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Nickel Effects on Phosphate Uptake, Alkaline Phosphatase, and ATPase of a Cyanobacterium

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The ever increasing input of Ni in the environment either through geological or anthropogenic activities could lead drastic alterations in aquatic ecosystems (IARC 1976), and since cyanobacteria constitute the vital component of biosphere, the effects of Ni as a pollutant on such microbes seems to be of primary concern. Ni at very low concentration (0.125 ppm) proved lethal to planktonic cyanobacterium Anabaena inaequalis (Stratton and Corke 1979). As per Indian Environmental Conservation Rules, the upper limit for Ni in effluents from electroplating establishments has been 3 ppm. However, still higher Ni concentrations in effluents from various electroplating industries around Varanasi, necessitated studies on Ni effect using cyanobacterium as the target organism. Phosphorus nutrition in cyanobacteria is regulated by divalent cations (Healey 1973). Elements like Al and Cd are known to inhibit cellular phosphate metabolism in cyanobacteria (Pettersson et al. 1988; Singh and Yadava 1984). The metalloenzyme, alkaline phosphatase (Kuenzler and Perras 1965) is sensitive to various cations (Ihlenfeldt and Gibson 1975; Doonan and Jensen 1979). Likewise the membrane bound ATPases having active role(s) in nutrient transport, are also sensitive to vanadate (Lockau and Pfeffer 1982).

In this paper, we present the effect of Ni on growth, phosphate uptake, alkaline phosphatase and membrane bound Ca^{2+} and Mg^{2+}-dependent ATPases in Nostoc muscorum ISU.

MATERIALS AND METHODS

Nostoc muscorum (ISU) was grown axenically in Erlenmeyer flasks containing 200 ml of combined nitrogen free Chu 10 medium (Gerloff et al. 1950) at 24±1°C in a culture room illuminated with fluorescent light

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(intensity 12 Watt/m^2) with 18:6 h light/dark cycle.

Growth was measured in terms of optical density of cultures (650 nm) in Spectronic-20 colorimeter (Bausch and Lomb, USA). The curves represent the mean of three independent observations.

Cellular protein was estimated by the method adopted by Lowry et al. (1951), modified by Herbert et al. (1971) using lysozyme as standard.

For PO_4^{3-} - uptake experiments, the log phase N. muscorum cells were starved for 12 h in PO_4^{3-} free medium and uptake monitored according to the method of Fiske and Subba Row (1925). As 2.0 mM K_2HPO_4 was saturating for uptake, this concentration was subsequently employed in further experiments with different Ni concentrations (50-200 µM).

Alkaline phosphatase activity was measured following the procedure of Ihlenfeldt and Gibson (1975). Pi-deficient algal cells were exposed to Ni (1-2000 µM) up to 8 h followed by the alkaline phosphatase activity measurements in cell suspensions adjusted to a density of 350 µg protein/ml. Specific activity has been expressed as nmol pNPP hydrolyzed/µg protein/min.

ATPase activity was assayed according to the method of Lockau and Pfeffer (1982) with little modification. Log phase cyanobacterial cells were harvested by centrifugation (5,000 x g), washed and resuspended in extraction buffer (30 mM Tris-HCl, pH 8.1). The cells were ruptured by ultrasonicator (MSE MK-2) followed by centrifugation (10,000 x g, 30 min) and the supernatant thus obtained, was dialyzed for 3 h against 10 mM Tris-HCl buffer (pH 8.1). The resulting preparation was used as crude enzyme extract. All such operations were carried out at 4°C.

Mg^{2+}-dependent ATPase was assayed as adopted by Ohnisi et al. (1975) and the amount of inorganic phosphate liberated was determined by the method of Fiske and Subba Row (1925).

Ca^{2+}-dependent ATPase of the enzyme preparation attributed to the coupling factor of photophosphorylation (Owers-Narhi et al. 1979), was activated prior to assay. The cell-free extract was treated with trypsin (0.5 mg/ml, Sigma) for 10 min, followed by addition of 0.75 mg/ml of trypsin-inhibitor (Sigma). Ca^{2+}-dependent assay was performed as above except that $MgCl_2$ was replaced by 6 mM $CaCl_2$.

