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Heavy Metal Uptake in the Cyanobacterium *Nostoc calcicola*

S.P.Singh & S.K.Verma
Centre of Advanced Study in Botany Banaras Hindu University
Varanasi - 221005

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Cu uptake pattern in *Nostoc calcicola* was a curvilinear function of time, and it followed a Michaelis-Menten type of kinetics with saturating concentrations at 40 μ M. A Line weaver-Burk plot of Cu uptake, at different concentrations of Cu (2.5 μ M to 40.0 μ M) followed a monophasic isotherm with Km 25.0 μ M and V max 3.0 nmol. mg *Protein*$^{-1}$. *min.*$^{-1}$. A positive correlation existed between Cu uptake and growth inhibition at various pH (from 4 to 10); maximum Cu uptake andtoxicity was observed at low pH. Cu uptake seemed to be dependent on light and metabolic energy. The inhibition of Cu uptake by DCCD, also suggests the involvement of ATP hydrolysis in regularing Cu transport across the plasma membrane. A complete inhibition of Cu uptake by PCMB suggests the role of sulfhydryl groups in the transmembrane movement of Cu in the cyanobacterium.

Key Words - Growth Hydrolysis Inhibition Metal Transport Up take

The literature on the inhibitory effect of copper on growth (Les & Walker, 1984), photosynthesis (Shioi *et al.*, 1978) and nitrogen fixation (Rai & Raizada, 1985) in algae and higher plants is impressive. Heavy metals inhibit the uptake and assimilation of nutrients (Singh & Yadava, 1983, 1984). Mechanisms governing the metal uptake process in microorganisms (Bhattacharjee, 1986) and in cyanobacteria (Singh & Yadava, 1985) are investigated. The possibility of involvement of carrier(s) and energy in the metal transport across the cell membranes has been suggested (Veltrup, 1977). The toxicity of heavy metal to algae (Hargreaves & Whitton, 1976 a,b; Singh & Pandey 1981) is influenced by environmental factors like pH, light and complexans. However, there have been only a few studies on the effect of environmental factors on copper uptake in cyanobacteria. The present investigation reports on the copper uptake kinetics and the role of various factors on *Nostoc calcicola.*

MATERIALS & METHODS

N.calcicola was a local isolate from rice field, and axenic culture was multiplied in Allen and Arnon's nitrogen free medium (Allen & Arnon, 1955) with A_5 trace elements devoid of copper. The culture was grown in a culture room, illuminated with cool day light fluorescent tubes (14.4 Watt. m -2) for 16 h as photoperiod and 8 h as nyctoperiod at 24 ±1 C

Exponentially growing cells of *N.calcicola* were harvested and re-suspended in sodium phosphate buffer (0.01M; pH 7.0) to a final cell density of 400 μ g protein ml^{-1}. Cu uptake was followed at concentrations of Cu from 2.5 to 60 μ M as $CuSO_4.5H_2O$ to account for the saturation level. The general methodology adopted was that of Singh & Yadava (1985) unless otherwise specified. To analyse the cellular Cu level, 10 ml samples were removed at regular interval of 10 min, centrifuged (3000 g. 75 sec.) and washed with EDTA (10 μ M). Cell pellet was dried and digested in 1 ml of HNO_3 : HClO (10 : 1 v/v) mixture in a hot water bath for 30 min. After cooling, samples were diluted to 5 ml with triple glass distilled water. A further low speed centrifugation removed the undigested material, and the resulting clear supernatant was analysed for Cu using Perkin-Elmer 2380, Atomic absorption spectrophotometer at 324.7 nm. Slope of the curves was used to calculate Cu uptake rate. The kinetic constants (km and Vmax) were calculated from Lineweaver-Burk plots (Lineweaver & Burk, 1934). The pH of the assay medium was adjusted in the range of 4 to 10 to account for the role of pH in metal uptake.

Changes in protein were measured by the method of Lowry *et al.* (1951) modified by Herbert *et al.* (1971) using lysozyme (Sigma) as the standard. The specific growth rate constant (k) was calculated by the equations of Kratz & Myres (1955).

Inhibitors, N'N'-dicyclohexyl carbodimide (DCCD), parachloro mercuribenzoate (PCMB) 3-3, 4-dichloropheny 1-N-dimethyl

Table 1 – The Effect of Various Inhibitors on Cu Uptake in *N. calcicola*.

