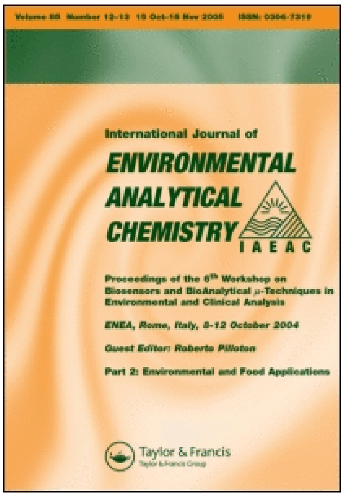

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Accumulation of Copper and Cobalt by Blue-Green Algae at Different Temperatures

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Two species of blue-green algae *Anacystis nidulans* and *Spirulina platensis*, grown in artificial aqueous culture medium, were treated with Cu and Co at different temperatures in order to study accumulation of these elements by the organisms. Metal concentrations used were 0.5, 1.0, 2.0 and 4.0 ppm at different temperatures viz, 25, 30, 35, 40 and 45 °C. Both these species showed high capacity to accumulate the metals. Concentration factors (CF) were in the range of 10^3–10^4. Metal accumulation followed the relation $m = KC^n$ where m = molar concentration of metal in the algae, C = molar concentration of initial total metal in the medium, K and n = constants. Metal accumulation by algae was higher at high temperature than at lower temperatures. A negative correlation between metal accumulated and the survival ratio of the algae when treated with different metals was observed, indicating the importance of passive absorption mechanism.

KEY WORDS: Concentration factor, cyanobacteria, survival ratio, temperature, copper, cobalt.

INTRODUCTION

Algae show high capacity of metal uptake, bioaccumulation and biotransfer. They show high bioconcentration factor in relation to their biomass mainly due to high surface area to biomass ratio.[1,2] Micro algal cells have diameter in microns and specific geometric surface area in the order of m^2/g fresh weight.[3] Davies[4] suggested that the cellular surface of phytoplankton consists of a mosaic of cationic and anionic exchange sites. The net charge on the surface layer determines to a great extent the accumulation of heavy metals by algal cells. Hence the physicochemical characteristics of the medium influence metal uptake by algae significantly. Uptake of metals by algae involve two processes. The first non-specific binding of metal to the cell surface, slime layers and extra-cellular matrices, and the second metabolism dependent intra-cellular uptake mechanisms.[5]

Many industrial installations exhaust a vast amount of waste heat to different water bodies. Thermal power plants are classic examples for the same.[6] Alterations in the temperature leads to drastic changes in the activities of organisms. It is reported that processes like metal uptake,[7,2] and toxicity[8–11] are influenced by temperature. The present study was undertaken to explore the influence of temperature variations on metal uptake by blue-green algae. It is in continuation of the study performed on the impact of thermal effluents from Indraprastha Thermal Power Plant, New Delhi, India on River Yamuna (recipient water body) phytoplankton community.[8]

EXPERIMENTAL

Materials and methods

Two species of cyanobacteria viz. *Spirulina platensis* and *Anacystis nidulans* were selected for this study. Pure culture of the experimental species were grown in artificial aqueous medium. The medium contains per liter of $NaHCO_3$ 18 g; K_2HPO_4 0.5 g; $NaNO_3$ 2.5 g; K_2SO_4 1.0 g; NaCl 1.0 g; $MgSO_4$ 0.2 g; $CaCl_2$ 0.04 g; $FeSO_4$ 0.01 g and A_5 solution 1.0 ml. A_5 solution was prepared by dissolving H_3BO_3 2.9 g; $MnCl_2$ 1.8 g; $ZnCl_2$ 0.11 g; $CuSO_4$ 0.08 g and $(NH_4)_2MoO_4$ 0.18 g in 1.0 liter of distilled water. Experiments were conducted in 100 ml flask containing 60 ml culture medium. Cu and Co were added as $CuSO_4 . 5H_2O$ and $CoCl_2 . 6H_2O$ respectively. All chemicals used were of AR grade. Required quantities of metal stock solutions (1000 ppm) were aded to the experimental culture to have final concentrations of 0.5, 1.0, 2.0 and 4.0 ppm in the experimental culture. Culture flasks were kept at five different temperature regimes

