

## **Stress and toxicity of biologically important transition metals (Co, Ni, Cu and Zn) on phytoplankton in a tropical freshwater system: An investigation with pigment analysis by HPLC**

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### **Abstract**

Stress and toxicity of four biologically important transition metals (Co, Ni, Cu and Zn) on phytoplankton in Godavari River (a tropical freshwater system) were studied to understand the fate of phytoplankton of freshwater if it receives metal contaminated water imposed by these four metals. Shift in community structure of phytoplankton and their different tolerance levels for different metals were also investigated. It was found that the variation of metal concentrations at lower level ( $1 \times 10^{-9}$  to  $1 \times 10^{-8}$  M) did not show a dramatic change in the total biomass or concentration of the pigment markers. At concentrations of  $1 \times 10^{-7}$  M of metal, Cu acted as a nutrient and helped to increase the biomass followed by Co, Ni and Zn. The variation in biomass in the freshwater system under exposure to different metals at high concentrations of  $1 \times 10^{-6}$  M indicates that Cu had strongest interactions with biotic ligand and was taken up by phytoplankton and acted as the most toxic metals followed by Zn, Co and Ni. Phytoplankton communities in Godavari River have different tolerance levels for different metals. Cu and Zn were found to be lethal at high concentration for both green algae and cyanobacteria. Cyanobacteria were found to be very sensitive to slight variation in Ni concentration and Co was found to be less toxic than Cu and Zn even at high exposed concentration.

**Key words:** Trace metal stress, toxicity, pigment analysis, Tropical freshwater phytoplankton, phytoplankton community

## 1. Introduction

One of the most necessary subjects of investigations among environmental chemists and ecotoxicologists is to understand the behavior and effects of trace metals in the environment.

Trace metals are important causes of environmental pollution, being ubiquitous and readily dissolved and transported in water. They are readily taken up by aquatic organisms. Trace metals can act as a nutrient but at an elevated concentration they can interact with proteins and can change the structure and enzymatic activities within the cell of an aquatic organism and can display its toxic effects at the whole organism level (Hodson 1988, Viarengo 1985, and Magdaleno et al 1997).

It has been reported that toxicity of metals is dependent on their speciation. Much of our current knowledge on trace metal toxicity to phytoplankton are based on laboratory based studies using cultured algae (e.g. Wong and Beaver 1979, Stauber and Florence 1989, Rodgher and Espindola 2008, Vasseur and Pandard 2006), and cyanobacteria (Wilson and Pearson 1985, Vymazal 2006) as a representative of natural phytoplankton. However, number of studies on stress and toxicity of trace metals at wild phytoplankton in natural system is scarce.

Regulatory authorities responsible for environmental protection are frequently referring to results from 'new research' in their efforts to regulate or restrict uses of metals and metal-containing materials. Thus, it is extremely important to have relevant information about the properties, behavior and effects of trace metals on phytoplankton community in natural environment.

The major thrust of this research was to understand the effects of varying concentrations of four biologically important transition metals (such as Co, Ni, Cu and Zn) on phytoplankton in Godavari River (a major peninsular river of India) and how the varying concentration of these metals can affect the total biomass and phytoplankton community structure in a tropical freshwater system. It is very important to know the effects of these metals on phytoplankton in a freshwater system as it is at or near the bottom of the food chain which might therefore influence the ecology.

This study is significantly important to provide a better scientific understanding to regulatory bodies of water resources for decision-making, taking into consideration the fate of phytoplankton of freshwater if it receives metal contaminated water imposed by Co, Ni, Cu and Zn.

It has been reported that photosynthetic pigments of phytoplankton can be used for characterization of the phytoplankton community status in the freshwater environment (Jeffrey et al. 1997, Barlow *et al.*, 1997). The distribution of, and variations in, the phytoplankton community after exposing to different metals at different concentrations was assessed by quantitative determination of their class specific

marker pigments by using High Performance Liquid Chromatography (HPLC) (Mantoura and Llewellyn, 1983; Klein and Sournia, 1987; Wright *et al.*, 1991) Chlorophyll *a*- was used as an indicator for total biomass, lutein and zeaxanthin was used as an indicator for green algae, and cyanobacteria respectively. This methodology is based on the premise that different algal classes have specific signature, or marker, pigments.

### *Study Area*

Godavari is the third largest river in India. It has formed two major distributaries; Goutami and Vasista before flowing into the Bay of Bengal, east coast of India as shown in Fig 1. Freshwater samples were collected from Gautami Godavari as it carries majority of the discharge. Sampling stations was located at Alamuru which is about 40 km upstream of Yanam estuary. The samples collected at Alamuru represent typical freshwater having salinity of 0.02 PSU. During monsoon, Godavari at Alamuru station receives a large quantity of discharge of freshwater from Dowleswaram Dam.