The data for various metal treatments and exposure time were verified for their significance at a

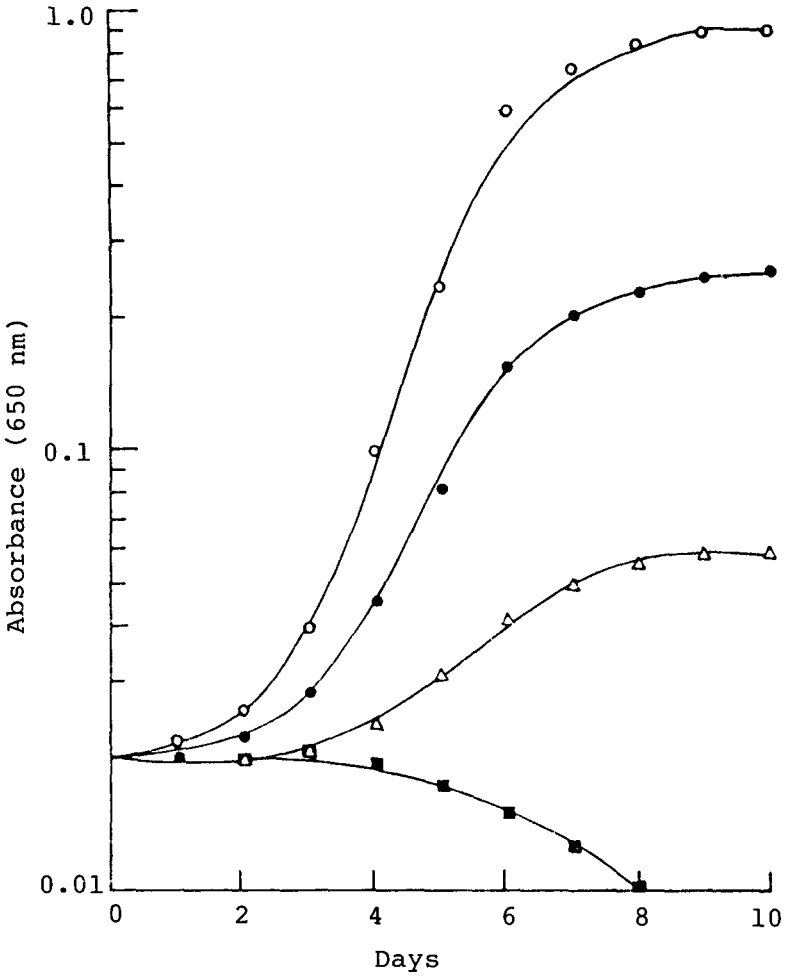

Figure 1. Growth of N. muscorum cells exposed to graded Ni concentrations: Ni-less control (o—o), 5 µM (●—●), 10 µM (Δ—Δ) and 15 uM (■—■); $F_{Ni\ 3,30}=3.164$ and $F_{days\ 10,30}=4.09$; $p<0.025$.

particular probability level, and the variance ratio (F) calculated as:

$$F = \frac{\text{Treatment mean square}}{\text{Residual mean square}}$$

RESULTS AND DISCUSSION

Ni significantly inhibited photoautotrophic growth of the cyanobacterium at 15 µM (Fig. 1). Similarly, Anabaena inaequalis cells exposed to Ni responded in terms of increase in the lag phase, culture doubling time and a faster retardation of growth, possibly due to poisoning of intracellular enzyme systems by Ni

(Stratton and Corke 1979). The toxicity response by Anacystis nidulans also came within the same range with a slight tolerance upto 38 µM (Whitton and Shehata 1982). In a separate study, Spencer and Greene (1981) observed significant reduction in growth of Anabaena flos-aquae and Anabaena cylindrica at 10.2 µM l^{-1} Ni. The plate count based observations by Rai and Raizada (1985), however, reported high sensitivity of N. muscorum to Ni (5.0 µM). The apparent lag in algal growth for all the concentrations (5-15 µM Ni) does indicate that 72 h lag will be first characteristic of Ni effect at higher metal concentrations followed by resumption of growth (tolerance) in 10 µM Ni, and a gradual decline in the absorbance value indicates the ultimate lethality in 15 µM Ni.

