

RESPONSE OF *CHLORELLA* SP. TO NICKEL POLLUTION

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ABSTRACT

Unicellular green microalgae are widely used these days for removing toxic heavy metals from the contaminated environment by virtue of their metal binding capacities confirming resistance mechanisms. Besides, they have several advantages over conventional physico-chemical methods such as ion exchange, precipitation with CaCO_3 , adsorption, flocculation, etc. The present study is a preliminary investigation regarding an attempt to provide low cost, efficient and an emerging eco-friendly technology for the removal of nickel from the nickel polluted environment by the use of wild type (WT) *Chlorella* sp. and one of the isolated cell lines, EMS-10 of the same species. Growth experiments show the effect of increasing Ni^{2+} concentration on the WT and EMS-10 cultures. However, the ID_{50} value of the EMS-10 revealed some degree of resistance to nickel. Kinetic experiments reveal effective metal binding capacity in EMS-10 and demonstrated appreciable resistance in response to the nickel toxicity as compared to the WT.

INTRODUCTION

Heavy metals comprise a rectangular block of elements in the periodic table flanked by titanium, hafnium, arsenic, and bismuth at its corners but also including selenium and tellurium. These are defined as the group of elements that have specific weights of higher than 5 g/cm^3 . There are about 40 elements that fall into this category (Hawkes, 1997). These elements are among the most toxic pollutants to living organisms and are always present in the aquatic and terrestrial environments. Among the metal pollutants, nickel has also become a serious environmental problem worldwide since its toxic effect to human and environment has been well recognized (ATSDR, 1988). Like many others, it is one of the essential heavy metals required at trace amount for life because it has important roles in metabolic processes taking place in living cells (Gadd, 1993). When the concentration of nickel in the environment exceeds a specific threshold level, the metal can act in a deleterious manner. This involves blocking essential functional groups, displacing essential metal ions, or modifying the active confirmation of biological molecules resulting in the inhibition of a variety of metabolic as well as enzyme activities in living organisms (Kotrba & Ruml, 2000). In human, it can cause allergic contact dermatitis, pulmonary asthma, conjunctivitis and inflammatory reactions. Toxic effects of oral exposure to nickel usually involve the kidneys with some evidence from animal studies

showing a possible developmental/reproductive toxicity effect. Inhalation exposure to some nickel compounds may cause toxic effects in the respiratory tract and immune system. Nickel and nickel compounds are also well recognized as carcinogens (ATSDR, 1988). The potential sources of nickel contamination in the environment are from industrial activities such as mining, electroplating and alloy production. Due to its serious toxic effects on human health and the environment, nickel is often regarded as one of the toxic heavy metal pollutants. That is why, the metal is treated as one of 13 metals on the list of 129 priority pollutants according to the US Environment Protection Agency (EPA, 1980).

The use of eukaryotic algae, especially *Chlorella* sp., is an eco-friendly technology for removal of toxic metals from the environment. At present, it has become an emerging, efficient and low cost technology due to the rich properties of the species. The *Chlorella* sp. is a unicellular photosynthetic organism, which is commonly present in abundance in wastewater and other surface water bodies. Since it possesses large surface area and chelating potential, it can maximize binding of toxic metal ions. The metal chelating to cell walls of the biomass has the ability to reduce metal concentration in aqueous to 1 ppm or less (Roy *et al.*, 1993). In addition, it is also used as a model organism to study metabolic processes in photosynthetic eukaryotic higher plants because of its similarity. Because the cell cultures are inexpensive, rapidly grow and easy to maintain in a simple mineral medium, the use of algae has been proposed and developed for numerous applications in different fields. The application includes water treatment, wastewater treatment, biological detoxification and heavy metal controls in natural and/or industrial waste streams (Gaur & Rai, 2001). Therefore, the *Chlorella* sp. was a proposed organism to use in the present work to study mechanisms confirming resistance to nickel toxicity. Keeping in view the present situation regarding environmental pollution due to nickel and also in an attempt to mitigate this problem, the present study was addressed on the following objectives:

1. To study comparatively the effects of nickel on growth of the living organisms such as the wild type (WT) of *Chlorella* sp. as a control and one isolated nickel resistant strain, EMS-10.
2. To study the metal detoxification mechanisms involving adsorption and removal on the selected algal cells.