## **2. Sampling and analysis**

### **2.1. Sample collection**

Surface water sample from Godavari River was collected from Alamuru station by using 5-L Niskin bottles and finally transferring it to 20 L (Nalgene) bottles. The pH of the sample was measured immediately after collecting the sample by Metrohm auto titration unit and found to be 8.15. The pH of the sample was measured prior to the incubation experiment and after the incubation of 60 hrs. No variation in the pH value was observed. The total organic carbon (TOC) concentration was found to be  $12.0 \pm 0.6 \text{ mg. L}^{-1}$ . The major ion concentrations such as  $\text{NO}_2^{-1}$ ,  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^{+}$  ions were found to be  $4.1 \times 10^{-6} \text{ M}$ ,  $6.0 \times 10^{-7} \text{ M}$ , and  $3.4 \times 10^{-6} \text{ M}$  respectively. The total concentrations of Na, K, Ca and Mg obtained from literature were  $(0.70 \pm 0.17) \times 10^{-3} \text{ M}$ ,  $(0.18 \pm 0.05) \times 10^{-3} \text{ M}$ ,  $(0.63 \pm 0.13) \times 10^{-3} \text{ M}$ , and  $(0.41 \pm 0.12) \times 10^{-3} \text{ M}$  respectively in Godavari River. However, it is important to note that the concentrations of major cations presented here are the average values obtained from literature (Duggempudi, 1998, Duggempudi 1995, Biksham and Subramanian 1988).

Water samples were distributed into twenty eight 1L (Nalgene) bottles. These twenty eight bottles were divided into four sets. Six bottles in each set was manipulated with varying concentrations of each metal. All metals were spiked as metal nitrate. It was understandable that the variation of metal concentration in each bottle would change the nitrate concentration in different bottles. It is well known that nitrate can act as a nutrient for phytoplankton and can interfere with this experiment. In order to swamp out the

effect of variation in nitrate concentrations,  $5.0 \times 10^{-6}$  M of nitrate and  $3.2 \times 10^{-7}$  M of phosphate was added following the Redfield ratio in each bottle. The added concentrations of nitrate and phosphate in each bottle were environmentally realistic. Total metal concentrations in the natural sample were analyzed by ICP-MS (X SERIES II, Thermo Scientific) and all these metals were present in the range of nM concentration. This indicates that the water collected in Godavari River at Alamuru station (with no discharge from Dawleswaram Dam) was not contaminated with these four metals.

Different concentrations of Co, Ni, Cu and Zn, which ranged from  $1 \times 10^{-9}$  to  $2.5 \times 10^{-6}$  M, were added from ICP standards (Merck, Germany). NaOH of AR grade were used to adjust the pH immediately after the addition of different concentration of metals. Ultrapure water was used through out the experiment. Referring to the atmospheric bulk deposition values of metals, the exposed concentrations ranging from  $1 \times 10^{-9}$  to  $2.5 \times 10^{-6}$  M. were approximately realistic. Four 1L nalgene bottles with freshwater were kept as control. After adjusting the pH all the bottles were incubated for 5 days (120 hours).

## **2.2. Filtration and Extraction:**

After five days of incubation, 0.5L of samples were filtered through Whatman GF/F filter paper (0.7 $\mu$ m nominal pore size and 47mm diameter) under reduced vacuum. Filters were dabbed in tissue paper to remove water. Each filter was cut into small slivers and was placed in heavy-walled 10 ml amber coloured culture tubes (Shimadaju: P/N 638-41462). 4mL of 90% Acetone was added to the tubes using a dispensette. Each tube was covered and placed in an ultrasonic bath filled with ice slurry to prevent heat accumulation. Ultrasonification (Cole Parmer, Model: 08893-26) of samples enables disruption of cells and facilitates pigments to come into the extraction solvent. All the tubes were then placed in a freezer (-20 degree centigrade) for overnight. Sample slurry was then vortexed (GeNei India Pvt. Ltd.) and clarified by pushing the contents through a nylon HPLC syringe cartridge filter (PAL, P/N 4118) with 0.45 $\mu$ m pore size.

## **2.3. HPLC Analysis**

Analysis was performed by using Agilent 1200 HPLC system equipped with quaternary pump, autoinjector, Peltier column thermostat, temperature controlled autosampler and chemstation software. Pigments were detected with the diode array detector using 450 and 665 nm wavelength (20 nm bandwidth was used in both cases). 665 nm was used to quantify chlorophyll-a, divinyl chlorophyll-a, chlorophyllide-a, phaeophorbide-a and phaeophytin-a as they respond similarly and strongly in this

wavelength. All other carotenoids and xanthophylls were detected and quantified at 445nm (Heukelem et al. 2001).

An injector program was optimized to deliver sample extract and buffer composed of 28 mM aqueous tetrabutyl ammonium acetate (TBAA) (AR Grade, Fluka) at pH 6.5 and methanol( GC Assay 99.7% pure,Merck) in 90: 10 ratio. The sample extract and buffer was mixed automatically within the sample loop which enabled effective retention of early eluting chlorophylls and lessened peak distortion. Method proposed by (Heukelem et al. 2001) was adapted for pigment analysis during the study. The method is outlined below,

Column: ZORBAX eclipse XDB-C8, 4.6 × 150 mm (diameter by length); PN: 963967-906

Gradient: Binary gradient elution

A: 70/30 methanol/TBAA pH 6.5

B: methanol

Linear gradient from 5-95% solvent B in 22 min, isocratic hold of 95% solvent B

From 22-29 min, return to 5% solvent B at 31 minutes, equilibration for further 5 minutes (31-36 minutes)

Injection volume: 200 ul

Oven temperature: 60 degree celcius

Solvent flow rate: 1.1 ml/min

### **3. Results and discussions:**

#### **3.1. Effect of copper on biomass and pigments composition in *phytoplankton***

Concentrations of different pigments obtained from phytoplankton after incubation with different exposed concentrations of Cu was calculated from the chromatograms and the data are presented and summarized in Fig. 2 and Table 1.

