

Response of *Anabaena* PCC 7120 to Nickel and Cadmium Stress

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ABSTRACT

Anabaena PCC 7120 strain is sensitive to Ni²⁺ and Cd²⁺ and growth rates at various concentrations of these metals were assessed in terms of protein level. Tolerance limits for Ni²⁺ and Cd²⁺ are 1.2 mg L⁻¹ (39 % level in growth kinetics) and 1.2 mg L⁻¹ (48.99 % level in growth kinetics), respectively. Absorption/adsorption kinetics was estimated after 240 hrs of heavy metal treatments. For both the heavy metals, absorptions were higher than adsorption. Metal concentration and absorption were linearly related ($r = 0.99$; $p > 0.01$). Sugar and pigment exhibited reduction due to heavy metal stress.

Key Words: Absorption/adsorption, *Anabaena*, Cadmium, Nickel

INTRODUCTION

Heavy metal contamination is one of the major environmental problems today our global ecosystem is facing (Gong et al. 2005). The hazards of heavy metal pollution are evidenced by such episodes as the Minamata and Nigata Bay incidents and by the occurrence of itai-itai ('ouch-ouch') diseases, caused respectively by mercury and cadmium poisoning (Cain et al. 1980). Cadmium is relatively a rare element (Wood 1974) with little known function in the biological systems, however, Cd²⁺ as essential requirement has been reported in marine diatoms (Park et al. 2007).

The essentiality of nickel is now generally accepted, based on the numerous symptoms caused by nickel deficiency (mainly in terrestrial vertebrates) and its role in various enzymes (urease and hydrogenase metabolism) in bacteria and plants (Muysen et al. 2004). Nonetheless, excess of heavy metal is toxic to most plants and it is for more than a decade since the significance of Ni²⁺ as a very serious pollutant were recognized (Iljin 1991). It is known to be highly toxic to a wide range of algae (Rai and Mallick 1993).

Conventional techniques for removal of heavy metals like chemical precipitation, ion exchange or

electrochemical processes are neither economical nor effective especially when dealing with low concentrations of heavy metals. Hence, search begins to invent new separation methods that reduce heavy metal concentration to environmentally acceptable levels at affordable cost. Bioremediation has emerged as potential alternative tool to achieve this goal (Gong et al. 2005). The ability of micro-algae to remove heavy metals from aqueous solution has been known for some decades. The algae of chronically metal-contaminated localities tend to accumulate heavy metals to a dangerous extent. The metal content of algae can be used to predict the level of metal pollution in a water body (Abdallah et al. 2006). The high accumulation capacity can even be used for the enrichment or recycling of valuable metals (Can et al. 2006). Hence, the aim of this study was to determine the responses of cyanobacteria *Anabaena* PCC 7120 to Ni²⁺ and Cd²⁺ stress. Changes in growth rate and absorption/adsorption kinetics were established for this purpose.

MATERIALS AND METHODS

Anabaena PCC 7120, obtained through the courtesy of Dr R. K. Asthana, Banaras Hindu University, Varanasi,

India, was axenically cultivated in BG-11 medium (Rippka et al. 1979) and grown in culture room under continuous light, illuminated with cool fluorescent tubes (14.4 watt. m⁻²) at 24±1°C.

Changes in Growth Rate

One mL of algal cells with protein value 100 µg mL⁻¹ were withdrawn from exponentially growing homogeneous culture of alga and inoculated in 100 mL freshly prepared BG-11 medium containing 0 to 2.0 mg L⁻¹ of Ni²⁺ and Cd²⁺ (stock solutions prepared with NiSO₄·6H₂O and 3CdSO₄·8H₂O, respectively). Changes in protein content were measured by the method of Lowry et al. (1951) as modified by Herbert et al. (1971) using lysozyme (Sigma) as the standard. Protein content was measured after every 48 hr, to measure changes in growth rates as influenced by metal stress.