MATERIALS AND METHODS

ORGANISMS, CULTURE MEDIA AND GROWTH CONDITIONS

A wild type (WT) *Chlorella* sp. and a nickel resistant line, EMS-10 isolated from the same species by EMS (Ethyl Methane Sulphonate) mutagenesis (Sil & Chenevert, 1998), were grown in modified BG-11 liquid mineral medium. The cultures in 250 ml capacity Erlenmeyer flasks were continuously exposed to a light intensity of 20 – 50 μmol by cool white fluorescent lamps while incubated

in a gyratory shaker (180 rev./min) at 27^o C. Samples were periodically removed from the flasks to monitor growth of the algal cells.

EFFECTS OF NICKEL ON GROWTH RATE OF THE WT AND EMS-10 CULTURES

Sterilized Erlenmeyers each containing 100 ml of the liquid medium were added with nickel solution, (NiCl₂. 6H₂O) in calculated amount such that they were at a series of concentrations viz., 0, 1, 10, and 50μM, respectively. The cultures at the exponential phase of growth were then inoculated to the medium in such a way that the initial cell densities were in the range of 5.0-5.5 x 10⁵ cells/ml of the liquid medium. The algal growth was monitored by measuring the change in absorbance of the algal cells at 540 nm. The measurement was taken at the time of inoculation and each day thereafter until it reached the stationary phase. The growth rate of the algal cultures was determined between the 2nd and the 6th days by the following equation:

$$\mu = (\ln X_6 - \ln X_2) / (T_6 - T_2)$$

Where μ is the specific growth rate of the algal culture, X_6 is the $A_{540 \text{ nm}}$ of the algal culture at time T_6 , and X_2 is the $A_{540 \text{ nm}}$ of the algal culture at time T_2 .

KINETIC EXPERIMENTS ON ADSORPTION AND REMOVAL OF Ni²⁺ BY THE WT AND EMS-10 CULTURES

To study the adsorption kinetics at different time intervals, the metal solution was added to maintain 50 μM in each of the flasks containing separately 10⁹ cells per hundred milliliters of each culture. From each of the metal added flasks, a 10-ml sample was drawn immediately in order to represent a zero hour sampling, however it took 15 minutes to proceed through a complete treatment. Hence, the 15 minutes was regarded as the zero hour samples. In a similar way, the samples were drawn at half, one, two, four, eight, twelve, twenty-four and forty eight hours, respectively. The flasks were placed back to the shaker after each sample drawn. The samples, at each of these time intervals, were spun down in a bench centrifuge (3500 rpm, 10 min) and the supernatants collected separately for metal analysis. This supernatant yields the residual metal left over the medium. The cell pellets were then washed with 5-ml of EDTA (10 g/l) three times. Each time, the cells were spun down (3500 rpm, 10 min) and the supernatants containing EDTA were collected for metal analysis. This analysis yields the concentration of Ni²⁺ adsorbed to the cell surface at varying time intervals.

PRESENTATION OF THE DATA

Each experiment was conducted in triplicate and the mean values were presented with their standard deviations. The data were subjected to student's T-tests at 95% level of confidence or at 5% level of significance.