Figure 2 shows that *chl-a* concentration (which is a representative of biomass) gradually increased with the increasing concentration of Cu from  $1 \times 10^{-9}$  to  $5 \times 10^{-7}$  M. *chl-a* concentration reached its maximum value of 8.00 mg.  $M^{-3}$  when the phytoplankton were exposed to Cu concentration of  $1 \times 10^{-7}$  M and remained constant with further increase in Cu concentration to  $5 \times 10^{-7}$  M as shown in Fig 2 and Table 1, which indicates that Cu probably acted as a nutrient and enhanced the total biomass up to  $5 \times 10^{-7}$  M.

However, further increase in Cu concentration to  $1 \times 10^{-6}$  M in the medium drastically decreased *chl-a* concentration from 8.00 to 0.90 mg.  $M^{-3}$ . This observation indicates that the elevated concentration

( $1 \times 10^{-6}$  M) of Cu reduced the biosynthesis of *chl-a* and probably phytoplankton was under stress in the system.

Further increase in Cu concentration to  $2.5 \times 10^{-6}$  M completely destroyed *chl-a* production of phytoplankton at Alamuru station in Godavari River. This is probably because of the fact that higher concentration of Cu ( $> 5 \times 10^{-7}$  M.) might have inhibited *chl-a* biosynthesis in the system as reported by Varda Caspi et al (Varda Caspi et al 1999).

It is very interesting to note that *chl-b* concentration increased with the increasing Cu concentration from  $1 \times 10^{-7}$  M to  $5 \times 10^{-7}$  M and decreased dramatically when phytoplankton were exposed to  $1 \times 10^{-6}$  M of Cu in the system (Please see chromatograms, which are given as supporting documents) . This observation suggests that the phytoplankton were stressed when they were exposed to  $5 \times 10^{-7}$  M of Cu. Under stressed condition, *chl-b* usually increases because the metals cause the transformation of *chl-a* to *-b* (Chettri et al. 1998). Oxidation of the methyl group, present on ring II of *chl-a*, to the aldehyde causes the formation of *chl-b* (Bidwell 1979).

However, it is impossible to understand the effect of varying concentration of Cu on a specific phytoplankton community within the system by simply looking at the variation of biomass. Photosynthetic pigments of phytoplankton were used for characterization of the phytoplankton community status in the system. Zeaxanthin and lutein concentration were used to quantify cyanobacteria and green algae respectively to understand the effect on phytoplankton at different exposed Cu concentrations.

Figure 2 shows the variation of zeaxanthin (which is a marker pigment for cyanobacteria) with varying concentration of Cu. It indicates that Cu acted as a nutrient to cyanobacteria and as a result zeaxanthin concentration increased from 3.45 to 9.00  $mg. M^{-3}$  with the increasing concentration of Cu from  $1 \times 10^{-9}$  to  $1 \times 10^{-7}$  M in the system. Zeaxanthin production decreased and reached a concentration of 2.50  $mg. M^{-3}$  in presence of  $5 \times 10^{-7}$  M of Cu, and completely disappeared with further increase in concentration of Cu to  $1 \times 10^{-6}$  M and  $2.5 \times 10^{-6}$  M within the system..

This indicates that cyanobacteria from Godavari River are quite sensitive to high Cu concentration and it is probably because of the fact that high Cu concentrations can inhibit cell defense mechanisms for the destruction of  $H_2O_2$ , leading to the production of free radicals and oxidative destruction of membrane lipids (Sandmann & Boger 1980). This metal ion may react with sulphhydryl groups to lower intracellular thiol concentration, or it may interfere with electron transport. Therefore, the detrimental effects of Cu

may changes in enzyme activity and can demolish the existence of cyanobacteria at  $1 \times 10^{-6}$  M concentration.

It is evident from Fig 2 that lutein (which is a marker pigment for green algae) concentration gradually increased with the increasing concentration of Cu from  $1 \times 10^{-9}$  M to  $5 \times 10^{-7}$  M. This trend was very similar to the change observed for *chl-a* concentration against the varying concentration of Cu. Lutein concentration was highest of  $11.00 \text{ mg. M}^{-3}$  in presence of Cu concentration of  $5 \times 10^{-7}$  M, whereas zeaxanthin concentration dramatically decreased at this concentration which clearly indicates that chlorophytes (green algae) were more resistant to Cu toxicity than cyanobacteria in Godavari River at the sampling station.

However, further increase in Cu concentration to  $1 \times 10^{-6}$  M, decreased the concentration of lutein in the system to  $2.50 \text{ mg. M}^{-3}$ . Such decrease of photosynthetic pigments, including *chl-a* and accessory pigments, such as zeaxanthin and lutein, on exposure to Cu metal at high concentration have also been reported.

### **3.2. Effect of nickel on biomass and pigments composition in *phytoplankton***

Concentrations of different pigments obtained from phytoplankton after incubation with different exposed concentrations of Ni was calculated from the chromatograms and the data are presented and summarized in Fig. 3 and Table 1.

