Chapter 4

Metal Uptake Kinetics
Oxygenic, photosynthetic and nitrogen fixing cyanobacteria *Aulosira fertilissima* and *Mastigocladus laminosus* showed contrasting metal uptake phenomena. Our experiments on *ex situ* bioremediation on river water was successful with *Aulosira fertilissima*. Although algal bloom could be induced in *ex situ* situation with *Mastigocladus laminosus*, metal uptake capacity was limited to a very low extent. The various parameters analysed for river water was described in chapter 2 of section C. Data is given below.

pH 5-5.5
Alkalinity 90-155 mg/l
Acidity 0.6 mg/l
Nitrogen 0.8 –3.5 mg/l
Phosphate 0.2-0.03 mg/l
Dissolved oxygen 6-9.4 mg/l
Dissolved carbon dioxide 0-4 mg/l
Temperature 22-34 degree centigrade

In *ex situ* situation river water was converted into a simulated effluent by adding heavy metal potassium dichromate to get a final concentration of 50ppm. To make this simulated effluent amenable to cyanobacterial growth the following constituents were used to augment it.
media gm/L

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO₄</td>
<td>0.2</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.2</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.1</td>
</tr>
<tr>
<td>EDTA Fe Complex</td>
<td>1ml</td>
</tr>
<tr>
<td>Micro Nutrients</td>
<td>1ml</td>
</tr>
<tr>
<td>A5 solution (for micronutrients)</td>
<td></td>
</tr>
<tr>
<td>Boric acid</td>
<td>2.86</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>1.81</td>
</tr>
<tr>
<td>ZnSO₄ 7H₂O</td>
<td>0.222</td>
</tr>
<tr>
<td>NaMoO₄</td>
<td>0.0177</td>
</tr>
<tr>
<td>CuSO₄ 5H₂O</td>
<td>0.079</td>
</tr>
</tbody>
</table>

The criteria used in the formulation was to get chemical constitution similar to Fogg’s media at pH 7. The effluent was not autoclaved so as to simulate the natural environment. But light at a distance of 50cms from a source of 40W fluorescent tube was used to suit the laboratory conditions. The effluent was aerated with filtered air from the bottom of the bench scale fermenter and cyanobacterial inoculum of one gram fresh weight was added for bloom induction.

Cyanobacterial bloom could be induced in 10 liter tank fermenter with simulated effluent serving as support for growth for bacteria. It took 45 days to induce full bloom of cyanobacteria. Coincident with the multiplication of cyanobacteria the metal concentration in effluent decreased gradually and at the end of the 45th day a residual concentration of 1 ppm of chromium was left in the
simulated effluent with an initial concentration of 50ppm chromium, on seeding the fermenter with *Aulosira fertilissima*.

The effect of time of contact for four different concentrations of chromium using cyanobacteria equivalent to 200mg dry weight of adsorbent is given in figure 4.1 and 4.2. The amount of chromium in solution decreased with time until equilibrium was established between amount of chromium adsorbed on the adsorbent and remaining in the solution. The equilibration time remained constant for a given concentration of the metal implying only very limited increase of growth of cyanobacteria during experimentation on 5 day duration.

**Fig. 4.1:** Contact time and chromium uptake in *Aulosira fertilissima*
Data on the fraction of metal adsorbed on biomass with respect to equilibration time for chromium followed a hyperbola in *AulOSira fertilissima* explaining a biphasic metal adsorption phenomena Fig. 4.3 and 4.4. Biphasic metal adsorption phenomena can be explained in terms of active and passive transport mechanisms. It begins by the diffusion of metal into the surface of the microbial cell. Diffused metal on the surface of the microbial cell show affinity to it and bind on the available sites on the cell surface. This process contain a number of passive accumulation process and may include adsorption, ion exchange, co-ordination, complexation, chelation and microprecipitation. Generally this type of metal adsorption is fast, reversible and not a limiting factor in metal uptake phenomenon in with dispersed cells. This fast uptake of metal ion is followed by a slower metal binding process in which additional metal ion is bound (Huang *et al* 1990, Xue and Sigg 1990). This slower
process can be due to diffusion in to the cell interior, crystallisation on the cell surface, covalent binding, surface precipitation and binding to protein and other intracellular sites. Statistically small increment in the amount of metal ion adsorbed on unit biomass could be observed even after equilibration at saturation point. It never attained a maximum limit at LC$_{50}$ concentration and below it with the advancement of time. From this observation it can be stated that it is possible to remove total metal ions in the concentration range selected at LC$_{50}$ concentration and below it for experimentation on biosorption. Further our pilot scale experimentation substantiate this fact.