N. muscorum cells pre-incubated under PO_4^{3-} - free condition in light (12 h) responded rapidly to PO_4^{3-} addition without lag. PO_4^{3-} uptake in Ni-free cells increased linearly up to 4 h attaining a maximum of 0.8 µ mol PO_4^{3-}/µg protein. However, Ni treatment of such cultures caused a concentration-dependent inhibition of PO_4^{3-} uptake (Fig. 2). It is noteworthy that PO_4^{3-} uptake still occurred in the highest Ni concentration used (200 µM) while 100 µM Ni could bring about 50% inhibition of the process. Therefore, 100 µM Ni was subsequently used to study the mode of Ni action of PO_4^{3-} uptake kinetics (Fig. 3). The reduced V_{max} (0.13 µmol/µg protein/h) accompanied by the unaltered K_m (1.15 mM), suggested a non-competitive type of interaction between Ni and PO_4^{3-}.

The continuously increasing rate of PO_4^{3-} uptake in the pre-starved cells even up to 5 h indicates the operation of enzyme polyphosphate synthetase as reported in other cyanobacteria (Grillo and Gibson 1979). The present observations on the Ni inhibition of PO_4^{3-} uptake in N. muscorum are similar to those observed with other cations in cyanobacteria (Singh and Yadava 1984) and PO_4^{3-} uptake as well as photosynthesis of planktonic communities in selected precambrian lakes (Nalewajko and Paul 1985). The non-competitive nature of Ni inhibition of PO_4^{3-} uptake in the present case, rules out the possibility of a direct competition between Ni and phosphate ions. In recent report, Pettersson et al. (1988) observed that Al severely affected growth of Anabaena cylindrica and induced symptoms indicating phosphorus-starvation although the cation did not inhibit PO_4^{3-} uptake. The rapid accumulation of polyphosphate granules in cells exposed to Al in such cases, also established that Al did not disturb phosphate incorporation in contrast to lowering of acid phosphatase activity and mobilization of polyphosphate as well.

