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Lead Toxicity, Adsorption and Resistance in *Chlorella* Sp.

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Abstract
These days, unicellular green microalgae have been widely used for removal of toxic heavy metals from contaminated soil and water bodies. In the present investigation, a lead resistant PbR-11 strain isolated from *Chlorella* sp. by EMS mutagenesis was compared against the wild type (WT) of the same species for Pb^{2+} accumulating capacity. Growth of both the tested algal cells, PbR-11 and WT (control) were found to be retarded with increasing Pb^{2+} concentrations in the medium. However, higher ID_{50} value of the PbR-11 exhibited some degree of resistance to the metal toxicity. When exposed to the liquid medium containing 50 µM Pb^{2+}, kinetic experiments showed rapid removal and adsorption of the metal ions in both the algal cells during the first few hours. Compared to WT, the PbR-11 showed significantly higher percentage removal and adsorption of Pb^{2+} at 15 minutes and 48 hours interval of time respectively. Extracellular Pb^{2+} adsorption was found significantly higher than intracellular uptake in the tested algal cells although both the processes occurred simultaneously. Total Pb^{2+}accumulation and distribution between the external and internal cell fractions of the PbR-11 were significantly higher to that of the WT. Thus, the strain appeared more useful for remediation of contaminated sites.

Key words: EMS mutagenesis, heavy metals, *Chlorella* sp., adsorption, lead toxicity

Introduction
Release of toxic metal pollutants in the environment has increased enormously over the past several decades as a result of anthropogenic activities (Ajmal and Khan, 1985). Among the metal pollutants, lead has become a serious worldwide environmental problem since its toxic effect to human and environment has been well recognized (U.S. ATSDR, 2005). The metal is toxic even at very low exposure levels (Fernando *et al*., 1981). Important releases of lead are from natural as well as anthropogenic sources. Natural releases of lead are from the natural mobilization of naturally occurring lead from the Earth's crust and mantle, such as volcanic activity and the weathering of rocks. Anthropogenic releases of lead are from mining and processing activities, manufacturing, use, disposal, recycling and reclamation; incineration and installations for municipal waste, open burning and emissions from leaded petrol (Harrison and Laxen, 1980).

In human, neurological, cardiovascular, renal, gastrointestinal, haematological and reproductive effects are due to lead toxicity (U.S. ATSDR, 2005). Lead is accumulated in bone and may serve as a source of exposure later in life. Organo-lead compounds, such as tri-alkyl-lead and tetra-alkyl-lead compounds, are more toxic than inorganic forms of lead (Corrin and Natusch, 1977). In the environment, lead is toxic to plants, animals and micro-organisms (Fernando, 1995). It bio-accumulates in most organisms. In surface

waters, residence times of biological particles containing lead have been estimated at up to two years (Corrin and Natusch, 1977). Although lead is not very mobile in soil, lead may enter surface waters as a result of the erosion of lead-containing soil particles and the dumping of waste containing lead products.

Heavy metals like lead, cadmium, mercury etc., cannot be degraded or destroyed by biological or chemical means unlike many other organic toxic pollutants; however rendering toxic forms into less toxic or harmless is the only way to bring about the metal detoxification. Many conventional physico-chemical methods such as excavation, precipitation with lime, adsorption, flocculation, filtration etc., are costly and inefficient for remediation of toxic metals from contaminated sites (Kamnev and Van der Lelie, 2000). Therefore, there is a growing realization to clean up the metal contaminated soil and water bodies using microorganisms, algae or plants since they are cost effective, efficient and eco-friendly in nature (Salt *et al.*, 1998).

Algae are capable of accumulating heavy metals to concentrations several orders of magnitudes higher than in the surrounding medium (Beker, 1986) and have therefore been used for their removal from contaminated sites (Sandau *et al*., 1996; Vilchez *et al*., 1997). Their high accumulating potential can even be used for the enrichment, recovery or recycling of traces of valuable metals like uranium, gold and silver from nature (Borowitzka and Borowitzka, 1988; Lopez-Suarez *et al*., 2000; Kessler 1986). Many algae growing in metal-polluted environments display an ability to tolerate high concentrations of toxic metals (De Filippis and Pallaghy,1994). Different groups of algae exhibit varying levels of tolerance to heavy metals.

