^{2+} pollution, particularly in aquatic system, is complicated by microbial conversion of Hg^{2+} compounds into more toxic forms (Tonomura *et al*., 1972). The effects of Hg^{2+} have been studied on algal growth (Ben-Bassat *et al*., 1972; Blinn *et al*., 1977; Davies, 1974; Kamp-Nielsen, 1971; Nuzzi, 1972; Tompkins and Blinn, 1976) on metabolic activities including photosynthesis (Harriss *et al*., 1970) and on ATP levels (Brezonik *et al*., 1975). Organic Hg^{2+} compounds were in general more inhibitory than inorganic ones. Sensitivity appears to be strongly species related. Hg^{2+} present in low concentration may have no obvious effect on organisms, especially if all other environmental conditions remain optimum. However, if some other variable such as temperature is rapidly changed, there may be synergistic interaction causing death or debility of organisms (Vernberg and Vernberg, 1972). Since phytoplanktons are at the base of food web, any adverse effects on the algae could directly affect their consumers and indirectly the other trophic levels of the aquatic community. At the high concentrations of Hg^{2+}, lysis of the vesicles enclosing the young colonies, an enzymatic hydrolysis was found delayed (Gawlik and Millington, 1969).

The detoxification mechanisms in the algae and cyanobacteria changes in their metabolism on exposure to heavy metals are to be understood in order to develop strategies for the treatment of industrial effluents, recycling of waste water and recovery of essential metals using these organisms. The present study was attempted as carotenoids are integral constituents of the protein-pigment complexes of the light-harvesting antennae in cyanobacteria; they are also important components of the photosynthetic reaction centers.

MATERIALS AND METHODS

Physiography of the study area: The sites selected for the present investigation are paddy fields of Warangal west region (Lat. 18°0' 0" N, Long. 79°34' 48" E) which is located 148 km (92 mi) northeast of Hyderabad.

Isolation and mass culture of cyanobacteria: *A. oryzae* and *T. tenuis* were collected and isolated (Venkataraman, 1969) from paddy fields of Warangal district Andhra Pradesh, India. Media selection studies have shown that modified BG-11 medium (Rippka *et al*., 1979) at pH 7.5 was the best suited for *A. oryzae* and *T. tenuis*. One single trichome from each colony was isolated and transferred to fresh solid media and subjected to repeated sub-culturing before transferring to sterilized liquid BG-11 medium. Filaments from actively growing healthy cultures were transferred to fresh medium one day prior to treatment with heavy metals.

Experimental methodology: An aliquot of 30 mL of exponentially growing cyanobacterial cells were transferred to sterilized Erlenmeyer flasks supplemented with 300 mL of nutrient medium containing separately 1 ppm, 10 and 100 ppm of cobalt, zinc, copper and mercury. A set of four flasks was used as replicate for each concentration and also one set as a control. All cultures were maintained at 26±2 °C temperature and 1500 Lux light intensity provided with 16/8 L/D cycle. The treated filaments were observed for short-term duration from the 2nd day and then continued for the period of 12 days (at 2, 4, 6, 8, 10 and 12 days) to screen the carotenoid contents.

Spectroscopic analysis of carotenoids: Absorption spectrum was recorded on the UV-SL-171 spectrophotometer at 461 and 664 nm for estimating carotenoids using pure di-methyl formamide as blank (Chamovitz *et al*., 1993):

$$\text{Carotenoid } (\mu g\ mL^{-1}) = (O.D._{461} - 0.046 \times O.D._{664}) \times 4$$

Statistical analysis: Correlation coefficient is used to determine the relationship between control and test samples and also find confidence interval for population mean. Fisher transformation and t-test are used for comparison.

RESULTS

Effect of different concentrations of cobalt (III), zinc (II), copper (II) and mercury (II) on the carotenoid content in *A. oryzae* and *T. tenuis* was significantly varied. In control, the amount of carotenoid content in *A. oryzae* have shown (0.967, 1.934, 2.663, 3.392, 2.687 and 1.982 μg mL^{-1}) carotenoid content at various durations (2nd, 4th, 6th, 8th, 10th and 12th day).