RESULTS AND DISCUSSION

EFFECT OF Ni²⁺ ON GROWTH RATE OF THE WT AND EMS-10 STRAIN

The presence of Ni²⁺ in the medium retarded the growth of both wild type (WT) and EMS-10. The effect was more pronounced with the increasing concentration of the metal ion (Fig. 1). Table 1 shows no inhibitory effect on growth rate of the WT and EMS-10 strain in presence of 1 μM Ni²⁺ while growth inhibition of the algal cells was 36% and 25%, respectively in presence of 10 μM Ni²⁺. The presence of 50 μM Ni²⁺ had significantly arrested the growth rate of both the algal cells in which the EMS-10 sustained less inhibitory effect (56%) compared to that of the WT (80%). This shows that the EMS-10 strain could survive comparatively better than that of the WT in various Ni²⁺ concentrations. Based on the growth rates, inhibition of 50% growth rate (ID₅₀) was also calculated. The ID₅₀ was obtained as follows:

$$\text{EMS-10 (32 } \mu\text{M)} > \text{WT (20 } \mu\text{M)}$$

This indicates that the EMS-10 possesses a certain degree of resistance to Ni²⁺ since it has higher ID₅₀ values than the WT. These results are in agreement with the results presented in many research reports, which also indicate that the algal growth is affected by the presence of heavy metals and the inhibitory effect on the growth rate is more pronounced with increasing metal concentrations in the medium. However, their resistance to the metal toxicity may vary with algal species (Wong & Wong, 1990; Macfie & Welbourn, 2000). Although both the algal cells responded with inhibitory effects in order of increasing metal concentration but comparatively, the EMS-10 strain exhibited better growth rate (Table 1), which may plausibly be due to EMS mutagenesis. The ID₅₀ value of the strain was also higher to that of the WT showing that the strain might be expected to possess a certain degree of resistance to the metal toxicity.

Table 1: Percentage growth rate of the WT and EMS-10 in various Ni²⁺ concentrations.

Cultures	Ni ²⁺ concentration (μM)			
	0 (control)	1	10	50
WT	100	94	64	20
EMS-10	100	96	75	44

KINETIC EXPERIMENTS ON ADSORPTION AND REMOVAL OF Ni²⁺ BY THE WT AND EMS-10 CULTURES

Figure 2 (A&B) shows the kinetics of Ni²⁺ adsorption and removal by the WT. On exposure to 50 µM Ni²⁺, the rate of the metal ion removal was rapid during the first few hours, increased gradually until 12 hours and then reached a steady state thereafter (Fig. 2B). Decreasing residual Ni²⁺ concentration in the medium with time indicated the amount of Ni²⁺ being removed from the medium simultaneously (Fig. 2A). During the first hour of treatment, Ni²⁺ removal from the medium was 44% (Fig. 2B). Correspondingly, adsorption of Ni²⁺ occurred side by side, being rapid in the first half-hour and then remained unaltered until 2 hours (Fig. 2A). Nickel adsorbed by the WT cell surfaces during the first hour was 36%. Further, a gradual increase in Ni²⁺ adsorption was observed until 12 hours and then reached the equilibrium point after this treatment hour. Initially, the removal of Ni²⁺ from the medium was 26% at 15 minutes. Finally 71% of the total nickel supplemented was found removed at 48 hours of which 54% was externally bound to the cell walls.

Apparently, the EMS-10 strain exhibited a distinct kinetics of Ni²⁺ adsorption and removal (Figs. 3A&B) compared to the WT. On exposure to 50 µM Ni²⁺, the rate of Ni²⁺ removal from the medium was very rapid during the first few hours unlike that of the WT. The removal was more than 80% within few hours. In other words, residual concentration of Ni²⁺ in the medium was less than 20% within the first few hours of treatment (Fig. 3A). Until 48 hours treatment, the strain showed 96% removal of the total metal ions from the medium (Fig. 3B). Correspondingly, a very rapid increase in the metal adsorption was found in the strain unlike the WT. The adsorption of Ni²⁺ to the cell surface was very rapid during the first few minutes, remained almost constant until 2 hours and gradually increased up to 8 hours (Fig. 3A). The adsorption attained a point of saturation after 8 hours. At 15 minutes, Ni²⁺ removal from the growth medium was 67% contributing 50% to the extracellular adsorption alone. This figure shows difference significantly from that of the WT in terms of Ni²⁺ removal and adsorption at 15 minutes. Similarly, of the 96% Ni²⁺ removal at 48 hours treatment, 66% was found externally adsorbed to the cell walls of the strain, which is higher to that of the WT.