Absorption/Adsorption Kinetics

One mL of algal cells with protein value 100 µg mL⁻¹ were withdrawn from exponentially growing homogeneous culture of alga and inoculated in 100 mL freshly prepared BG-11 medium containing different Ni²⁺ and Cd²⁺ ranges (0, 0.4, 0.8 and 1.2 mg L⁻¹). After 240 hrs of inoculation, 10 mL of algal sample was harvested from homogeneous cultures, centrifuged (4000g, 15 minutes) and supernatant media were separated. The algal samples in the sediment were mixed individually with 10 mL of EDTA (10 µM) solution and gently shaken. Samples were once again centrifuged (4000g, 15 min.). Supernatant EDTA was taken out for measuring the adsorbed ionic concentration. All three parts i.e. media, EDTA and algal pellets from each sample were dried, digested with double acid [HNO₃: HClO₄ mixture (10:1, v/v)] in boiling water bath for 1 hr. After cooling, the samples were diluted to 10 mL with triple glass distilled water and analyzed for metal level by Atomic Absorption Spectrophotometer (Perkin Elmer model 373).

Adsorbed here refers to extra-cellular metal (i.e., metal not transported into the algal cells) that exchanged to EDTA, and absorbed refers to intra-cellular metal (i.e., metal transported into the interior of the cell), represented in method as algal pellets.

Other biochemical parameters

The soluble and insoluble sugars were estimated using

the method of Plummer (1971). Chlorophyll and carotenoids were extracted in methanol and their relative amounts were calculated according to Mackinney (1941). Sugar and pigments were estimated 240 hr after the different ranges of heavy metal treatment.

All the experiments were triplicated and the results were statistically analyzed for variance (ANOVA) and cause effect relationship (Snedecor and Cochran 1967). Growth rate experiment involved two factors (concentration of metal and period of growth) and performed as per strip-plot design, whereas in rest of the experiments concentration of heavy metal is the only factor, accordingly they were carried out following randomized block design.

RESULTS AND DISCUSSION

Growth Changes Measured by Protein Value

The Ni²⁺ and Cd²⁺ concentrations affected growth rate (Figures 1 and 2). Tolerance limit (sub-lethal concentration) for Ni²⁺ and Cd²⁺ are 1.2 mg L⁻¹ (39 % level in growth kinetics) and 1.2 mg L⁻¹ (48.99 % level in growth kinetics), respectively. The effect of sub-lethal concentrations of these metals on cultures appears to delay the onset of exponential phase. Such marked increase in lag phase of the growth has been manifested earlier also (Anand et al. 2006). The results of factorial analysis of variance suggested that toxicity to growth rate is a consequence of metal dosage, duration of exposure and their strong interaction (p>0.01). Similar results have been reported with cultures of *Scenedesmus* and *Ankistrodesmus* (Jin et al. 1996; Lin and Jiang 2000). The results indicated that algal growth becomes oppressive as metal concentration increases, and ultimately inhibited at higher concentration (> 1.2 mg L⁻¹). Resistant cells, that survive the lag phase, are then able to enter the exponential phase of growth. Alternatively, the cells may release secondary metabolites which chelate the toxic ions (Gardea-Torresday et al. 1990) or resistance may be genetic, rather than physiological (Rubinelli et al. 2002).

Absorption/Adsorption Kinetics

Anabaena is not only just a tolerant species but also found to be metal hyperaccumulator with maximum accumulation factor (AF = 1.34) found at 0.4 mg L⁻¹ of Ni²⁺ and Cd²⁺ (AF = 1.25) treatments (Table 1). The adsorptions were comparatively lower than absorption