Figure 3, shows that *chl-a* concentration gradually increased from  $3.90$  to  $6.40 \text{ mg. M}^{-3}$  with the increasing concentration of Ni from  $1 \times 10^{-9}$  M up to  $1 \times 10^{-6}$  M. This indicates that Ni acted as a nutrient up to  $1 \times 10^{-6}$  M. However, further increase in Ni concentration to  $2.5 \times 10^{-6}$  M started to reduce *chl-a* concentration. This finding indicates that phytoplankton in freshwater of Godavari River at Alamuru station can tolerate Ni to higher concentration than Cu.

Figure 3 shows the variation in zeaxanthin concentration against different concentration of Ni. Zeaxanthin concentration reached its highest value of  $10.0 \text{ mg. M}^{-3}$  when phytoplankton was exposed to  $1 \times 10^{-9}$  M of Ni. Further increase in Ni concentration to  $1 \times 10^{-8}$  M, reduced zeaxanthin production tremendously to  $1.15 \text{ mg. M}^{-3}$ . Not much change was observed on the concentration of zeaxanthin with further increase in Ni concentration to  $1 \times 10^{-7}$  M in the medium. This observation suggests that cyanobacteria obtained in Godavari River at Alamuru station are quite sensitive to Ni toxicity. It is has been reported that Ni toxicity in cyanobacteria is due to poisoning of intracellular enzyme systems by Ni ions at low concentration (Asthana et al 1992).

However, green algae were quite adaptable to this variation of Ni concentration. It is shown in Fig 3 that Ni acted as a nutrient for green algae and as a result lutein concentration steadily increased from 0.80 to 9.40  $mg. M^{-3}$  with the increasing Ni concentration from  $1 \times 10^{-9}$  M to  $1 \times 10^{-6}$  M. This observation suggests that green algae are more resistant to Ni toxicity than cyanobacteria present in Godavari River. It has been reported that the growth of cyanobacteria is generally more sensitive to Ni toxicity than for green algae (Susan and Mark 2008).

It is interesting to note that Ni toxicity is dependent on pH and the nature and binding capacity of humic substances present in the system. It has been reported that most algal species showed maximum accumulation of Ni at about pH 8. This would be consistent with surface binding of Ni to functional groups on extracellular mucopolysaccharides. The pH of the freshwater sample used in this study was 8.3. Basic condition ( $>pH 7$ ) would facilitate the ionization of more oxygen-donor ligands and amino groups at the cell surface to provide a large number of Ni-binding sites on green algae. Thus, Ni initially accumulates on the cell wall of green algae and can not show its lethal effect immediately. However, Ni damages intracellular enzyme systems in cyanobacteria which make this metal highly toxic to cyanobacteria than green algae. This indicates that the changes in Ni concentration may have serious consequences on cyanobacterial community in Godavari River at Alamuru station.

### **3.3. Effect of cobalt on biomass and pigments composition in *phytoplankton***

Figure 4 represents the concentrations of different pigments obtained from phytoplankton which was exposed to different concentrations of Co (ranging from  $1 \times 10^{-9}$  to  $2.5 \times 10^{-6}$  M) in Godavari freshwater system. The data are summarized in Table 1.

Variation of Co concentration and its effect on *chl-a* production is shown in Fig 4. It is very evident that *chl-a* concentration that is the total biomass gradually increased from 3.40 to 5.20  $mg. M^{-3}$  with the increasing exposed Co concentration from  $1 \times 10^{-9}$  to  $1 \times 10^{-7}$  M. Low concentrations of Co led to an increase in the total biomass of phytoplankton and probably acted as a nutrient. Concentration of *chl-a* reached its maximum value of 5.20  $mg.M^{-3}$  when phytoplankton was incubated with  $1 \times 10^{-7}$  M. of Co. Further increase in Co concentration started to show its toxic effect as shown in Fig 4 and Table 1. It is also interesting to note that a much slower decrease in biomass was observed with the increasing concentration of Co compared to Cu and Ni (Table 1).

Variation in zeaxanthin concentration with varying concentration of Co indicates that cyanobacteria were stressed at higher Co concentration ( $>5 \times 10^{-7}$  M). However, cyanobacteria can tolerate higher



concentration of Co than Cu and Ni. It has been reported that cyanobacteria can adapt to changes in the Co concentration quite readily (Reuter and Muller, 1993)

It is interesting to note that lutein concentration gradually increased from 1.00 to 5.30  $mg. M^{-3}$  with the increase in Co concentration from  $1 \times 10^{-9}$  to  $1 \times 10^{-7}$  M and further increase in Co concentration reduced lutein production.

### 3.4. Effect of zinc on biomass and pigments composition in phytoplankton

Figure-5 shows the variation of *chl-a* concentrations with respect to total Zn concentration in the freshwater system. *chl-a* concentration reached its maximum value of 4.10  $mg. M^{-3}$  in presence of  $1 \times 10^{-7}$  M of Zn and remained constant with the increasing Zn concentration to  $5 \times 10^{-7}$  M. However, further increase in Zn concentration reduced *chl-a* production which indicates that phytoplankton communities in Godavari river can not tolerate more than  $5 \times 10^{-7}$  M of Zn and probably were under stress as shown in Fig 5 and Table 1.

Figure 5 shows that zeaxanthin concentration increased from 2.80 to 4.00  $mg. M^{-3}$  with the increasing concentration of Zn from  $1 \times 10^{-9}$  to  $1 \times 10^{-7}$  M and further increase in Zn concentration decreased the concentration of zeaxanthin to 3.15  $mg. M^{-3}$  and completely disappeared from the system in presence of  $2.5 \times 10^{-6}$  M concentration of Zn. It is important to mention that Zn is more toxic than Co at  $2.5 \times 10^{-6}$  M concentration even though. Zn is a more commonly available and present relatively in excess compared to other three metals.

