Biotechnological potential of naturally occurring and laboratory-grown *Microcystis* in biosorption of Ni^{2+} and Cd^{2+}

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In this article we provide information on the biosorption of Cd^{2+} and Ni^{2+} by capsulated nuisance cyanobacterium *Microcystis* both from field and laboratory. Compared to laboratory, the naturally occurring cells show higher efficiency both for Ni^{2+} (14%) and Cd^{2+} (9%) biosorption. Freundlich and Langmuir constants revealed that *Microcystis* is not only an excellent biosorbent for metal removal but it has greater affinity for Cd^{2+} than Ni^{2+}. Freundlich isotherm was found to explain the biosorption mechanism more explicitly than Langmuir isotherm, both for single metal and for the bimetallic combination. Freundlich mathematical model further revealed that the biosorption would follow different courses at low and high concentrations. The failure of Freundlich and suitability of BET isotherms at high metal concentration demonstrated a multilayer binding of metals by *Microcystis*.

INCREASING contamination of aquatic resources with a host of pollutants including heavy metals, is not only endangering the aquatic biota but creating a world-wide shortage of recreational and drinking waters. This has aroused concern in the minds of public health engineers and biotechnologists to find out economically viable strategies which could help in the restoration of such abused ecosystems. It is befitting to state that physicochemical methods available for metal removal require large capital and energy investments. For this reason biosorption is emerging as an effective alternative technology to overcome the problems associated with physicochemical methods. Biosorption consists of two phases: (i) passive adsorption which is generally a rapid cell surface binding, the efficiency of which is dependent on the cell wall structural organization[1] and metal solution chemistry, and (ii) active phase which is an energy-dependent slow process[2].

Several species of microalgae including the green alga *Chlorella*[3], blue-green alga *Anabaena*[4] , marine algae[5], bacteria[6], mossess[7], and macrophytes[8] have been used for heavy metal removal. However, monospecificity and good operational conditions are some of the prerequisites, difficult to maintain, that limit the practical application of these organisms.

Microcystis, an abundantly occurring nuisance cyanobacterium in many eutrophic ponds and reservoirs of India and other tropical countries, is responsible for unpleasant odour (upon death), fish kill due to sharp decrease of oxygen content, and death of wild birds and cattle due to ingestion of the toxin produced by certain strains. It appears to be a potential candidate for use as metal biosorbent. This cyanobacterium occurs in naturally immobilized state due to the presence of capsule or slime layer around the cell. The structure of capsule has been studied by Nakagawa *et al.*[9] and Plude *et al.*[10] Nakagawa *et al.*[9] and Doers and Parker[11] demonstrated that *Microcystis* slime or capsule has tremendous potential for interaction with cations. Parker *et al.*[12] further demonstrated that cations play major role in regulation of the viscous nature of capsular polysaccharide.

Taking recourse to the above characteristics, we thought of exploring the possibility of using *Microcystis* as a suitable biosorbent for restoration of metal contaminated aquatics. Nevertheless, this study is the first of its kind to make analytical comparison to evaluate the biosorption efficiency of naturally occurring and laboratory-grown, capsulated and decapsulated *Microcystis* for biotechnological application in metal removal. Different adsorption isotherm models have been employed to explain metal biosorption by *Microcystis* in single metal and the bimetallic system.

Microcystis collected from Luxmi Kund in Varanasi was isolated in pure form and cultured in Parker's medium[13] pH 9.2 at $29 \pm 20°C$ under continuous illumination of 72 µmole photon $m^{-2}\ s^{-1}$ light intensity. Exponentially grown *Microcystis* cells were harvested by centrifugation, washed twice with Mili 'Q' water, and known quantities of homogenized cells (0.80 mg dry wt) were added to flasks containing known doses (1–32 µg ml^{-1}) of test metals ($NiCl_2.6H_2O$ and $CdCl_2.2H_2O$) in 10 ml Mili 'Q' water (preadjusted pH 9.2 by 1 N NaOH or 1 N HCl). The flasks were agitated at 300 rpm in continuous light at $29 \pm 2°C$ in an environmental shaker model 3597-ICOGMPR USA. Samples were withdrawn at known time intervals to measure the residual metal (Ni, Cd) content in the aqueous solution by Perkin–Elmer atomic absorption spectrophotometer model 2380 respectively at 232 and 228.8 nm. Sorbent from the solution was separated by vacuum filtration using 0.45 µm cellulose acetate Sartorius membrane filters.