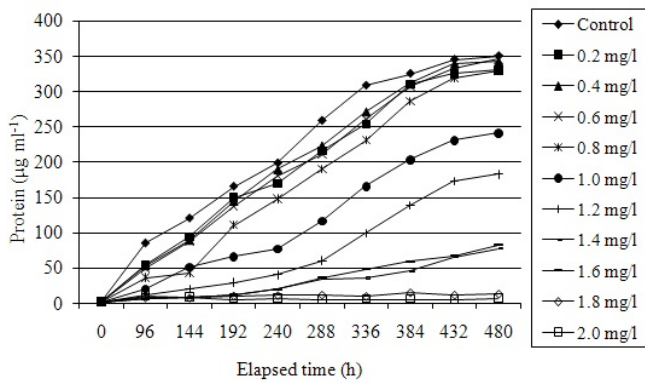


Figure 1. Effect of Ni²⁺ on growth rate as measured by protein content in *Anabaena* PCC 7120

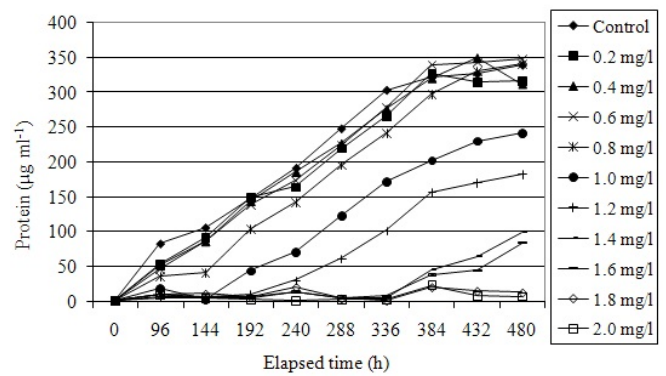


Figure 2. Effect of Cd²⁺ on growth rate as measured by protein content in *Anabaena* PCC 7120

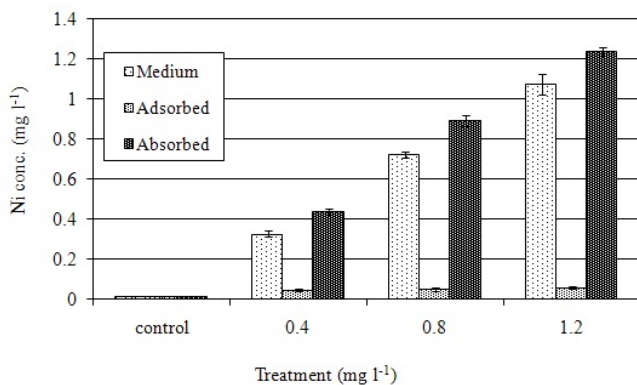


Figure 3. Nickel absorption/adsorption kinetics of *Anabaena* PCC 7120

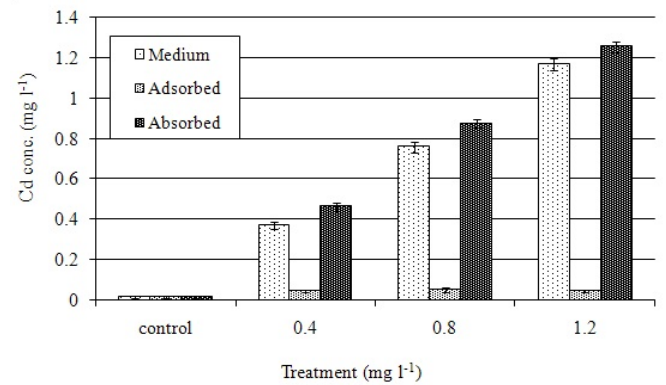


Figure 4. Cadmium absorption/adsorption kinetics of *Anabaena* PCC 7120.

Table 1. Accumulation Factor in *Anabaena*

Ni ²⁺ & Cd ²⁺ Concentration (mg L ⁻¹)	AF value	
	Ni ²⁺	Cd ²⁺
0	1	1
0.4	1.34	1.25
0.8	1.23	1.15
1.2	1.15	1.07

(Figures 3 and 4). However, both adsorption and absorption levels (Y) were linearly related with Ni²⁺ [Y = 0.008 + 0.013 X; r² = 0.79 for adsorption; Y = -0.386 + 0.412 X; r² = 0.99 for absorption] and Cd²⁺ concentrations [Y = 0.012 + 0.011 X; r² = 0.66 for adsorption; Y = -0.378 + 0.412 X; r² = 0.99 for

absorption]. The variance analysis revealed that metal concentration contributed significantly to adsorption (F ratio 17.56; p>0.01 for Ni²⁺ and 18.12; p>0.01 for Cd²⁺ concentrations) and absorption (F ratio 2035.53; p>0.01 for Ni²⁺ and 1999.51; p>0.01 for Cd²⁺ concentrations) levels. However, variations among the replicates were non-significant.