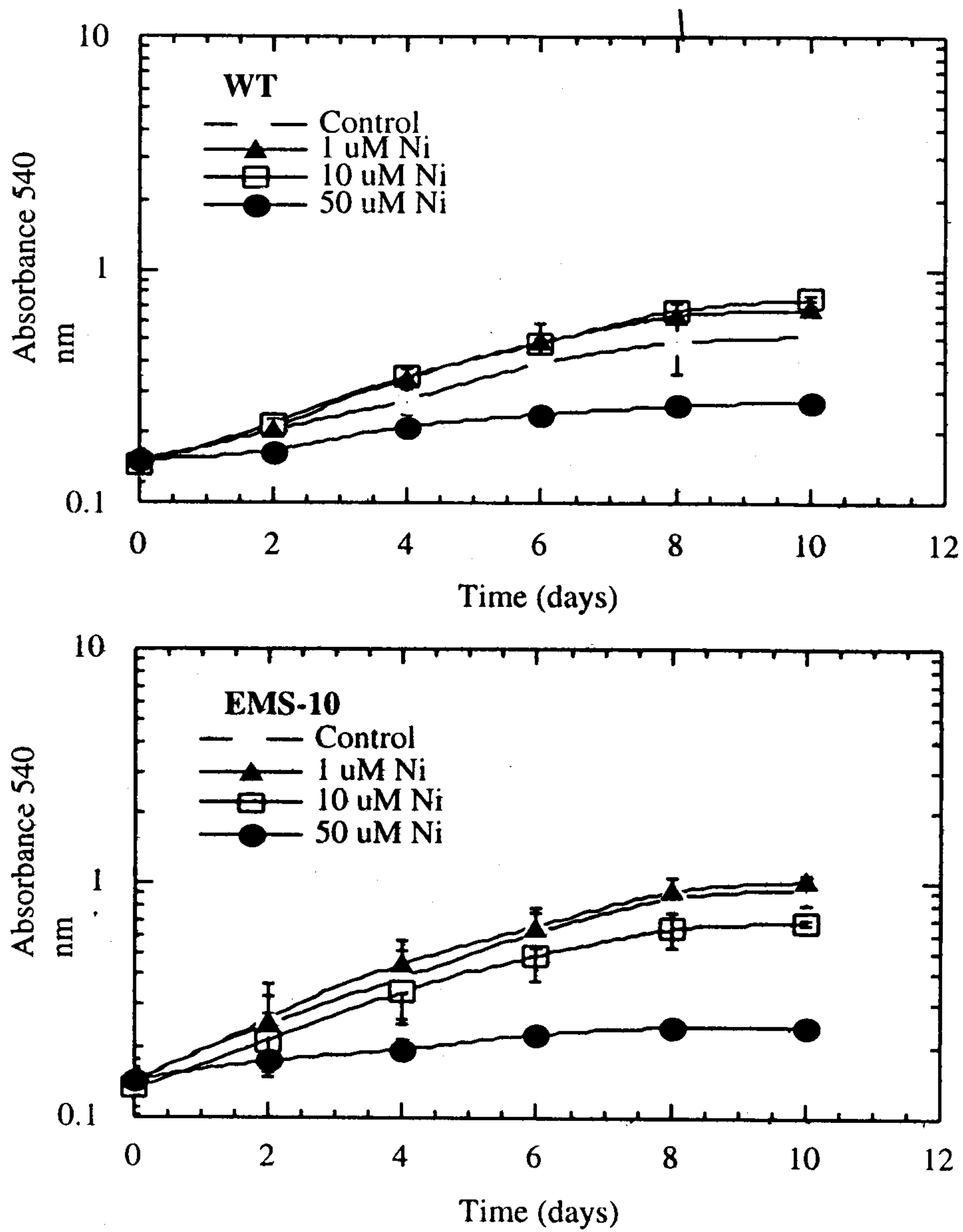


Fig. 1: Growth curves of WT and EMS-10 in the presence of Ni²⁺. Each point in the curves is the mean \pm SD of three experiments.