Literature reveal that different *Chlorella* species have been isolated from highly polluted domestic, industrial and metal contaminated lakes (Lopez-Suarez *et al*., 2000; Kessler, 1986; Kaplan *et al*., 1995; Wong *et al*., 2000). Many studies have been addressed on metal detoxification of various *Chlorella* spp.; however little information is available regarding the lead toxicity and resistance mechanisms in *Chlorella* sp. Therefore, a unicellular green microalga, *Chlorella* sp. has been used in the present investigation since it resembles all characteristics to that of the common algal groups. The species is abundant in wastewater and surface water bodies and may be used as a model organism to study metabolic processes in photosynthetic eukaryotic higher plants because of its similarity. Hence, the present study is an attempt to isolate lead resistant *Chlorella* strains from the wild type (WT) culture and then characterize the mechanism(s) confirming resistance to lead toxicity.

Materials and methods

Growth conditions

Lead resistant strains from the wild type *Chlorella* sp. were isolated by EMS (Ethylmethane Sulphonate) mutagenesis using the Herskowitz Lab Protocol (Sil and Chenevert, 1998). So far, fifty five-lead resistant strains (those survived and grew only) were isolated from the procedure described. The EMS mutagenized strains were designated as *PbR* with numerals. All the strains were maintained in modified BG-11 liquid mineral medium containing 25 µM Pb^{2+}. The cultures 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°C. A 250 ml capacity Erlenmeyer flask containing a volume of 100 ml of the liquid medium was used for performing all experiments. Samples were periodically removed from the flasks to monitor growth of the algal cells. When the cultures reached the stationary phase of growth, they were further inoculated into fresh liquid medium to keep growing unless otherwise mentioned. One of the fifty five lead resistant strains, PbR-11 was selected for the present investigation.

Preparation of stock metal solution

Stock solution (0.1M) of lead salt was prepared by dissolving the calculated amount of $Pb(NO_3)_2$ in ultra pure water and filter sterilized. The solution was divided into 4-ml aliquots and stored frozen at minus 20^0C. The metal solution was defrosted and used when required.

Calibration of absorbance (optical density) vs. cell numbers

Growth of the cultures was monitored by measuring absorbance (optical density) in Spectrophotometer at 540 nm or counting cell numbers by a Hemacytometer. Initially, the absorbance was calibrated against the cell numbers. The calibration was done with a purpose of calculating cell numbers from the absorbance or vice versa as necessary.

Growth experiments

Cultures of the selected PbR-11 and *Chlorella* sp. (WT) at the exponential phase of growth were used to study growth pattern in presence of lead. Erlenmeyers each containing 100 ml of BG-11 medium were firstly sterilized in autoclave and then cooled at room temperature keeping inside the laminar flow hood so as to avoid any kind of contamination. The stock lead solution was added to the autoclaved medium in calculated amount such that they were at concentrations of 0, 1, 10, 50 and 100 μM respectively. The cultures 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 10^5 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. Cells were also counted using a Hemacytometer. 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 $A_{540\ nm}$ of the algal culture at time T_6, and X_2 is the $A_{540\ nm}$ of the algal culture at time T_2 (Ajmal and Khan, 1985). All the experiments were carried out in triplicate.

Kinetic experiments on adsorption of Pb^{2+} by the WT and PbR-11 cultures

Firstly, the selected lead resistant strain, PbR-11 initially maintained in the medium containing 25 μM Pb^{2+} was inoculated into the fresh liquid medium without lead and allowing them to grow for 5 days. The process was repeated three times to ensure that the cells were completely free from the metal ions. Then, the PbR-11 cells at the stationary phase of growth were collected by centrifugation (10^0 C, 8000 g for 15 min). The pelleted cells were re-suspended in 10 ml of fresh liquid medium and counted in the Hemacytometer to note the actual cell

numbers. Three flasks containing fresh liquid medium were inoculated with the dense cell suspension such that each flask contained 10^9 cells per hundred milliliters. The WT culture was also used as control in parallel.

To study the adsorption kinetics at different time intervals, the metal solution was added to each of the flasks maintaining a final concentration of 50 µM. 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 1/2, 1, 2, 4, 8, 12, 24 and 48 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/lit.) three times (Roy *et al*., 1993). Each time, the cells were spun down (3500 rpm, 10 min) and the supernatants containing EDTA were collected for metal analysis. The experiment was carried out in triplicate. This analysis yields the concentration of Pb^{2+} adsorbed to the cell surface at varying time intervals.