For comparing the biosorption potential of *Microcystis* (both from naturally occurring and laboratory-grown) in single metal and the bimetallic combination of Ni and Cd, a similar set of experiment was carried out. The dry weight was measured by filtering cells through preweighed membrane filters (0.45 µm) and drying them at 80°C for 1 h.

Figure 1 shows equilibrium mass isotherm of Ni^{2+} and Cd^{2+} biosorption by naturally occurring and laboratory-grown *Microcystis*. Two saturation values were observed at equilibrium concentration. The first and second saturation values obtained for Cd^{2+} biosorption

with naturally occurring and laboratory-grown *Microcystis* were respectively at 3.0, 12.3 and 3.2, 12.5 μg ml^{-1} equilibrium free metal concentration. At these concentrations, Cd^{2+} biosorption was maximum, i.e. 24.2, 54.4 and 22.1, 53.5 μg Cd^{2+} mg^{-1} dry wt respectively for the first and second saturations. Likewise, the saturation values for Ni^{2+} biosorption were observed at 5.1, 21.0 and 5.3, 21.7 μg ml^{-1} equilibrium concentration. Maximum Ni^{2+} binding capacity of *Microcystis* both from naturally occurring and laboratory-grown was respectively 18.1, 90.0 and 16.0, 80.2 μg Ni^{2+} mg^{-1} dry wt. This figure shows an initial 27% higher biosorption of Cd^{2+} than Ni^{2+} on a weight basis. After the first saturation, Ni^{2+} biosorption registered a sudden 36% increase over Cd^{2+}. The biosorption of Cd^{2+} and Ni^{2+} by capsulated *Microcystis* could be due to the presence of galacturonic acid (major constituent) and carbohydrates in the cell wall. The carboxyl groups (–COOH) of galacturonic acids are the main metal-sequestering sites[14].

$$R\text{–}COOH \text{---} RCOO^- + H^+.$$

The initial rapid Cd^{2+} binding may be due to its greater affinity (higher K_f and n value) for cell surface than Ni^{2+}. A sudden increase in Ni^{2+} biosorption in the second saturation may be due to an increased cell permeability at high concentration, resulting presumably into simultaneous operation of both active and passive uptake[15]. The biosorption rate of both the metals was dependent on initial concentration, i.e. biosorption of Ni^{2+} and Cd^{2+} increased at increasing initial metal concentration (Table 1)[16]. Naturally occurring *Microcystis* showed approximately 14% and 9% higher biosorption efficiency respectively for Ni^{2+} and Cd^{2+} than the laboratory-grown cells. A comparatively reduced uptake of metals by laboratory-grown cells may be due to decrease in the slime layer of such cells[17]. Capsulated cells, however, showed 40–50% higher biosorption efficiency than decapsulated cells (data not shown). This may be due to the larger surface area of capsulated than the decapsulated cells.

Figure 2 shows a comparison of Ni^{2+} and Cd^{2+} biosorption by the test cyanobacterium in the bimetallic combination of test metals. Even in the combined state Cd^{2+} showed single ion-like behaviour (see Figure 1), and Ni^{2+} showed an indifferent behaviour. Up to 6.5 μg ml^{-1} concentration Ni^{2+} registered fluctuation, i.e. an increase or decrease in biosorption, which could be due to adsorption and desorption of metals. Since nickel is highly mobile, it is generally adsorbed to a small extent only[18]. The observed rapid increase in Ni^{2+} biosorption could be due to increased cell wall permeability. Figure 2 further revealed that in the bimetallic combination Cd^{2+} biosorption was less than its single metal condition. It can be inferred that Ni^{2+} antagonistically affects Cd^{2+} biosorption because both are bivalent cations and compete for the common binding sites[19]. However, at high concentration because of increased permeability, both ions

Table 1. Effect of initial metal concentration on biosorption rate of Ni^{2+} and Cd^{2+}

Initial metal concentration (μg ml^{-1})	μg metal mg^{-1} dry wt min^{-1}			
	Ni^{2+}		Cd^{2+}	
	Naturally occurring	Lab. grown	Naturally occurring	Lab. grown
1.510	0.016	0.012	0.063	0.051
4.036	0.024	0.021	0.100	0.095
8.080	0.030	0.026	0.185	0.172
14.320	0.105	0.083	0.244	0.227
20.150	0.124	0.120	0.272	0.262
32.355	0.149	0.134	0.489	0.346