Effect of Co^{2+} on the 2nd, 4th, 6th and 8th day at concentrations 1, 10 and 100 ppm on the carotenoid content was observed (Fig. 1). There was decrease in carotenoid content on 12th day at 100 ppm. Zinc showed gradual increase in the carotenoid content at 1 ppm on every alternate day form 2nd day to 10th day. Similarly, at 10 ppm on 2nd, 4th and 6th day and at 100 ppm on the 2nd and 4th day carotenoid content was higher than control. Relatively, higher concentration of 100 ppm on the 6th day and for 10 and 100 ppm on the 8th day and 10th day and at all the test concentrations on the 12th day 1, 10 and 100 ppm the carotenoid content decreased. Copper on the 2nd, 4th, 6th and 8th at 1, 10 and 100 ppm stimulated more carotenoid content than the control. 10th day at 10 and 100 ppm and 12th day at 1, 10 and 100 ppm the carotenoid content was negligible of all the metals studied. Copper at 1 ppm on the 8th day showed maximum carotenoid content (6.652 μg mL^{-1}) nearly double than the control (3.392 μg mL^{-1}). Mercury caused less carotenoid content than the control.

In control, the amount of carotenoid content in *T. tenuis* varied from 1.540, 1, 1.647, 0.955, 0.818, 0.127 and 0.481 μg mL^{-1}, respectively, at 2nd, 4th, 6th, 8th, 10th and 12th day.

Effect of cobalt Co^{2+}, Zn^{2+} and Cu^{2+} caused decrease in carotenoid content. Mercury at 1 ppm on the 2nd and 6th day caused more carotenoid content than at 10 and 100 ppm (Fig. 2).

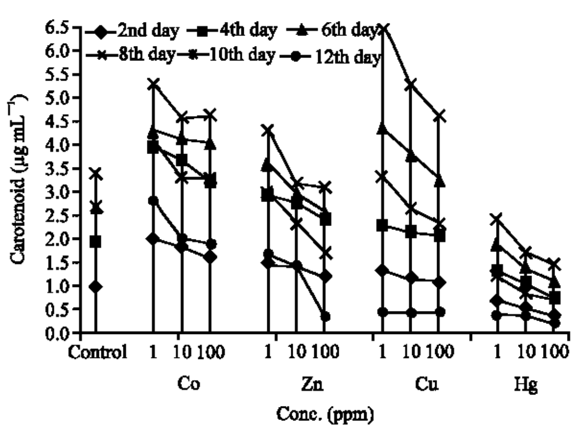


Fig. 1: Effect of heavy metals on carotenoid content of *Anabaena oryzae*

Statistical analysis: The statistical data that will give information about goodness or fitness of model is regression (R^2), the coefficient of determination is a statistical measurement of how the regression line approximately equals to the real data points. Almost all the metal treated samples showed inverse linear relationship with the control in the two species studied. It was observed in the analysis that direct linear regression line positively and perfectly fits the data in Co^{2+} and Cu^{2+} treated samples (correlation), and there is perfect inverse linear relationship with Zn^{2+} as r^2 (t-test) equals to 1 (Fig. 3). In Co^{2+} and Zn^{2+} samples there is high degree of inverse linear relationship as the Linest (R^2) is equals to 1