Sample digestion and preparation for lead determination

The cell pellets after EDTA treatment were finally subjected to the experiments involving the intracellular Pb^{2+} uptake. For this, each cell pellet samples were re-suspended in 10 ml of double distilled water by gentle vortex. The cells were spun down and re-suspended in distilled water repeatedly for three times following the process of washing. Each of the washed cells was then re-suspended in 1 ml of double distilled water and transferred to digestion tubes. The cell suspensions in the digestion tubes were treated with 2 ml of conc. nitric acid. The mixture was placed in a chemical hood overnight so as to ensure a complete dissolution and prevent foaming during subsequent digestion processes. The sample was digested at 100^0C for 1 hour followed by gradually increasing the temperature up to 230^0C (Foster, 1982). The digestion was carried out for approximately 3 h until the solution became completely clear and transparent. After the digestion was completed, the digest was cooled, diluted and adjusted to a final volume of 5 ml with double distilled water. Then, the metal was determined using a Perkin Elmer 1100 B atomic absorption spectrophotometer. This analysis reveals the total lead accumulated inside the cells at different time intervals i.e., intracellular lead uptake or absorption.

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

Effect of Pb^{2+} on growth of the WT and PbR-11

The effect of increasing Pb^{2+} concentration on growth of the WT and PbR-11 is given in table 1. Under the same experimental condition, the presence of 1µM Pb^{2+} had no effect on growth rate of the WT and PbR-11

while growth inhibition of the algal cells was observed by 36% and 25% respectively in presence of 10µM Pb^{2+}. Similarly, the PbR-11 sustained less inhibitory effect (56%) compared to that of the WT (80%) in the presence of 50µM Pb^{2+}. The presence of 100µM Pb^{2+} had significantly arrested the growth rate of both the algal cells. Under the condition, the PbR-11 sustained inhibitory effect by 90% while the WT was by 96%. This shows that the PbR-11 could survive to some extent even at higher Pb^{2+} concentration compared to the WT. Based on the growth rates, inhibition of 50% growth rate (ID_{50}) was also calculated on the basis of the growth rates. The ID_{50} was obtained as: PbR-11 (34 µM) and WT (20 µM). Thus, higher ID_{50} value of the PbR-11 compared to the WT indicated a certain degree of resistance to Pb^{2+}.

Table 1. Percentage growth rate of WT and PbR-11 at different Pb^{2+} concentration

Strain	Pb^{2+} concentration (µM)				
	0 (control)	1	10	50	100
WT	100	94	64	20	4
PbR-11	100	96	75	44	10

Cell counts of the WT and PbR-11

Table 2 shows the initial and final cell counts of the WT and PbR-11 in the liquid growth medium. Both the cultures were exposed to the medium containing 50 µM Pb^{2+} up to 48 treatment hours. The final cell numbers of both the cultures even after their exposure to 48 hours in 50 µM Pb^{2+} did not show significantly different compared to that of the initial cell counting. Besides, the difference of initial and final (48 hours of treatment with Pb^{2+}) cell counting between the tested cell lines was not found significant (t-test, $p>0.05$). Therefore, the same cell numbers were presumed for all time intervals throughout the kinetic experiment and for further calculations as well.

Table 2. Cell numbers per 100 ml of liquid growth medium at initial (before addition of Pb) and final (48 hours after addition of Pb) hours of treatment; the WT and PbR-11 were treated with 50 µM Pb^{2+}

Strain	Cell numbers per 100 ml of growth medium	
	Initial	Final
WT	6.1×10^9	6.3×10^9
PbR-11	7.0×10^9	7.7×10^9

Kinetics of Pb^{2+} removal and adsorption from the growth medium by WT

Figure 1 (A and B) shows the kinetics of Pb^{2+} extracellular adsorption and removal by the WT. On exposure to 50 µM Pb^{2+}, 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. 1B). Decreasing residual Pb^{2+} concentration in the medium with time indicated the amount of Pb^{2+} being removed from the medium simultaneously (Fig. 1A). During the first hour of treatment, Pb^{2+} removal from the medium was 44% (Fig. 1B). Correspondingly, extracellular adsorption of Pb^{2+} occurred side by side, being rapid in the first half-hour and then remained unaltered until 2 hours (Fig. 1A). Lead adsorbed by the WT cell surfaces during the first hour was 36%. Further, a gradual increase in Pb^{2+} adsorption was observed until 12 hours and then reached the equilibrium point after this treatment hour. Initially, the removal of Pb^{2+}

from the medium was 26% at 15 minutes. Finally 71% of the total lead supplemented was found to be removed at 48 hours of which 54% was externally bound to the cell walls.