(25, 30, 35, 40 and 45 °C) under a light hood (light intensity–2500 lux) with light and dark cycle of 16 and 8 hours. Control experiments were also run simultaneously with algal culture devoid of metals. These temperature regimes were selected to stimulate the thermal variations caused in River Yamuna water by effluents from Indraprastha Thermal Power Plant. Four replicates were taken for each treatment. At every twelve hours interval 10 ml culture medium was collected from each flask and algal cells were harvested on pre-weighed glass fiber filter paper. Cells were washed with distilled water twice, dried and weighed to determine the dry weight biomass. The samples were transferred to a mixture of HNO_3 and $HClO_4$ in the ratio of 3:1. After 24 hours the solution was filtered and the filtrate was made up to 10 ml. Metal concentrations in these digested samples were determined using Varian Tetron AA 6 atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Both of the algal species showed high capacity to accumulate Cu and Co. Concentration factors ($CF = C'/C$ where C' is the metal concentration in algal cells and C is the initial total metal concentration in the medium) were in the range of 10^3–10^4 (Figures 1 and 2). In the present study *CF* values estimated were based on the initial concentration of the metal added to the medium. But the equilibrium concentration of the metal in the dissolved phase will be considerably lower than the initial concentration, for the metal gets sorbed on the surface of experimental vessel and is also removed by organisms. Hence *CF* values reported here will always be biased to the lower side. *CF* varied with the type of metal, the concentration of the metal present in the medium and also with the algal species. *CF* varying in the order of thousands with the variation in these factors were reported by a number of workers.[1,12] A reduction in *CF* with increasing metal concentration in the medium was observed in the present study. Dongmann and Nurnburg[13]; Geisweid and Urbach[14] and Les and Walker[15] also reported about the *CF* showing an inverse relation with the metal concentration in the medium, which indicates a saturating effect of metal absorption by algae.

Metal accumulation by *S. platensis* and *A. nidulans* followed the relation $m = KC^n$ (where m is the molar concentration of metal in the algae, C is the molar concentration of the initial total metal in the medium, K and n are constants). Similar relations for Cu and Cd uptake by various species of algae were also observed by different workers.[12,14,16] However Rebhum and Ben-Amotz[16] in case of *Chlorella stigmatophora* observed the exponential constant 'n' to be more than one indicating increase in metal uptake with metal concentration in the medium. '*n*' reported by Geisweid and Urbach[14] in case of *Chlorella vulgaris, Ankistrodesmus braunii* and *Eremospheara viridis* were well below one and hence they conclude that the metal uptake by algae followed a non-Langmuir type of sorption.

In the present study the exponential constants (Table 1) were always lower than that reported by Geisweid and Urbach.[14] This can be attributed to species specific variations and/or also to variation in experimental conditions like culture medium composition. Factors like ionic strength of the medium alters the uptake of metal.[17] In the case of present study the artificial culture medium used was of ionic strength $I = 0.287$.[18]

S. platensis showed higher ability to accumulate metals than *A. nidulans*. Both the species showed higher accumulation capacity towards Co than Cu (Figures 1 and 2). The filamentous species *S.*

Table 1 Constants (k and n) of the metal concentration (10^{-6} M) in the medium vs metal concentration (10^{-5} M/g) in algal biomass plot ($m = KC^n$) when algae were treated with Cu and Co

Species	*Metal*	*k*	n^a	r^b	p^c
S. platensis	Cu	−5.392	0.480	0.480	0.001
	Co	−2.674	0.584 ±0.174	0.576	0.001
A. nidulans	Cu	−7.551	0.266 ±0.176	0.373	0.001
	Co	−4.436	0.539 ±0.272	0.389	0.001

[a] ±95% confidence level.
[b] Correlation coefficient.
[c] Significance of r.