Exposure of algae to elevated concentrations of Ni²⁺ and Cd²⁺ may lead to intra-cellular accumulation of high concentrations of the metals (Suresh and Ravishankar 2004). Comparatively higher level of absorption than adsorption in our study and its comparison with other such study suggests that different species may have different mechanism to deal with excess amount of heavy metals. Some may responds by absorption (intra-cellular) strategy (Axtell et al. 2003) while other may with adsorption (extra-cellular; Anand et al. 2006). Energetic advantage of

both the mechanisms is questionable and needs further exploration, since absorption may have metabolic requirement for active intra-cellular transport of the heavy metal or even if it is passive, species may have energy requirement for its conjugation/detoxification (in active mechanism also; Perales-Vela et al. 2006), while, on the other hand, greater amount of adsorption of heavy metal may consume more energy for synthesis of higher amount of extra-cellular biomass that chelates to metals (Singh et al. 1999).

Other Biochemical Parameter:

The different range of metal treatments decreased both insoluble and soluble sugars and variations were significantly contributed by Ni²⁺ (F = 553.96; p>0.01 and 4709.3; p>0.01, respectively) and Cd²⁺ concentrations (F = 1346.78; p>0.01 and 1237.57; p>0.01, respectively) only (Table 2 and 3). Soluble sugar was found to be more than insoluble sugar. Our results are in conformity of earlier trend observed for such study (Fathi et al. 2005).

Chlorophyll and carotenoid content reduced significantly with increase in Ni²⁺ (F = 529.12; p>0.01 and 2971.30; p>0.01, respectively; Table 2) and Cd²⁺ concentration (F = 664.79; p>0.01 and 2221.47; p>0.01, respectively; Table 3). Concentration dependent reduction of pigment was similar to those obtained earlier (Doke et al. 2005).

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Table 2. Level of sugar and pigment in *Anabaena* PCC 7120 due to nickel treatment.

Ni ²⁺ Concentration (mg L ⁻¹)	Sugar (mg mL ⁻¹)		Pigment (µg mL ⁻¹)	
	Insoluble	Soluble	Chlorophyll	Carotenoids
0 (control)	0.033±0.002	0.065±0.0006	7.967±0.079	129.533±1.026
0.4	0.025±0.001	0.052±0.0009	6.782±0.068	104.533±1.137
0.8	0.010±0.001	0.019±0.0002	4.567±0.343	81.867±1.222
1.2	0.0002±0.001	0.009±0.0006	2.769±0.074	43.933±0.757
CD (at 0.05 level)	8.70E-03	5.40E-03	2.59E-02	1.15E-02

Table 3. Level of sugar and pigment in *Anabaena* PCC 7120 due to cadmium treatment.

Cd ²⁺ Concentration (mg L ⁻¹)	Sugar (mg mL ⁻¹)		Pigment (µg mL ⁻¹)	
	Insoluble	Soluble	Chlorophyll	Carotenoids
0 (control)	0.032±0.0008	0.063±0.002	7.864±0.215	132.267±1.007
0.4	0.022±0.0003	0.053±0.002	6.527±0.087	103.2±2.358
0.8	0.009±0.0009	0.018±0.001	3.767±0.179	81.933±0.416
1.2	0.0001±0.0002	0.006±0.001	2.402±0.023	43.2±0.917
CD (at 0.05 level)	5.24E-03	1.09E-02	2.50E-02	1.38E-02

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