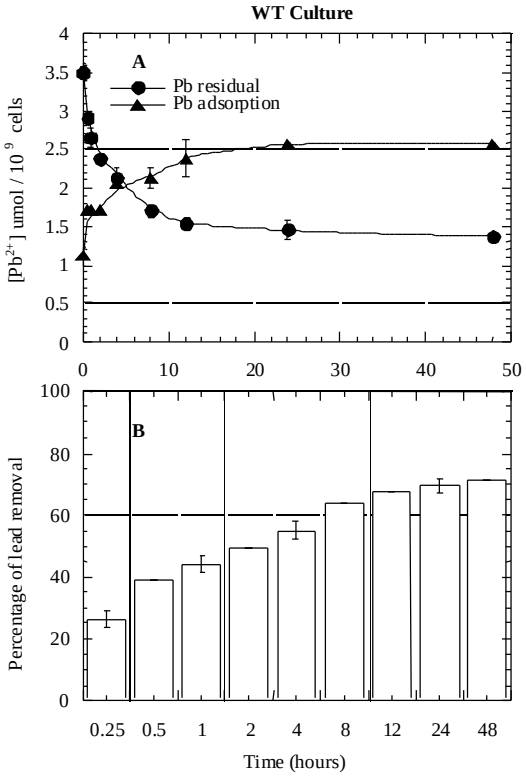

Figure 1. Extracellular adsorption and residual concentration of Pb^{2+} at different time intervals (A) and percentage removal of Pb^{2+} from the medium (B) in WT culture; mean (standard deviation; n=3)

Total lead accumulation and lead in external and internal cell fractions of WT

The WT showed a gradual accumulation of Pb^{2+} with increasing time of exposure to the medium containing 50 µM Pb^{2+} (Tab. 3). Total Pb^{2+} accumulation reached a steady state after about 12 hours while the accumulation was rapid during the first few hours. The distribution of Pb^{2+} between the external and internal cell fractions also increased with duration of exposure indicating that the process of adsorption and absorption occurred simultaneously. The amount of Pb^{2+} associated with the external cell fractions was higher than the internal at each time interval. While the extracellular adsorption was found gradually increased, intracellular uptake appeared fluctuating at different time intervals although the tendency of Pb^{2+} association was of increasing order. Of the total Pb^{2+} accumulated (3.38 µmol per 10^9 cells) at 48 hours, 76% was externally bound to the cell surface whereas only 24% was found inside the cells.

Table 3. Total Pb^{2+} accumulation and distribution between external and internal cell fractions of the WT at different time intervals; mean (standard deviation; n = 3)

Strain	Time Int. (hr.)	Lead Association (µmol / 10^9 cells)		
		External	Internal	Total Pb^{2+} accumulated
WT	0.25	1.11 (0.10)	0.14 (0.00)	1.25 (0.69)
	0.5	1.70 (0.00)	0.14 (0.00)	1.84 (1.10)
	1.0	1.70 (0.00)	0.40 (0.10)	2.10 (0.92)
	2.0	1.70 (0.00)	0.65 (0.00)	2.35 (0.74)
	4.0	2.04 (0.00)	0.57 (0.10)	2.61 (1.04)
	8.0	2.13 (0.10)	0.91 (0.10)	3.04 (0.86)
	12.0	2.39 (0.25)	0.82 (0.24)	3.21 (1.11)
	24.0	2.56 (0.00)	0.74 (0.10)	3.30 (1.29)
	48.0	2.56 (0.00)	0.82 (0.00)	3.38 (1.22)

Kinetics of Pb^{2+} removal and adsorption from the growth medium by PbR-11

Apparently, PbR-11 exhibited a distinct kinetics of Pb^{2+} extracellular adsorption and

removal (Figs. 2 A and B) compared to the WT. On exposure to 50 µM Pb^{2+}, the rate of