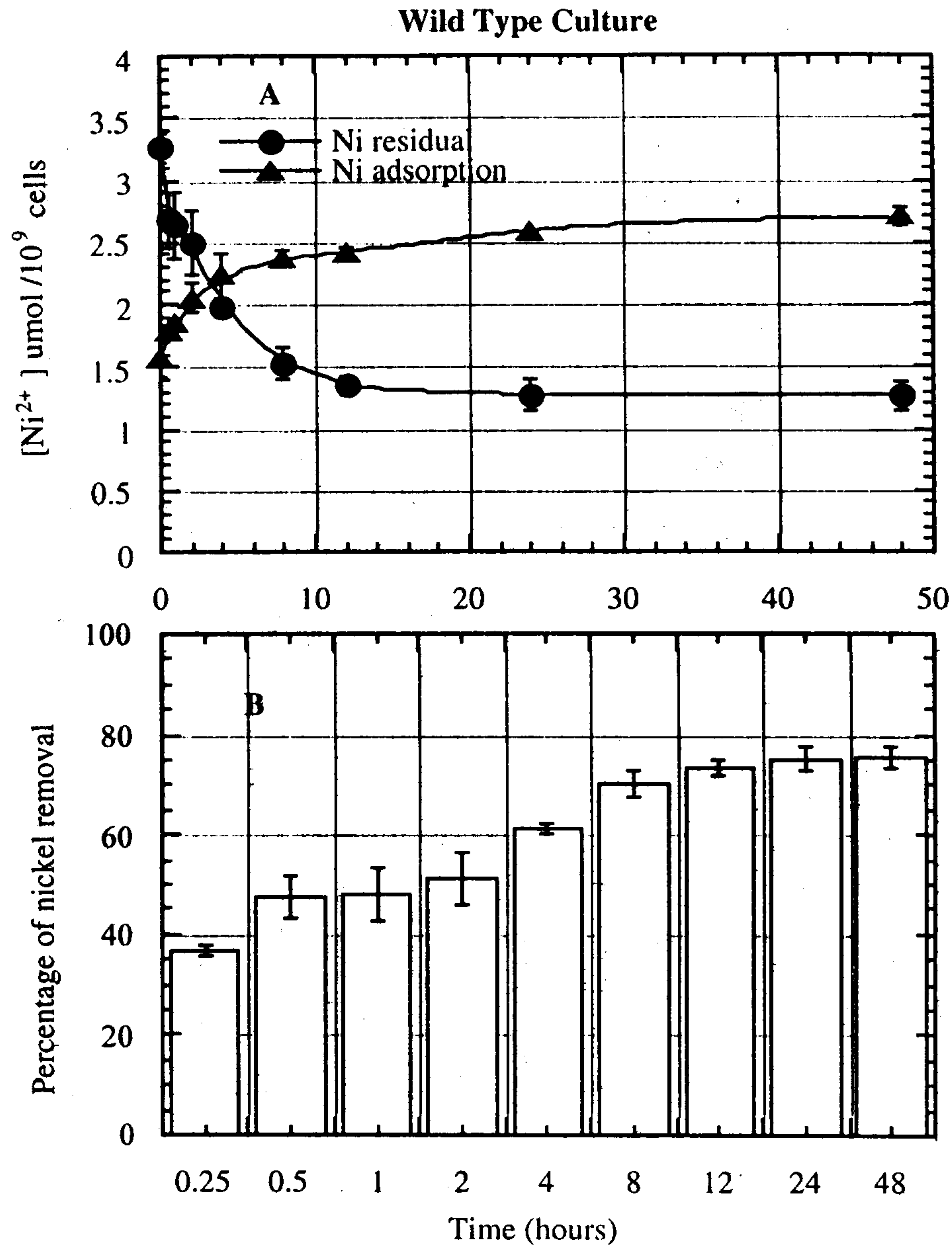


Fig. 2: Extracellular adsorption and residual concentration of Ni²⁺ by the WT at different time intervals (A); and percentage removal of Ni²⁺ from the medium (b). Each value in the curves and bars is the mean \pm SD of three experiments.