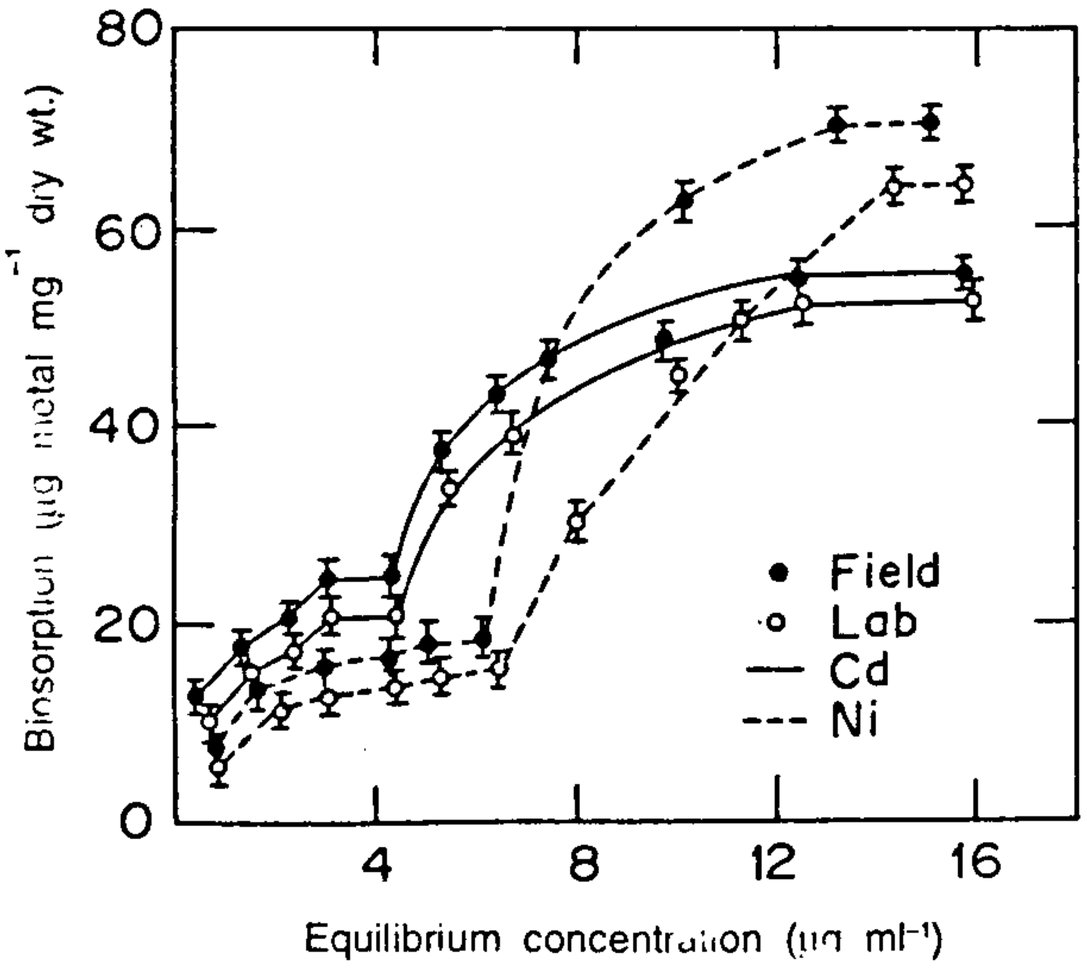

Figure 1. Equilibrium mass isotherm for Ni^{2+} and Cd^{2+} biosorption by naturally occurring and laboratory-grown *Microcystis* in the single ion system.