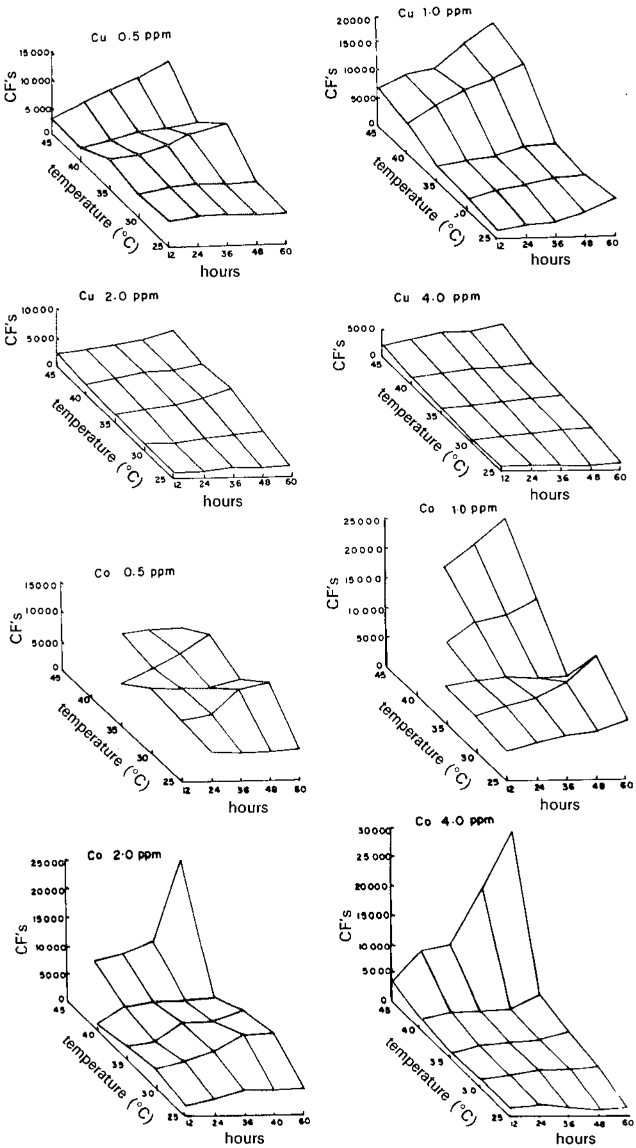

Figure 1 Uptake of Cu and Co shown as values of concentrations factors with different incubation temperatures by *S. platensis*.