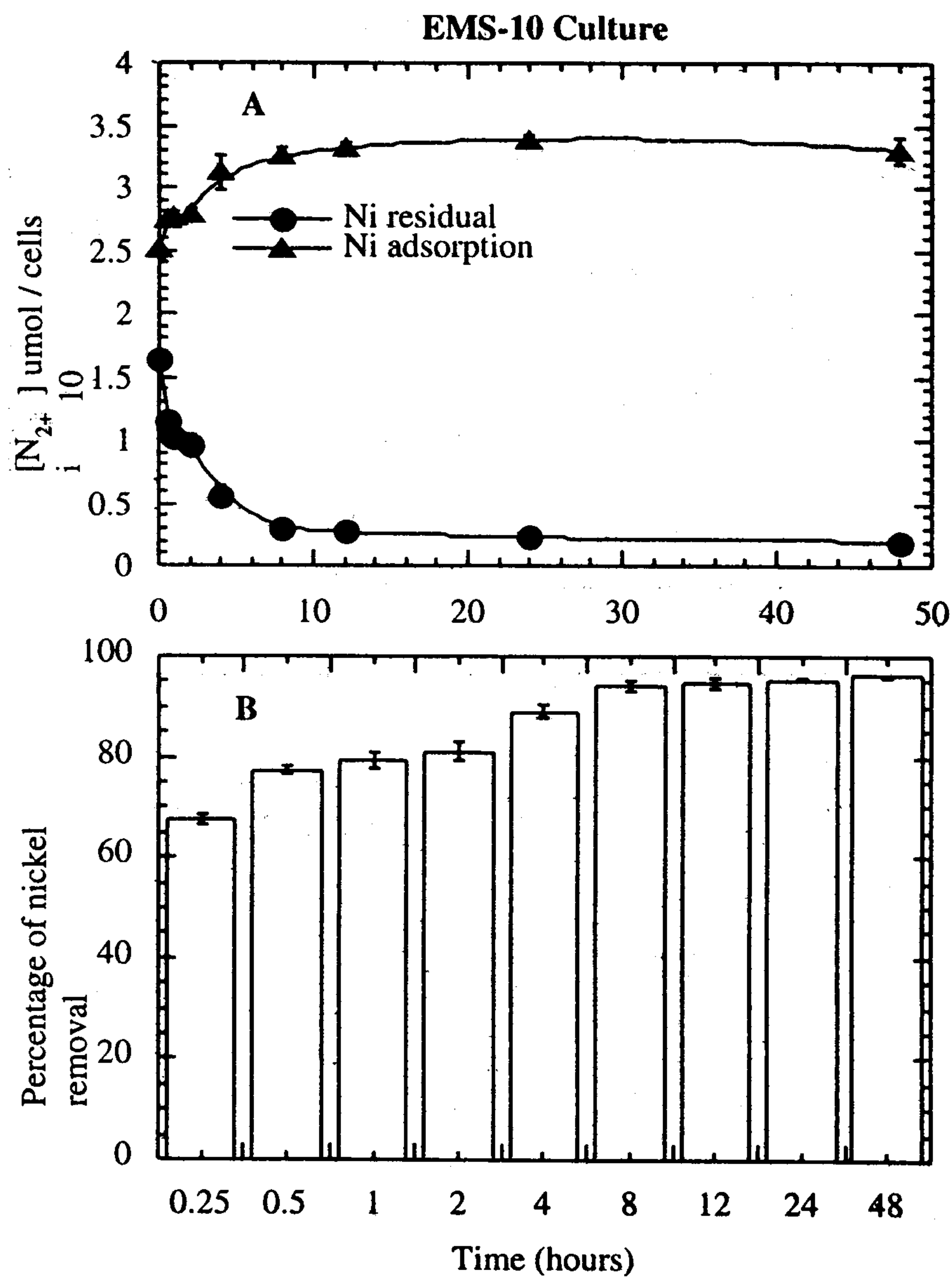


Fig. 3: Extracellular adsorption and residual concentration of Ni^{2+} by the EMS-10 strain at different time intervals (A) and percentage removal of Ni^{2+} from the medium (b). Each value in the curves and bars is the mean \pm SD of three experiments.

A variety of resistance mechanisms are exhibited by microalgae in response to metal toxicity. In general, two mechanisms are taken into account for the removal of metal ions. One is metabolically independent passive surface adsorption or biosorption, while the other, active uptake of the metal ions into the cells, is metabolically dependent. Both mechanisms work simultaneously in algal cells in which adsorption is very rapid and occurs in few minutes as reported by several studies (Crist *et al.*, 1988; Wang & Wong, 1984). The present study supports the above findings while analyzed with the kinetic experiments on extracellular adsorption but results on intracellular uptake are not included here. The result also shows that most of the metal ion was bound to the cell walls in the early 15 minutes of treatment. The rapid adsorption to the algal cell surface may be due to the availability of specific binding sites to which the metal ions are bound until all the sites are saturated. Furthermore, the difference in the magnitude of metal binding capacity to the surface between the WT and EMS-10 at different time intervals may be due to different affinities of the algal cells towards the metal ions. However, presence of other metal ions in the growth medium, light, temperature, time of exposure to metal ions and pH are some of the dependent and sensitive parameters of the processes (Hamdy, 2000).