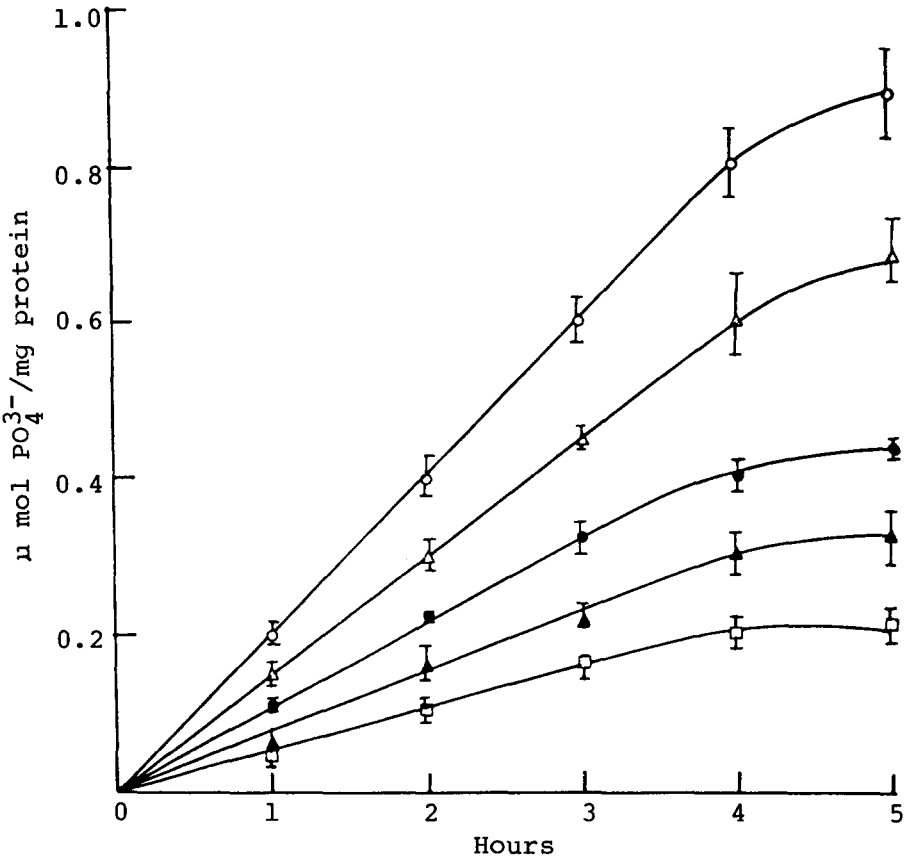

Figure 2. Pattern of phosphate uptake in N. muscorum cells exposed to Ni (50-200 µM); Ni-less control (o—o), 50 µM (Δ—Δ), 100 µM (●—●), 150 µM (▲—▲) and 200 µM Ni (□—□). $F_{Ni\ 4,\ 16}=19.179$ and $F_{hours\ 4,\ 16}=16.487$; $P<0.025$.

Alkaline phosphatase provides inorganic phosphate by hydrolyzing extracellular organic phosphates dissolved in the ambient medium (Ihlenfeldt and Gibson 1975). It is, therefore, expected that the enzyme has considerable bearing in ecology of algae in nature. The PO_4^{3-} starved cells of N. muscorum developed optimum level of alkaline phosphatase activity (2.20 nmol pNPP hydrolyzed/µg protein/min, data expressed as percentage). In order to examine the effect of Ni on the in vivo alkaline phosphatase activity, the cells pre-starved of PO_4^{3-} as described earlier, were transferred to the fresh PO_4^{3-}-free medium containing graded Ni concentrations (1-2000 µM). The data compare Ni effect on the in vivo alkaline phosphatase activity in cells exposed to a common duration of 8 h for each Ni dose (Fig. 4). Ni (1 µM) caused an