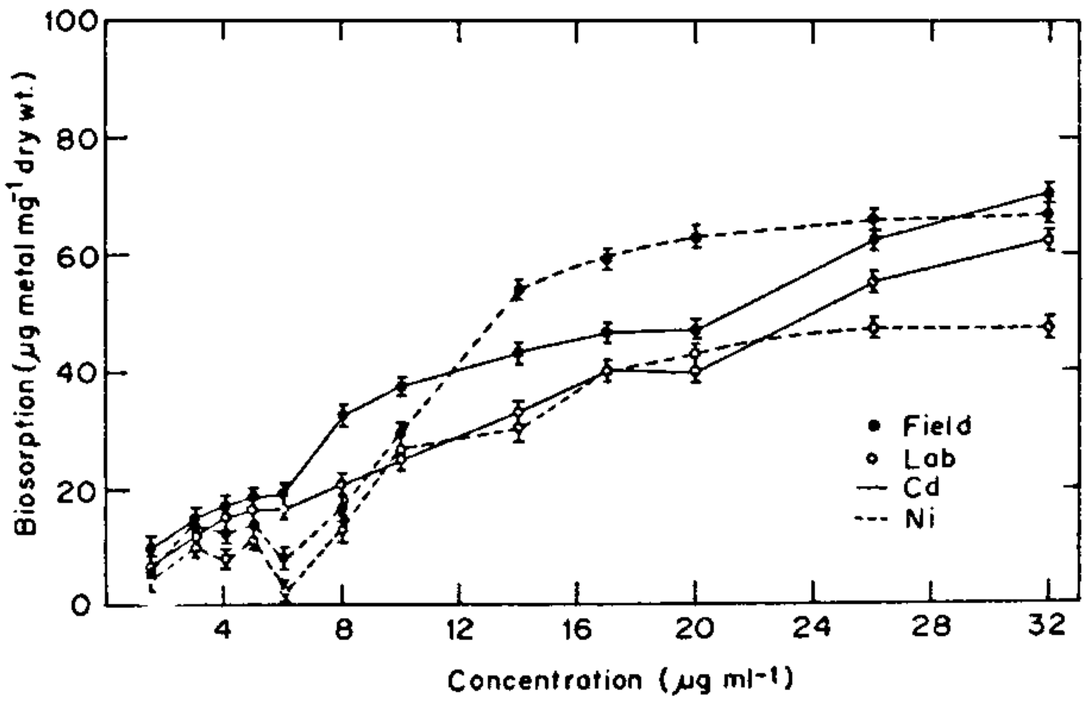

Figure 2. Ni^{2+} and Cd^{2+} biosorption by naturally occurring and laboratory-grown *Microcystis* from the bimetallic combination.

Table 2. Comparison of Freundlich and Langmuir constants for Cd^{2+} and Ni^{2+} biosorption for single metal and for the bimetallic combination

			Freundlich			Langmuir		
Metal	Condition	Combination	K_f	n	R^2	K_1	K_2	R^2
Cd^{2+}	Cap (F)	Single	0.152	1.876	0.911	0.097	11.235	0.771
	Cap (L)		0.111	1.912	0.971	0.119	8.771	0.730
Ni^{2+}	Cap (F)	Single	0.052	1.404	0.847	0.023	25.000	0.170
	Cap (L)		0.028	1.240	0.892	0.011	37.030	0.074
Cd^{2+}	Cap (F)	Bimetallic	0.049	1.821	0.940	0.090	9.174	0.827
	Cap (L)		0.080	1.712	0.966	0.069	8.547	0.802
Ni^{2+}	Cap (F)	Bimetallic	0.190	1.356	0.669	0.020	20.000	0.081
	Cap (L)		0.341	1.464	0.539	0.001	166.600	0.000