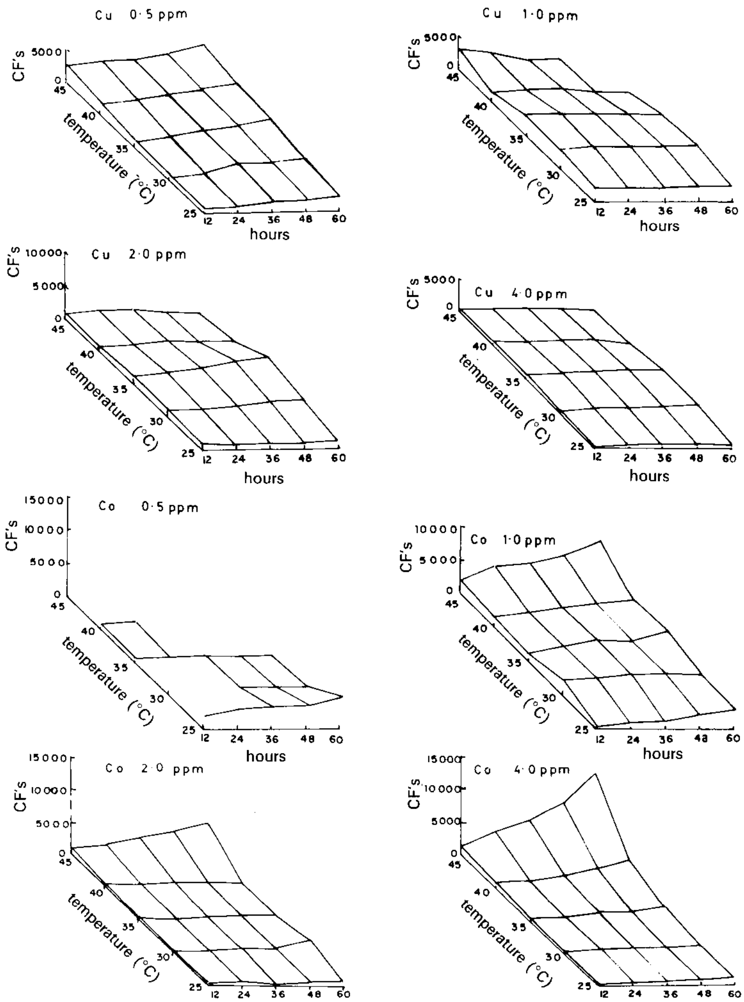

Figure 2 Uptake of Cu and Co shown as values of concentration factors with different incubation temperatures by *A. nidulans*.

platensis accumulated more Cu or Co than the non-filamentous one *A. nidulans*. In the case of Cu, the metal accumulation by *S. platensis* was at least twice that of by *A. nidulans*. Similar was the case with Co accumulation by these two species.

The present study showed that with increase in exposure time metal concentrations in algae also rose (Figures 1 and 2). When algae were exposed to metals at different temperatures, Cu accumulation increased with temperature increase. Accumulation of Co also showed the same trend with some exceptions. For instance, except when Co was applied in concentration of 4.0 ppm the metal accumulation by *S. platensis* was higher at 30 °C than either at 25 ° or 35 °C. However at 45 °C the accumulation in case of both the species and metals was significantly higher than at any other temperature regimes. Along with temperature increase as there is in general a trend of increase in *CF* (Figures 1 and 2) it emphasizes the importance of the passive absorption mechanism in metal uptake.

Significant negative correlation (except in case of *A. nidulans* with Cu) between the survival ratio of the algae under heavy metal stress and the metal uptake also corroborates the above inference (Table 2). The survival ratio estimated as $SR = (X'/X) \times 100$ (where X' = biomass of the algae with different treatments at time t and X = biomass in the control culture at the same time t) is used as an index of toxicity. The exception observed in case of *A. nidulans* with Cu, i.e., the correlation being negligible, might be due to the higher sensitivity of the species to temperature and the metal.[8] Bentley-Mowat and Reid;[19] Geisweid and Urbach[14] Tobin *et al*[20] and

Table 2 Correlation between the metal concentration and the survival ratio of the algae when treated with Cu and Co

Species	*Metal*	*r*	*n*[a]	*p*
S. platensis	Cu	−0.74	48	0.001
	Co	−0.74	38	0.001
A. nidulans	Cu	−0.30	40	0.1
	Co	−0.64	36	0.001

[a]Number of observations.

Bollag and Duszota[5] also reported that dead cells accumulate more than living cells. In the process of metal uptake the attachment of metal ions to the cell surface is followed by intra-lamellar and intracellular transport. Simultaneously the toxic metals are sequestered to make them inert which involves the synthesis of special types of protein like metallothionein and poly-phosphate bodies.[22–27] In addition to sequestration, processes to control metal uptake also commence. Hawkins and Griffiths[10,11] Hall[28] and Lolkema and Vooijis[29] have reported on the capacity of tolerant strains of organisms to restrict Cu uptake. Hence the high and ongoing metal uptake observed in the present study at high temperature might be due to the possible inactivation of metabolism dependent exclusion mechanism effective in living organisms.

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