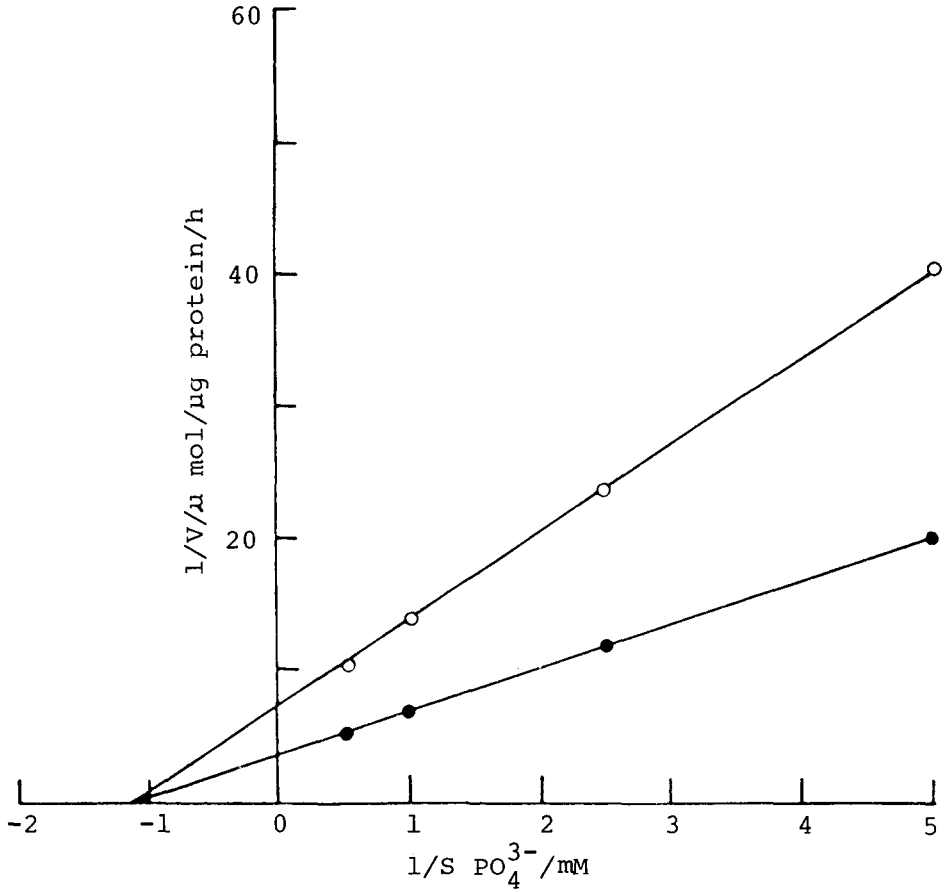

Figure 3. A Lineweaver-Burk plot for phosphate uptake in N. muscorum cells as Ni-less control (●—●) and cells dosed with 100 µM Ni (o—o).

increase in the enzyme activity (by 23% over the Ni-free control), however the subsequent Ni concentrations proved inhibitory with a maximum of 75% inhibition at 2000 µM Ni. The amount of alkaline phosphatase in algae, is controlled by their nutritional status as phosphorus replete cells do not synthesize this enzyme. Such phosphatases are usually zinc metalloenzymes, and the metal may also be replaced by Cu with the resultant loss of enzyme activity (Vallee 1959; Foy et al. 1978). A general survey of the reports suggests that only essential cations have been tried to assess their impact on alkaline phosphatase in cyanobacteria (Healey 1973; Ihlenfeldt and Gibson 1975; Doonan and Jensen 1979). Apart from the general inhibition, many cations also proved stimulatory. Similar concentration-dependent differential behaviour of such cations towards