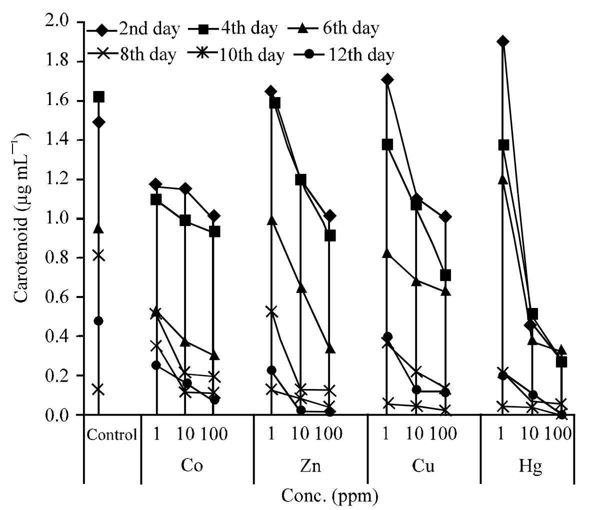
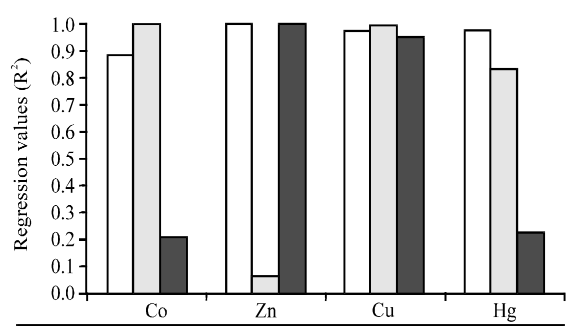
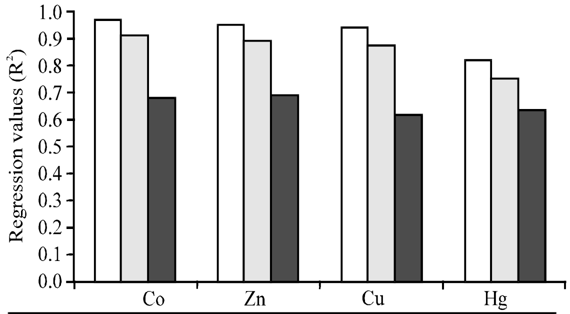


Fig. 2: Effect of heavy metals on carotenoid content of *Tolypothrix tenuis*



	Co	Zn	Cu	Hg
Linest	0.889	0.992	0.972	0.976
T-test	0.996	0.066	0.999	0.834
Correlation	0.211	0.996	0.949	0.228

Fig. 3: Simple Linear regression, t-test and correlation statistics of *Anabaena oryzae*



	Co	Zn	Cu	Hg
Linest	0.986	0.957	0.956	0.839
T-test	0.929	0.906	0.888	0.762
Correlation	0.693	0.699	0.623	0.647

Fig. 4: Simple Linear regression, t-test and correlation statistics of *Tolypothrix tenuis*

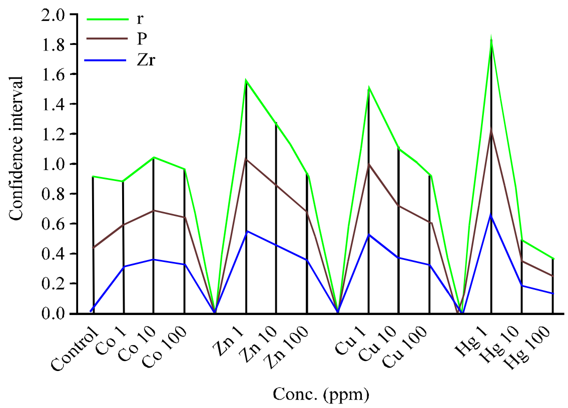


Fig. 5: Confidence interval for P in *Anabaena oryzae*, r: Sample confidence interval, P: Population confidence interval, Zr: Fisher transformation

(Fig. 4). It can be inferred that the use of simple linear regression analysis t-statistic computed is appropriate to test null hypothesis, when population p = 0. From (Fig. 5, 6) in case of P≠0, R^2 (correlation coefficient) is transformed to Zr (Fisher transformation). So, in order to find the confidence interval for P (control) the data range of P should be in between r (sample confidence observations) and Zr (Fisher transformation). Then we will not reject the hypothesis if P is in the confidence interval and our observations regarding the test organisms have 95% confidence.