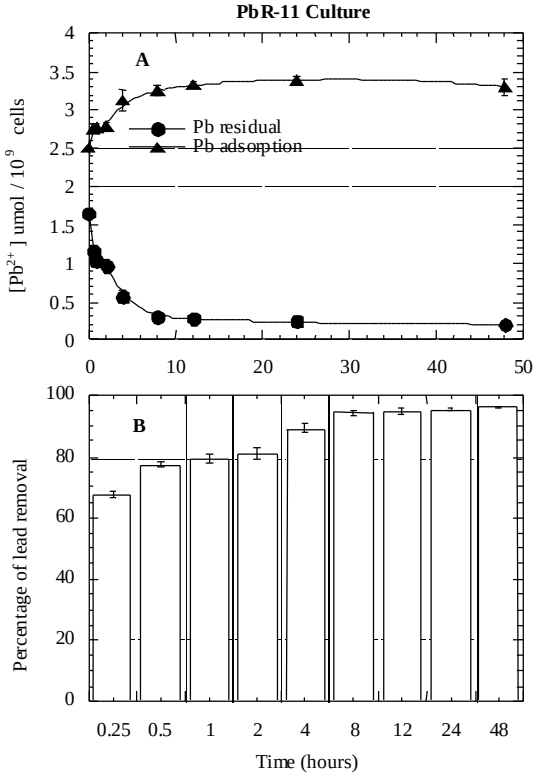

Figure 2. Extracellular adsorption and residual concentration of Pb^{2+} at different time intervals (A) and percentage removal of Pb^{2+} from the medium (B) in PbR-11 culture; mean (standard deviation; n=3)

Pb^{2+} 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 Pb^{2+} in the medium was less than 20% within the first few hours of treatment (Fig. 2A). Until 48 treatment hours, the strain showed 96% removal of the total metal ions from the medium (Fig. 2B). Correspondingly, a very rapid increase in the metal adsorption was found in the strain unlike the WT. The adsorption of Pb^{2+} 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. 2A). The adsorption attained a point of saturation after 8 hours. At 15 minutes, Pb^{2+} removal from the growth medium was 67% contributing 50% to the extracellular adsorption alone. This figure shows difference significantly to that of the WT in terms of Pb^{2+} removal and adsorption at 15 minutes. Similarly, of the 96% Pb^{2+} removal at 48 treatment hours, 66% was found externally adsorbed to the cell walls of the strain, which is higher to that of the WT.

Total lead accumulation and lead in external and internal cell fractions of PbR-11

The PbR-11 demonstrated a higher accumulation of Pb^{2+} per 10^9 cells compared

Table 4. Total Pb^{2+} accumulation and distribution between external and internal cell fractions of the PbR-11 at different time intervals; mean (standard deviation; n = 3)

Strain	Time Int. (hr.)	Lead Association (µmol / 10^9 cells)		
		External	Internal	Total Pb^{2+} accumulated
PbR-11	0.25	2.50 (0.01)	0.85 (0.01)	3.35 (1.10)
	0.5	2.75 (0.01)	1.15 (0.00)	3.90 (0.97)
	1.0	2.80 (0.01)	1.30 (0.01)	4.10 (0.85)
	2.0	2.81 (0.00)	1.31 (0.01)	4.10 (0.85)
	4.0	3.10 (0.00)	1.46 (0.01)	4.56 (1.10)
	8.0	3.30 (0.10)	1.47 (0.00)	4.77 (1.20)
	12.0	3.32 (0.25)	1.46 (0.10)	4.78 (1.29)
	24.0	3.34 (0.00)	1.44 (0.00)	4.78 (1.34)
	48.0	3.30 (0.00)	1.50 (0.10)	4.80 (1.23)

to that of the WT (Tab. 4). Besides, the distribution of Pb^{2+} between the external and internal cell fractions of the strain was also higher to the WT. The strain accumulated almost all of the metal supplemented within the few hours showing a saturation point at early treatment hours. The lead in external and internal cell fractions increased with duration of exposure to the metal solution. But they were found to be saturated with the metal during the first few hours showing that the strain promptly responded to the lead toxicity. This further indicates that the strain demonstrated more resistance to the toxic metal ion by defending in terms of adsorption and absorption mechanisms. Of the total metal accumulated (4.80 µmol /10^9 cells) by the strain at 48 hours, 69% and 31% of Pb^{2+} were externally bound and inside the cells respectively.