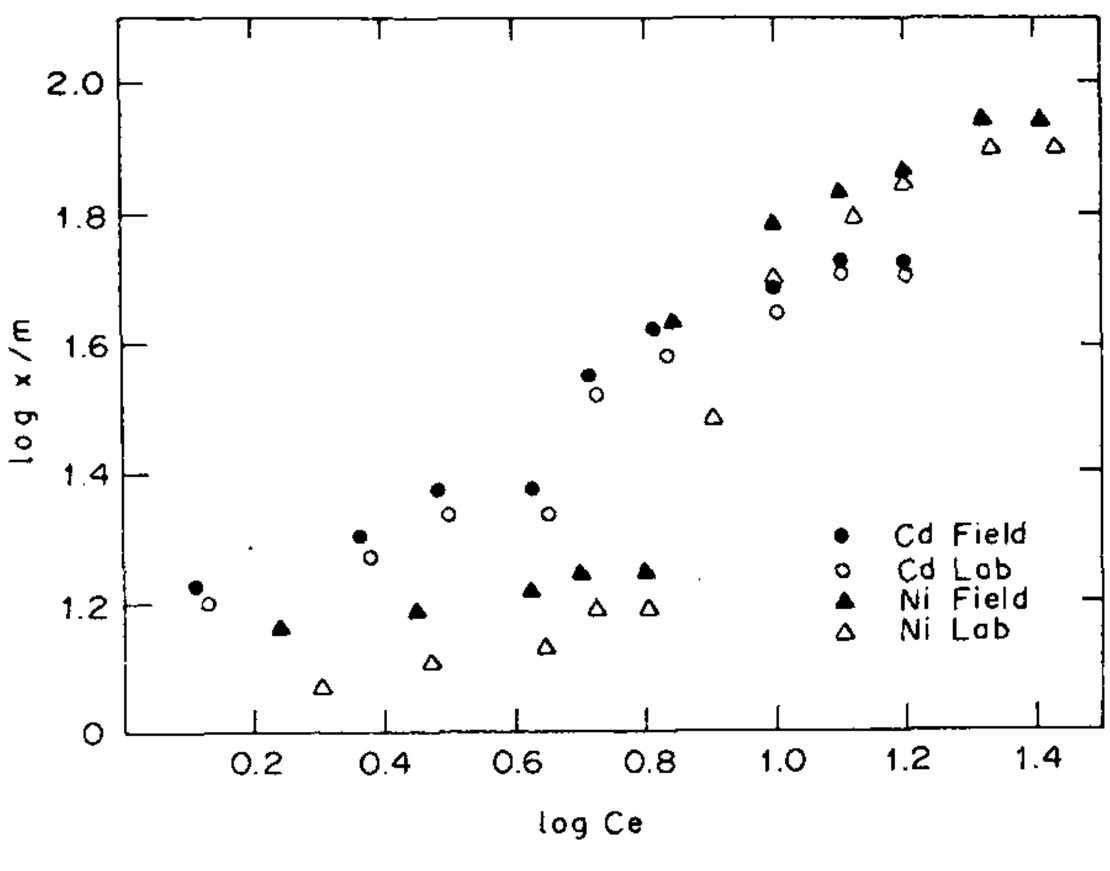

Figure 3. Freundlich isotherm for Ni^{2+} and Cd^{2+} biosorption by naturally occurring and laboratory-grown *Microcystis*.

find entry into the cell, thus inhibiting the biosorption of each other.

The Freundlich ($x/m = K_f C_e^{1/n}$) and Langmuir ($Ce/Q = 1/K_1K_2 + C_e/K_2$) isotherm constants and correlation coefficients (R^2) (Table 2) indicate a linear correlation for Cd^{2+} biosorption both in single metal and the bimetallic combination. This table further suggests that correlation coefficient (R^2) values for Freundlich in single metal (0.911, 0.971) and the bimetallic combination (0.940, 0.966) are better than Langmuir (0.771, 0.730 for single and 0.827, 0.802 for bimetallic) isotherm. In contrast to Cd^{2+}, Ni^{2+} biosorption showed a much linear correlation for Freundlich (0.847, 0.892) than Langmuir (0.170, 0.070) in the single ion system. A very poor performance of Freundlich isotherm is reflected by R^2 values (0.669, 0.539) for Ni^{2+} biosorption in the bimetallic combination. It is worth stating that the extremely low R^2 values (0.089, 0.000) for Ni^{2+} biosorption in the bimetallic condition ruled out the possibility of application of Langmuir isotherm. Table 2 further revealed (i) a higher biosorption of Cd^{2+} than Ni^{2+}, and (ii) a greater affinity of *Microcystis* for Cd^{2+} than Ni^{2+} in both

Table 3. BET isotherm constants for Cd^{2+} and Ni^{2+} biosorption

	BET		
Metal	V_m	c	R^2
Cd^{2+} (F)	2.092	21.727	0.946
Cd^{2+} (L)	1.855	21.560	0.948
Ni^{2+} (F)	2.150	9.686	0.864
Ni^{2+} (L)	2.178	5.810	0.816

for single metal and for the bimetallic combination. Besides, the n value clearly shows that *Microcystis* is a good biosorbent for Cd^{2+} and Ni^{2+} .

In order to check how far the correlation coefficient values support the linearity of Freundlich isotherm, the isothermal data ($\log x/m$ against $\log C_e$) were graphically represented in Figure 3. This figure showed two linear regions for Freundlich lines, one at low concentration and the other at high concentration. This not only points toward a change in the biosorption mechanism at low and high concentration but non-applicability of Freundlich model on the experimental data. Hence, we decided to study the biosorption behaviour at high concentration which can be best explained by BET isotherm (Table 3). This isotherm says that single layer formation at low concentration, is followed by the multiple layers at high concentration. Mathematical expression of BET isotherm can be expressed by: $C_e/V(C_0 - C_e) = 1/V_m c + (c-1)\ C_e/V_m c C_0$. The plot of $C_e/C_0/V(1 - C_e/C_0)$ against C_e/C_0 should be a straight line, where C_e is the equilibrium concentration; V the specific amount of sorbed cation; C_0 the saturated concentration of adsorbate; V_m the amount of cations to form monomolecular layer; c the constant.