DISCUSSION

Increase in carotenoid content in Co^{2+} and Zn^{2+} treated cells of *A. oryzae*, appears to be in response to metal detoxification mechanism. The requirement of Co^{2+}

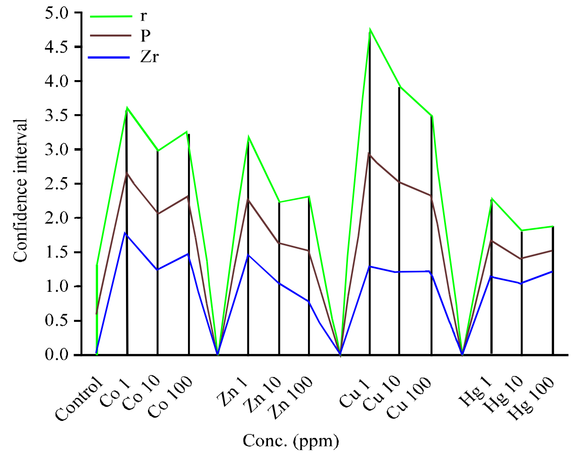


Fig. 6: Confidence interval for P in *Tolypothrix tenuis*, r: Sample confidence interval, P: Population confidence interval, Zr: Fisher transformation

for the growth of cyanobacteria at concentrations above 5 ppm was observed in *Nostoc muscorum*, *Calothrix parietina*, *Coccochloris peniocystis* and *Diplocystis aeruginosa* (Holm-Hansen *et al*., 1954). *T. tenuis* also was found to be sensitive to Co^{2+}. These observations were in accordance with Czerpak *et al*. (1994) who found that the concentration 5×10^{-6}-10^{-5} mol L^{-1} Co^{2+} exerted maximum stimulatory effect on total carotenoids (55-65% increase) of *Chlorella pyrenoidosa* cells at the exponential growth phase, when compared to control cultures. Growth of *Synechocystis* PCC 6803 cells in 10 mol L^{-1} $CoCl_2$ stimulated the PSII electron transport rates (Tiwari and Mohanty, 1993).

High Zn^{2+} accumulation in the cells was associated with decrease carotenoids in *Synechocystis aquatilis* (Andrade, 1997). The mechanism of Zn^{+2} toxicity of photosynthetic electron transport has been hypothesized to result from inhibition of either the donor or the acceptor side of PSII spinach thylakoids (Rashid *et al*., 1991). Copper treated cells of *A. oryzae* at (1 and 10 ppm) were found to be productive in 10 days unlike, *Anabaena* cells which were totally inhibited by Cu^{2+} at concentration between 0.6 and 6.0 ppm (Laube *et al*., 1980). Mercury was found to be more toxic compared to other metals. Stratton *et al*. (1979) found this in *Anabaena aequalis*. Similar effects of Hg^{2+} were reported in other algae (Hannerz, 1968; Gloosschenko, 1969; Matida *et al*., 1971; Matson *et al*., 1972; Richardson *et al*., 1975).

The relative toxicities of the test metals to both *A. oryzae* and *T. tenuis* were found to be in the increasing order of Co<Zn<Cu<Hg. Metal toxicities seemed to be in the decreasing order of Hg>Cu>Cd>Pb>Zn based on the photosynthetic response of natural phytoplanktons

(Hongve *et al.*, 1980). It is proposed that the differences in metal toxicities may be due to the differential affinity of the metal ions for the Sulphur complexation according to Sulphur affinity hypothesis (Fisher and Jones, 1981). Further the poisoning of the intracellular enzyme systems has also been said to be responsible for the toxicity of heavy metals. These metals were found to cause disruption of the thylakoid membranes in *Anabaena flos-aquae*, resulting in the degradation of the light harvesting pigment and thus decreasing their contents of the cells (Rai and Dubey, 1989). A greater inhibition of the total carotenoid pigment content observed in our experiment may be due to the higher sensitivity of the total carotenoid pigment towards the heavy metal ions.

CONCLUSION

The importance of carotenoids as biological indicators for monitoring the effects of heavy metals on the cyanobacteria is to be perceived. The obtained results contribute to the basic database in ascertaining the toxic potential of the metals and also in determining the sensitive cyanobacterial strains for bio-monitoring of metal pollution and in detoxification of polluted waters.

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