Figure 5 also shows that lutein concentration gradually increased from 0.90 to 6.60  $mg. M^{-3}$  with the increasing concentration of Zn from  $1 \times 10^{-9}$  to  $5 \times 10^{-7}$  M. However, further increase in Zn concentration to  $1.0 \times 10^{-6}$  M reduced lutein concentration to 3.70  $mg. M^{-3}$  in the system. Zn at  $2.5 \times 10^{-6}$  M concentration was also found to be lethal for green algae which demolished the existence of green algae from the system as indicated by the disappearance of lutein from the system.

These observations suggest that the toxicity of metals to freshwater phytoplankton varied greatly and phytoplankton have different tolerance levels for different metals. However, in order to understand and permit prediction of changes in metal toxicity, greater attempts are now being made to relate metal toxicity to speciation and the concentration of free metal ions.

Most studies in which the toxicity of metals to microorganisms has been varied by addition of organic complexing agents suggest that not only the free metal ion is toxic but also dynamic metal complexes may also become toxic (e.g. Anderson and Morel, 1978; Canterford and Canterford, 1980; Jackson and Morgan, 1978; Sunda and Gillespie, 1979). Attempts to study metal toxicity and speciation in natural

waters, however, are hampered by difficulties in estimating free metal ion concentrations. It is important to note that the concentration of free metal ion and bioavailable metal complexes depend upon metal to TOC ratio. In this study, strategically this ratio was varied (by changing the total metal concentration) to change the bioavailable metal fraction in the system. The TOC concentration was found to be  $12.0 \pm 0.6$  mg. L<sup>-1</sup> in the sample.

At low metal/TOC ratio, metals preferably undergo complexation reactions with the stronger binding sites of TOC and form thermodynamically stable complexes which does not dissociate in the vicinity of phytoplankton and remain as inert complexes. In that case, a metal neither act as a nutrient nor as a toxicant and not much variation in total biomass and concentrations of specific phytoplankton (as indicated by their pigment marker) is expected. Similar observation was found for all the metals. It was found that the variation of metal concentration at lower level ( $1 \times 10^{-9}$  to  $1 \times 10^{-8}$  M) did not show a dramatic change in the total biomass or concentration of the pigment markers (except Ni for zeaxanthin) with respect to the control. However, Ni showed its high toxicity to cyanobacteria. This indicates that cyanobacteria were quite sensitive to Ni toxicity and probably Ni-TOC complexes were taken up by cyanobacteria.

With increasing metal/TOC ratio, metal ions first occupy the stronger binding sites and then the rest of the metal ions move towards the weaker binding sites for complexation with TOC and rest of the metals remain uncomplexed and termed as bioavailable metal ion in the medium (Chakraborty and Chakrabarti 2006; Chakraborty et al 2006). Metal complexes formed by the weaker binding sites of TOC which can dissociate within the vicinity of biotic ligand (BL) (complexing sites present on cell wall of phytoplankton) may also become bioavailable. The dissociation of metal-TOC complexes in the vicinity of BL is dependent on the relative stability of metal-DOC complexes and metal-BL complexes. Thus, metals which form weaker complexes can act as a nutrient or toxicant depending upon their relative stability. It was found that variation of metal concentrations at higher level ( $1 \times 10^{-7}$  to  $2.5 \times 10^{-6}$  M) had dramatic effect on the biomass and concentration of marker pigments.

It is evident from the data that all metals acted as a nutrient when  $1 \times 10^{-7}$  M of metals were added. The variation in biomass in the freshwater system under exposure to different metals at concentrations of  $1 \times 10^{-7}$  M indicates that they follow the following order as a nutrient.

Cu>Co>Ni>Zn. This order clearly shows that Cu acted as a nutrient and helped to increase the biomass followed by Co, Ni and Zn. The order indicates that the bioavailable Cu strongly interacted with BL and was easily taken up by phytoplankton and acted as a nutrient followed by Co, Ni and Zn depending upon

their affinity towards BL. This statement is based on the assumption that bioavailable fractions of all these metals acted as a nutrient when  $1 \times 10^{-7}$  M of these metals was added.

However, metal at  $1 \times 10^{-6}$  M concentration, is expected to have elevated bioavailable fraction for phytoplankton in the freshwater system which may show its vulnerable effect as a toxicant. The variation in biomass in the freshwater system under exposure to different metals at concentrations of  $1 \times 10^{-6}$  M indicates that they follow the following order as toxicant.

Cu>Co>Zn>Ni. This order also suggest that Cu had strongest interactions with BL and was taken up by phytoplankton and acted as the most toxic metals followed by Co, Zn and Ni. It is very interesting to note that the toxicity of Zn was more than Ni at high concentration to the phytoplankton in Godavari River at Alamuru station. It has been reported that Ni is not usually taken up by phytoplankton unless there is a scarcity of nitrogen source. In this study, enough nutrients were present in the medium and less toxicity of Ni was observed.

In general it is found that phytoplankton communities in Godavari River station have different tolerance for different metals. Cu and Zn were found to be lethal at high concentration. Cyanobacteria were found to be very sensitive to slight variation in Ni concentration and Co was found to be less toxic than Cu but more toxic than Zn at high exposed concentration.