Inhibitor	Concentration (M)	% Cu uptake (n mol mg protein)
Light (Control)	–	100
Dark	–	17
Dark + ATP	10^{-5}	82
DCMU (Light)	10^{-5}	28
DCCD (Light)	10^{-4}	12
PCMB (Light)	10^{-5}	0

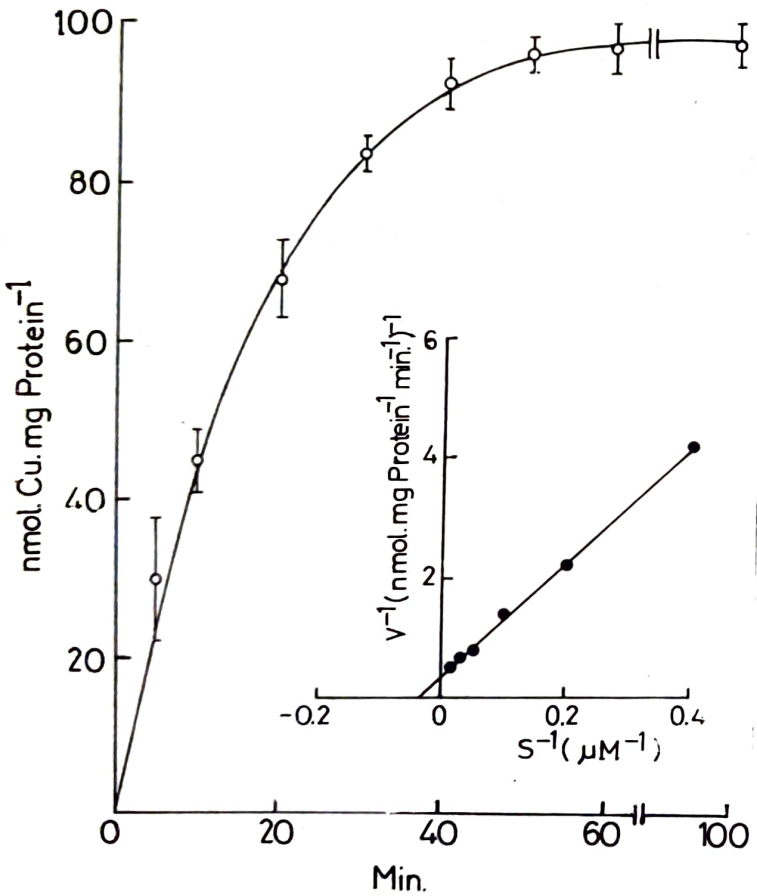

Fig. 1 Pattern of Cu uptake in *N. calcicola*, values are mean ± 1 S.E.

Inset - Double reciprocal plot of Cu uptake at graded concentrations of Cu.

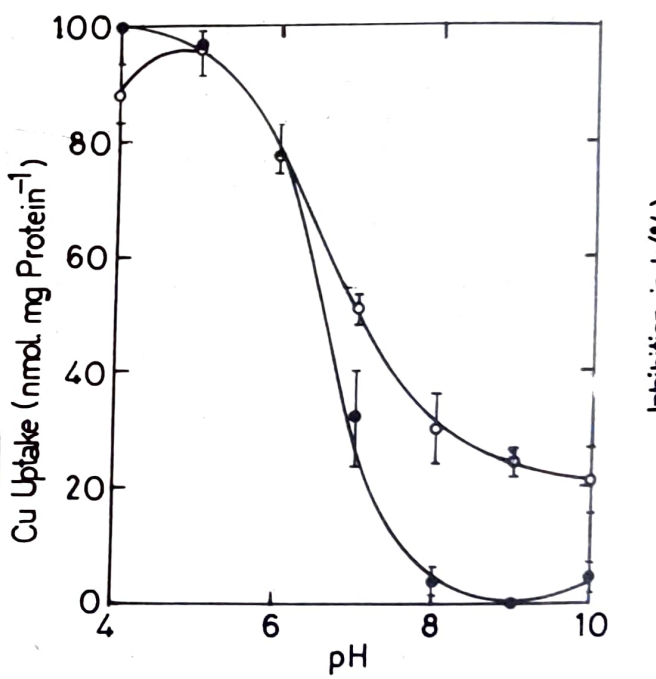

Fig. 2 Effect of pH on Cu uptake (○——○) and per cent inhibition in specific growth rate constant (●——●) in *N. calcicola*, values are mean ± 1 S.E.

urea (DCMU) and ATP were obtained from Sigma Chemicals (USA). Stock solutions of DCMU and DCCD were prepared in ethanol before diluting 100 fold in the growth medium to get desired levels. PCMB was dissolved in 0.1 N NaOH and ATP solution was prepared in sterile distilled water.