Discussion

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 (Wong and Wong, 1990; Macfie and Welbourn, 2000). However, their resistance to the metal toxicity may vary with algal species (Jin *et al.*, 1996b). The present study involving effects on growth rate of the WT and PbR-11 in presence of increasing Ni^{2+} concentrations (Tab. 1), agree well with their findings. Both the algal cells responded with inhibitory effects in order of increasing metal concentration but comparatively, the PbR-11 strain exhibited better growth rate (Tab. 1), which may plausibly be due to EMS mutagenesis. The ID_{50} value of the strain was also nearly double to that of the WT showing that the strain possesses some degree of resistance to the metal toxicity.

A variety of resistance mechanisms are exhibited by microalgae in response to metal toxicity. Possible mechanisms that govern heavy metal resistance are metal binding to the cell wall, reduced transport across the cell membrane, active efflux, compartmentalization and chelation (Prasad, 1995). 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; Honeyman and Santschi, 1988; Wang and Wood, 1984). The present study is in agreement with the above findings. Results reveal that the rate of Pb^{2+} adsorption was very rapid during the first few minutes, i.e., 15 minutes in the present study and the process gradually reached a steady state after few hours of treatment in both the tested cells (Figs. 1 and 2). However, the difference in terms of adsorption patterns occurred between them although their initial cell counts were almost in the same range (Tab. 2). It is important to mention here that the kinetic experiment for extracellular adsorption at zero hour sampling required about 15 minutes to complete the process of treatment. Therefore, zero hour is regarded as 15 minutes or vice versa (Figs. 1 and 2). 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 followed by a slow

intracellular uptake (Wang and Wood, 1984). Furthermore, the difference in the magnitude of metal binding capacity to the external cell fractions between the WT and PbR-11 at different time intervals may be due to different affinities of the algal cells towards the metal ion (Hamdy, 2000).

Active intracellular uptake comes into play once metal ions are bound by the cell wall. The membrane potential, which is negative on the inside of the plasma membrane (Kramer *et al*.,1996), provides a strong driving force for the uptake of metal ions through secondary transporters. In the present study, the intracellular Pb^{2+} uptake in the tested cells was significantly less compared to the extracellular adsorption (Tabs. 3 and 4). It may be because of the reason that when the binding sites of the algal cells became exhausted or nearly saturated, the cells began taking up the metal ion by active physiological mechanisms (Prasad, 1995). Inside the cell, metals are chelated and excess metal is sequestered by transport into the vacuole exhibiting intracellular detoxification mechanisms. (Clemens *et al.*, 2002). Mehta and Gaur (1999) noticed that the greater the toxicity of a metal, the greater is the intracellular concentration of proline in *Chlorella vulgaris*, which is induced to protect the alga from metal toxicity. A common response of organisms to metal toxicity is the synthesis of metallothioneins (Hamer, 1986) and phytochelatins (Kondo *et al.*, 1984), which may play a role in the intracellular detoxification of metal ions. But the present study could not investigate the fate of Pb^{2+} inside the cells. However, the total Pb^{2+} accumulation and distribution of the same between the external and internal cell fractions show that both the processes occur simultaneously in response to the metal toxicity. Indeed, the PbR-11 strain showed significant accumulation and distribution of the metal ions compared to that of the WT indicating its better resistance capacity. It was also noted that Pb^{2+} bound to the external cell surface was significantly high (t-test, $p>0.05$) compared to that of the metal inside the cells in both the tested cells (Tabs. 3 and 4). However, the 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 (Donmez *et al.*, 1999; Bajguz, 2000; Hamdy, 2000).

Conclusion

In conclusion, the PbR-11 strain possesses comparatively higher Pb^{2+} accumulating potential than the WT exhibiting a certain degree of resistance to the metal toxicity. The rapid removal of the metal followed by the simultaneous extracellular adsorption suggests that the strain plays important role in reducing the level of metal concentration from the medium. The findings also open wide prospects for further 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. Besides, the present study is also expected to provide baseline information regarding the status of metal 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. Moreover, a comprehensive understanding of physiological, biochemical and molecular mechanisms conferring Pb^{2+} resistance in *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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