The overall result of the present study indicates that the EMS-10 strain possesses comparatively higher nickel accumulating potential than the WT and exhibit resistance to the metal toxicity. The rapid removal of the metal followed by the simultaneous adsorption suggests that the strain plays important role in reducing the level of metal concentration from the medium. Besides, the findings also generate wide prospects for research regarding the nature and chemical compositions of the algal cell walls since the metal binding affinity also depends on the availability of various functional groups as described in several studies (Crist *et al.*, 1981; Donmez *et al.*, 1999). The purpose of acquiring nickel-resistant *Chlorella* cells will act as a guideline for a biological understanding of the living organisms to an extreme environment in one hand and also as a biological tool for the removal of the metal ions from the polluted environment on the other hand. Understanding the mechanisms of resistance may contribute much towards processing and purification of industrial and domestic wastewater as well as reduction of environmental contamination in soil and water bodies.

CONCLUSIONS

In conclusion, the present study seems to provide baseline information regarding the fate of metal in the contaminated soil and water bodies. The metal content in this alga can be a reflection of background concentrations of heavy metals contaminated in the sites. Furthermore, the preliminary screening of the isolated EMS-10 strain also reflects that the high capacity of the strain for metal binding would lead to research into its uses in a number of areas. This may include bioremediation of heavy metal pollution and precious metal recovery during industrial and mining operations. Besides, a comprehensive understanding of physiological, biochemical and molecular

mechanisms conferring Ni²⁺ resistance in the *Chlorella* sp. would enable the engineering of metal accumulating organisms such that they could serve as a tool in water treatment, wastewater treatment and controlling the environment from toxic metal pollution.

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