A correlation between Cu uptake rate and per cent inhibition in k value were calculated by using the following equation.

$$r = \frac{\Sigma XY - \Sigma X \Sigma Y/N}{\sqrt{\{\Sigma X^2 - (\Sigma X)^2/N\}\{\Sigma Y^2 (\Sigma Y)^2 N\}}}$$

RESULTS & DISCUSSION *Copper uptake kinetics*

The pattern of Cu uptake was a curvilinear function of time (Fig. 1) in contrast to the linear pattern reported earlier in *Anacystis nidulans* (Singh, 1985). It is quite apparent that the metal uptake rate was faster for the initial 5 min (6.0 nmol mg.protein -1.min-1.), followed by a slow uptake lasting up to 50 min at a rate of 2.12 nmol.mg protein -1.min.-1 (as calculated within a period of 5-30 min). A Lineweaver-Burk plot (Fig. 1, inset) shows a metal saturation with respect to its uptake, corresponding to 40 μM Cu. The overall pattern suggests that the metal uptake process strictly followed a monophasic pattern with a Km=25 μM and Vmax of 3.0 nmol.mg protein-1.min-1, in contrast to the biphasic and multiphasic isotherm reported in the aquatic plant *Elodea nutalli* (Werff & Ernst, 1979).

Role of pH Hydrogen ion concentration regulates metal toxicity uptake in microbes (Babich & Stotzky, 1982), and in algae (Whitton, 1970 ; Singh & Pandey, 1981; Singh & Yadava, 1985). The maximum copper uptake (96.69) nmol.mg protein-1) as observed at pH 5, did not accompany growth when tested under long-term growth experiments (Fig. 2) pH 4 although did not favour growth of the organism, the cells absorbed Cu in sufficient amount (73.08 nmol.mg protein-1). A decrease in growth inhibition (76%) at pH 6, also resulted in decreased Cu uptake (70.83 nmol mg. protein -1). In the alkaline range (pH 8-9), there was a positive correlation between Cu toxicity (% inhibition in k) and uptake, and enhanced algal growth resulted in reduced Cu intake. Still higher pH values (pH 10.0), neither favoured algal growth (k = O.O24) nor metal uptake. Reduced metal uptake at elevated pH levels is due to the formation of metal hydroxides (Babich & Stotzky, 1982) rendering such ions less available to the cell organelles because of their absorption on the cell surface (Les & Walker, 1984). However, there are reports on the enhanced metal uptake even in the alkaline range (Hargreaves & Whitton, 1976 a,b; Schecher & Driscoll, 1985).

Role of light and Metabolic Inhibitors

The inhibition of Cu uptake in dark as well as in presence of DCMU indicates the involvement of light generated energy or metabolism dependent metal uptake (Table 1). Since the metal uptake process was sensitive to the photosynthetic inhibitor DCMU, which permits only the operation of PS I and not PS II (Raven, 1969; Cadd & Cossar, 1978), the energy generated only through cyclic photophosphorylation would not have been sufficient to drive the uptake process in the DCMU added set. The enhancement of Cu uptake process in dark, at the expense of exogenously supplied ATP (10^{-5} M), suggests that the lowered uptake in dark could be mainly due to the limited energy prerequistic for Cu intake. The ATP-stimulated metal uptake process in light, indicates the Cu-induced depletion of energy, even if none of the phosphorylation was inhibited by any other agent. Further, the inhibition of Cu uptake by DCCD (10^{-4} M), a specific inhibitor of membrane bound ATPase (Scheirer & Haddock, 1972), indicates the possible involvement of ATP hydrolysis in Cu transport across the cell membrane (table). Biological surfaces contain a variety of functional sites such as carboxylic, amino, sulphydryl etc. The total inhibition of Cu uptake by non-growth inhibitory concentration of PCMB (10^{-5} M), suggests that the -SH group is the main active component involved in the trans-membrane movement of copper in *N.calcicola.*

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