It is important to note that the toxicity of a metal depends on environmental conditions and habitat types, thus any risk assessment of the potential effects of these metals on organisms must take into account local environmental conditions. Because these metals are essential elements, procedures to prevent toxic levels in the environment should not incorporate safety factors that result in recommended concentrations being below natural levels or causing deficiency symptoms.

### **Conclusions**

It is important to note that only few attempts have been made to correlate the dynamic speciation of metals and their toxicity in aquatic environment. In this investigation speciation, bioavailability and toxicity of four biologically important metals on phytoplankton communities have been qualitatively correlated for the first time in a tropical freshwater system. However, the major thrusts of further studies remain to correlate trace metal speciation with their biological effects (as a nutrient and toxicant) quantitatively.

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Author (P. Chakraborty) dedicate this work to the memory of his beloved supervisor Late Prof. C. L. Chakrabarti.

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Table 1: Variation in concentrations of *chl-a*, zeaxanthin and lutein obtained from phytoplankton in Godavari River with respect to the varying concentrations of Cu, Ni, Co and Zn.

[Cu] (M)	<i>chl-a</i> (mg. $M^{-3}$ )	Zeaxanthin(mg. $M^{-3}$ )	Lutein (mg. $M^{-3}$ )
Control	3.20 ± 0.20	2.50 ± 0.10	0.90 ± 0.04
1×10 <sup>-9</sup>	3.10 ± 0.20	3.45 ± 0.20	2.90 ± 0.20
1×10 <sup>-8</sup>	2.70 ± 0.10	4.00 ± 0.20	4.60 ± 0.20
1×10 <sup>-7</sup>	8.00 ± 0.4 0	9.00 ± 0.50	9.70 ± 0.50
5×10 <sup>-7</sup>	7.80 ± 0.40	2.50 ± 0.1 0	11.00 ± 0.60
1×10 <sup>-6</sup>	0.90 ± 0.05	0.00	2.50 ± 0.10
2.5×10 <sup>-6</sup>	0.00	0.00	0.00
[Ni] (M)	<i>chl-a</i> (mg. $M^{-3}$ )	Zeaxanthin (mg. $M^{-3}$ )	Lutein (mg. $M^{-3}$ )
Control	3.20 ± 0.20	2.50 ± 0.10	0.90 ± 0.04
1×10 <sup>-9</sup>	3.90 ± 0.20	10.0 ± 0.50	0.80 ± 0.04
1×10 <sup>-8</sup>	4.20 ± 0.20	1.15 ± 0.05	1.20 ± 0.06
1×10 <sup>-7</sup>	4.40 ± 0.20	1.50 ± 0.07	1.70 ± 0.10
5×10 <sup>-7</sup>	5.60 ± 0.30	0.00	7.60 ± 0.40
1×10 <sup>-6</sup>	6.40 ± 0.30	0.00	9.40 ± 0.50
2.5×10 <sup>-6</sup>	2.90 ± 0.10	0.00	4.80 ± 0.25
[Co] (M)	<i>chl-a</i> (mg. $M^{-3}$ )	Zeaxanthin(mg. $M^{-3}$ )	Lutein (mg. $M^{-3}$ )
Control	3.20 ± 0.20	2.50 ± 0.10	0.90 ± 0.04
1×10 <sup>-9</sup>	3.40 ± 0.20	7.60 ± 0.40	1.00 ± 0.05
1×10 <sup>-8</sup>	4.00 ± 0.20	4.70 ± 0.20	2.30 ± 0.10
1×10 <sup>-7</sup>	5.20 ± 0.30	4.00 ± 0.20	5.30 ± 0.30
5×10 <sup>-7</sup>	3.25 ± 0.20	3.80 ± 0.20	3.70 ± 0.20
1×10 <sup>-6</sup>	2.90 ± 0.10	1.20 ± 0.05	3.50 ± 0.20
2.5×10 <sup>-6</sup>	2.40 ± 0.10	1.00 ± 0.04	3.30 ± 0.20
[Zn] (M)	<i>chl-a</i> (mg. $M^{-3}$ )	Zeaxanthin (mg. $M^{-3}$ )	Lutein (mg. $M^{-3}$ )
Control	3.20 ± 0.20	2.50 ± 0.10	0.90 ± 0.04
1×10 <sup>-9</sup>	2.90 ± 0.20	2.80 ± 0.10	0.90 ± 0.10
1×10 <sup>-8</sup>	3.40 ± 0.20	3.80 ± 0.20	3.25 ± 0.20
1×10 <sup>-7</sup>	4.10 ± 0.20	4.00 ± 0.20	4.90 ± 0.20
5×10 <sup>-7</sup>	4.10 ± 0.20	3.15 ± 0.20	6.60 ± 0.30
1×10 <sup>-6</sup>	1.80 ± 0.10	0.60 ± 0.02	3.70 ± 0.20
2.5×10 <sup>-6</sup>	0.00	0.00	0.00

## Captions and legends

**Figure 1** Geographical location of the study area

**Figure 2.** Variation in *chl-a*, zeaxanthin and lutein concentrations obtained from phytoplankton in Godavari River with respect to the varying concentrations of Cu.

**Figure 3.** Variation in *chl-a*, zeaxanthin and lutein concentrations obtained from phytoplankton in Godavari River with respect to the varying concentrations of Ni.