A linear relationship obtained in Figure 4 suggests that biosorption not only follows the BET isotherm at higher concentration but also a multilayer binding. This study clearly revealed that adsorption may be both species- and metal-specific and variable under single metal and the bimetallic combination. Hence before recommending the test cyanobacterium for removal of specific metal, one should have information about the

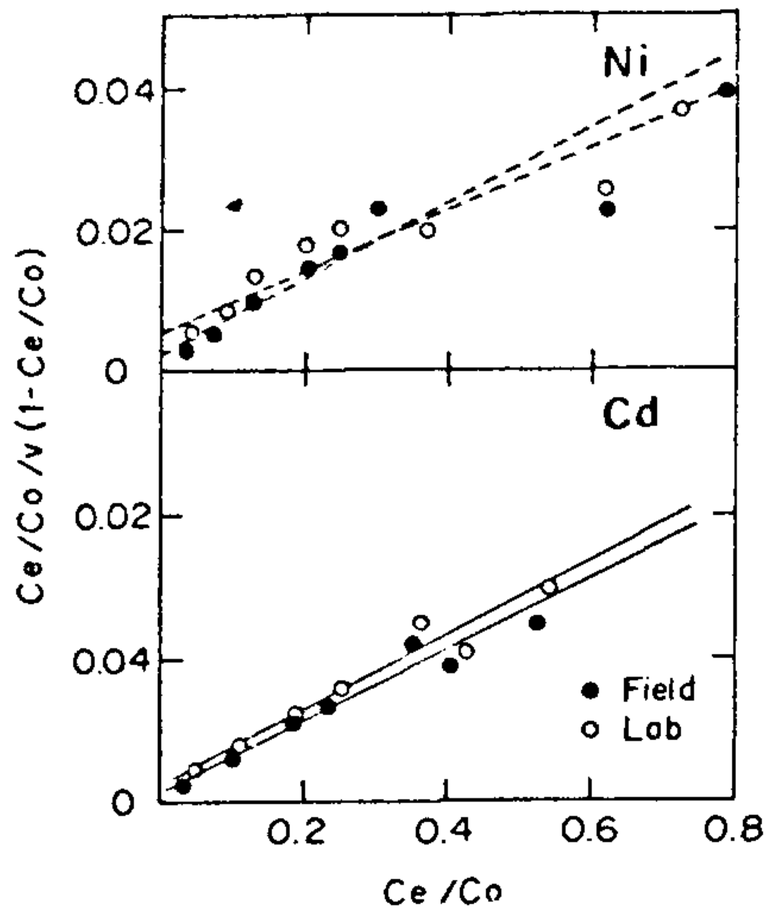

Figure 4. BET isotherm for Ni^{2+} and Cd^{2+} biosorption by naturally occurring and laboratory-grown *Microcystis*.

nature of biosorption of that particular metal in defined condition.

The application of mathematical models and constants has demonstrated that (i) *Microcystis* is an excellent biosorbent, (ii) the naturally grown cells are better than the laboratory-grown cells, (iii) biosorption is multilayer, and (iv) this biological biosorbent is quite similar to physical biosorbent, hence, it may replace the physical biosorbents. We are currently evaluating the potential of *Microcystis* as well as optimizing different environmental conditions for the use of this test cyanobacterium in removal of heavy metals from aquatics polluted with different metals.

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Solubilization of phosphorus by *Trichoderma viride*

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Solubilization of insoluble phosphates by *Trichoderma* spp. has been described and their solubilizing efficiency compared with that of certain known phosphate solubilizers. *T. viride* proved to be an efficient solubilizer of tricalcium phosphates.

Of the various macronutrients, phosphorus plays an important role in plant growth and reproduction. Further availability of phosphorus in soil is conditioned by various factors and is a major limiting factor for the growth of plants. Soil organisms, specifically bacteria[1] and fungi[2,3], growing in the root region of plants play an important role in supply of phosphorus. Kundu and Gaur[4] have reviewed the role of fungi in solubilization of rock phosphate in soil. The main problem in application of P as a plant nutrient in P-fixing tropical soils is its conversion to unavailable P in soil up to 85%. Therefore, for making P available to plants, several microorganisms are used as P solubilizers.

The soil-borne fungus *Trichoderma* is a very effective biocontrol organism used against several soil-borne plant pathogens. It is now widely used both in India and abroad under several trade names like BINAB and Trichodes.

Although phosphate solubilization by bacteria has been

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