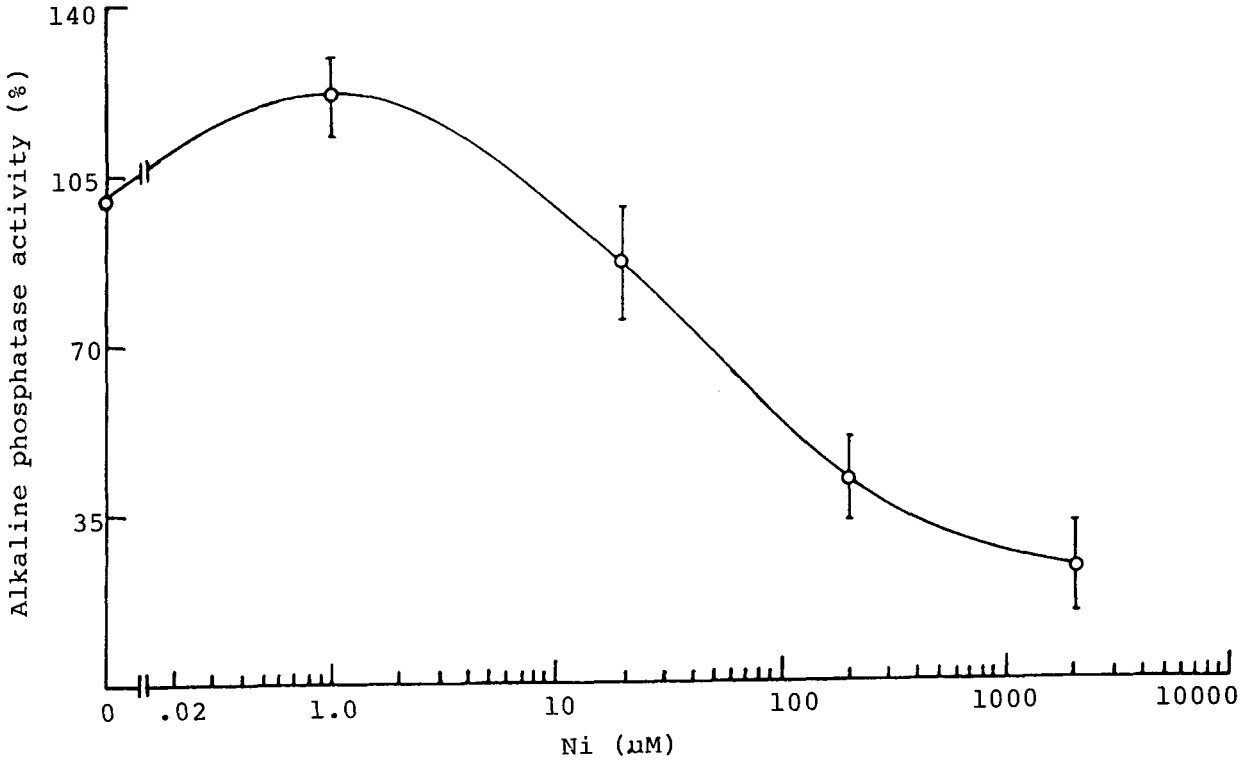

Figure 4. *In vivo* alkaline phosphatase activity in *N. muscorum* cells exposed to Ni (1-2000 µM) for a common duration of 8 h.

alkaline phosphatase in cyanobacteria, includes stimulation by Mo and Mn and inhibition by Zn (Ihlenfeldt and Gibson 1975) or with Co and Mn (Doonan and Jensen 1979). Ni has also been known to inhibit the activities of acid and alkaline phosphatases in soil associated microbes (Juma and Tabatabai 1977).

Role of membrane bound ATPases in regulating transport of nutrients, has not been extensively studied in cyanobacteria (Lockau and Pfeffer 1982). We therefore, attempted to find out Ni influence on two ATPases in N. muscorum. Accordingly, the photoautotrophically growing cyanobacterial cells were treated with Ni (25-100 µM) for a fixed duration of 1 h, and such cells were examined for estimating the activities of Mg^{2+}-dependent and Ca^{2+}-dependent ATPases (Table 1). Lower Ni dose (25 µM) invariably increased the activities of both the enzymes by nearly 1.5 fold. Subsequent higher Ni doses (50 and 100 µM) also stimulated the activities of both the enzymes with the difference that the extent of stimulation was not as high as observed for lower Ni doses.

Table 1. Mg^{2+}- and Ca^{2+}-dependent ATPase activities in N. muscorum (as determined after 1 h metal exposure to whole cells)

Ni	Mg^{2+}-dependent ATPase		Ca^{2+}-dependent ATPase	
	nmol Pi/mg protein/min Mean ± SE	% activity of control	nmol Pi/mg protein/min Mean ± SE	% activity of control
Metal-less control	29.76±2.080	100.0	34.00±0.60	100.0
25	46.00±4.600	154.5	52.38±2.095	154.0
50	36.90±3.321	123.9	44.04±4.180	129.5
100	34.52±2.934	115.9	38.09±2.285	112.0

We also compared the in vitro activity of the Mg^{2+}-dependent ATPase as influenced by Ni ions. Metal pre-treatment (1 h) was given to cells before isolating the enzyme as well as to the enzyme isolated from Ni-free cells. Ni activated enzyme activity under both the conditions with maximum activation at 25 µM and minimum at 100 µM (Table 2). It is, therefore, suggested that Ni activation of membrane bound ATPases might be a factor of considerable significance in regulating their response to Ni. The Ni stimulation of Ca^{2+}-dependent ATPase in N. muscorum points towards the hydrolysis of photosynthetically generated ATP, thereby causing limitation of energy for cyanobacterial growth and multiplication. Ni stimulation of Mg^{2+} dependent ATPase on the other hand, could lead to

the altered energetics in the cell membrane that controls the transmembrane movement of vital ions.

Table 2. Ni-sensitivity of Mg^{2+}-dependent ATPase activity (nmol Pi/mg protein/min) in whole cells and crude enzyme extracts of N. muscorum (as determined after 1 h metal exposure).

Ni (μM)	Whole cells Mean ± SE	Crude enzyme extract Mean ± SE
Metal-less		
Control	29.76±2.232	29.76±2.232
25	46.00±2.300	52.38±3.142
50	36.90±2.583	36.90±2.952
100	34.52±3.452	33.00±3.135

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