**Figure 4.** Variation in *chl-a*, zeaxanthin and lutein concentrations obtained from phytoplankton in Godavari River with respect to the varying concentrations of Co.

**Figure 5.** Variation in *chl-a*, zeaxanthin and lutein concentrations obtained from phytoplankton in Godavari River with respect to the varying concentrations of Zn.



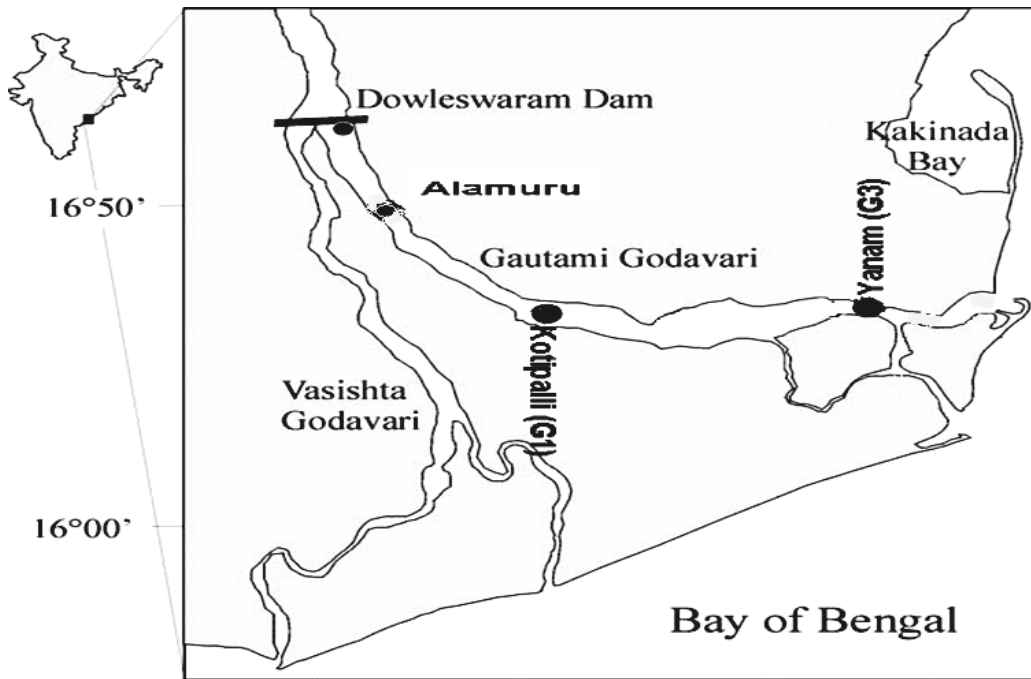


Figure 1

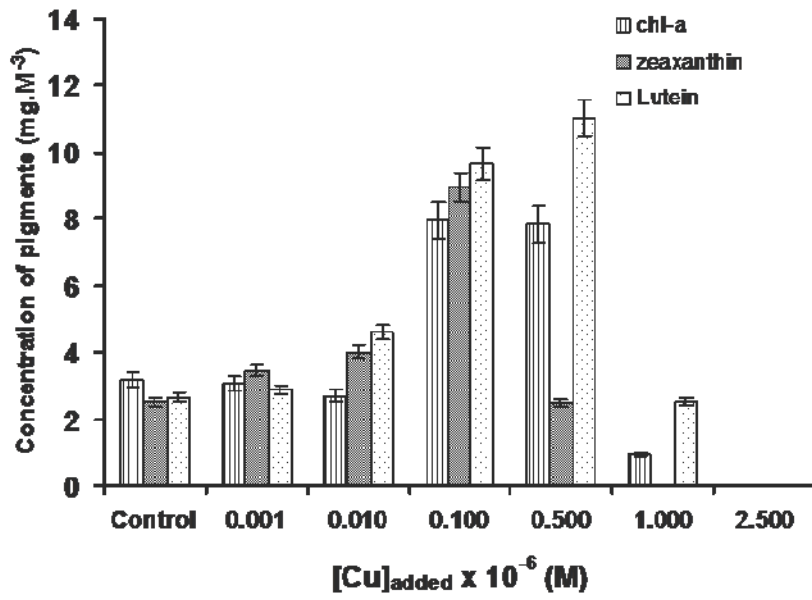


Figure 2

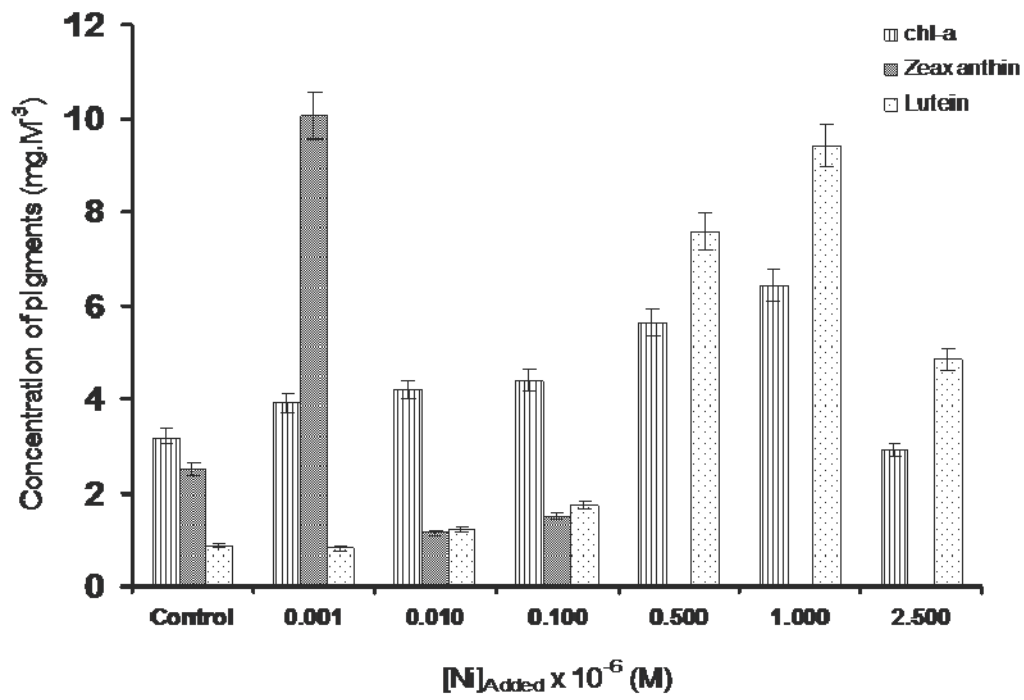
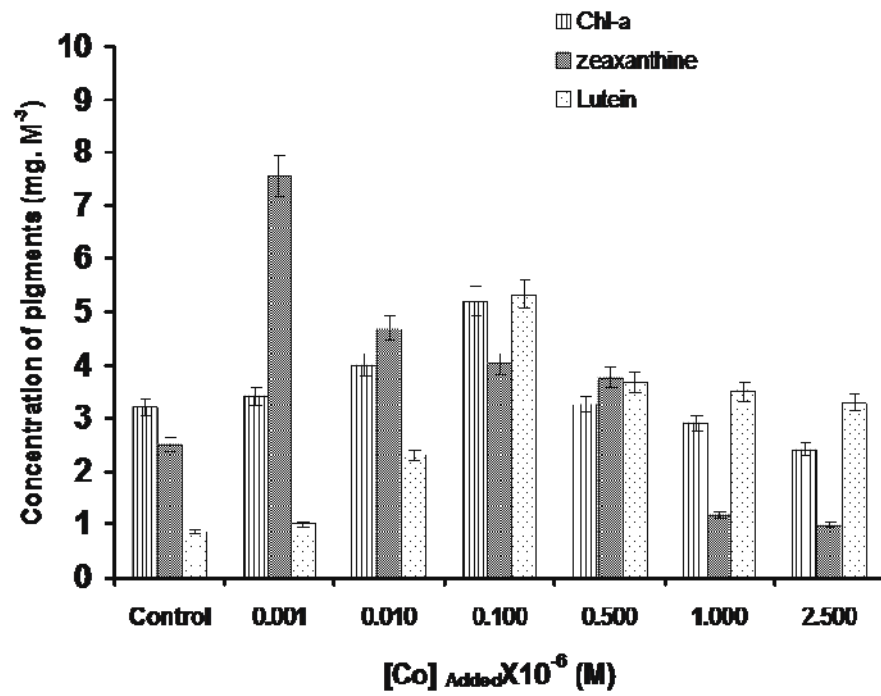


Figure 3



**Figure 4**

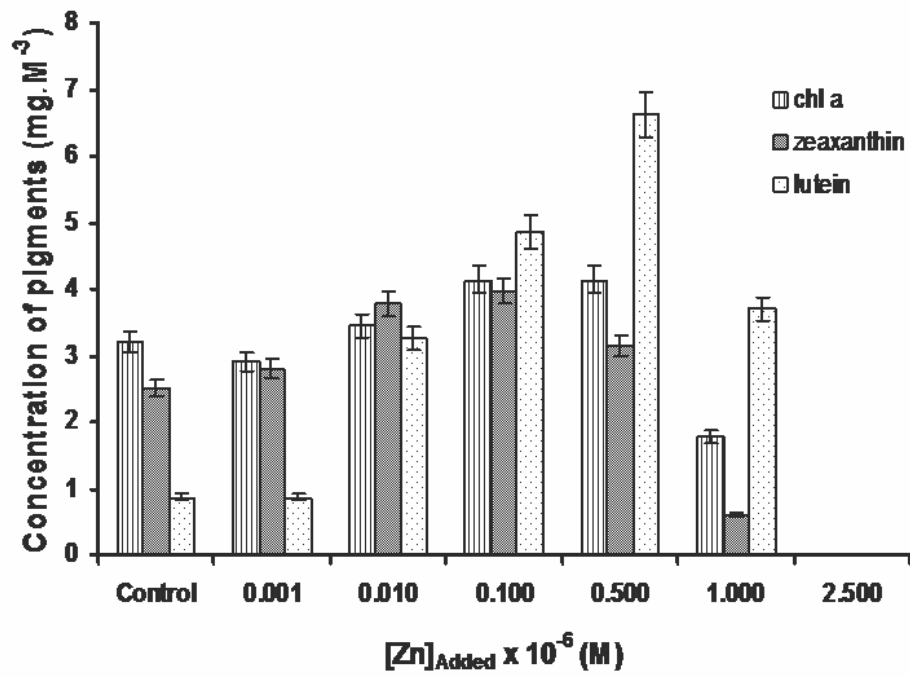


Figure 5