**Fig. 4.3: Chromium adsorption in *Mastigocladus laminosus***
Fig. 4.4: Chromium adsorption in *Aulosira fertilissima*

![Graph showing chromium adsorption](image)

The kinetic pattern followed in pilot scale was studied in detail in *Aulosira fertilissima* and *Mastigocladius laminosus* by taking different concentration of metals in the laboratory scale experimentation. All studies of adsorption have been evaluated in terms of either Langmuir or Freundlich isotherms. The equilibrium data analysed in the light of Langmuir adsorption isotherm was of the form (Eq 1).

\[
q_{eq} = \frac{Q^0 \times b \times C_{eq}}{1 + b \times C_{eq}} \tag{Eq 1}
\]

$Q^0$ is the maximum amount of metal ion per unit weight of alga to form a complete monolayer on the surface bound at high
equilibrium concentration, $C_{eq}$ (mg metal / g of dry biomass) of cyanobacteria and b is a constant related to the affinity of the binding sites. $Q^0$ indicates a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance.

In addition to Langmuir isotherm data was evaluated further with Freundlich isotherm. Freundlich equation based on adsorption is of the form

$$q_{eq} = K_F C_{eq}^{1/n} \quad \text{(Eq 2)}$$

where $K_F$ and $n$ are the Freundlich constants characteristic of the system. $K_F$ and $n$ indicates adsorption capacity and adsorption intensity respectively. This equation can be linearised by taking natural logarithm.

Log-log plot between concentration of different metals in solution and fraction of metals in the biomass of *Aulosira fertilissima* and *Mastigocladus laminosus* followed a straight line Fig (4.5-4.7). These organisms were selected for metal biosorption because they are nitrogen fixing and no report was available on the adsorption abilities of these organisms. The capacity of *Aulosira fertilissima* and *Mastigocladus laminosus* for metal adsorption was a function of metal concentration and type of the metal.
Fig. 4.5: Freundlich isotherm for biosorption of chromium in *Mastigocladus laminosus*

![Graph showing Freundlich isotherm for biosorption of chromium in *Mastigocladus laminosus*.](image1)

Fig. 4.6: Freundlich isotherm for biosorption of copper in *Mastigocladus laminosus*

![Graph showing Freundlich isotherm for biosorption of copper in *Mastigocladus laminosus*.](image2)
Langmuir isotherm studied for the uptake of metals in both cyanobacteria followed a straight line. The adsorption of metals on *Mastigocladus* took longer duration of time of 5 days to accumulate the low fraction of metals from the applied concentration ranges. *Aulosira fertilissima* proved to be a fast chelator of metal ions by giving the kinetic pattern given in Figure (4.8-4.10) in a short duration of time of one hour. Phosphate and calcium affected metal uptake phenomenon adversely in *Aulosira fertilissima*. 
Fig. 4.8: Langmuir isotherm for biosorption of chromium in *Aulosira fertilissima*

Fig. 4.9: Langmuir isotherm for biosorption of Copper in *Mastigocladus laminosus*
Fig. 4.10: Langmuir isotherm for biosorption of chromium in *Mastigocladus laminosus*

In laboratory scale experimentation comparatively high concentration of biomass was used than in pilot scale experimentation where 1 g of biomass for a 10 litre fermenter was used. Growth of cyanobacteria in pilot scale could be easily be divided into distinct phases for the whole duration of experimentation ie 45 days. But laboratory scale experimentation on sorption spanning 5 days and again due to the high biomass in simulated effluent growth could not be easily distinguishable even with several fold dilution of biomass for chlorophyll estimation.

However statistically insignificant increase in the amount of biomass could take place during the 5 day experimentation which account for the non attainement of saturation limit of metal binding at
LC$_{50}$ and concentration below it. The uptake of chromium and copper increased with increasingly applied concentration of metal, the highest uptake of 17.6 mg metal chromium 18.8 mg of copper per gram of dry biomass in the case of *Aulosira fertilissima* at the highest concentration of 200 ppm of respective metal. Where as in *Mastigocladus laminosus* a lower uptake was noticed with 0.6 mg/gram dry weight of biomass for metal chromium and 0.8mg/gram dry weight of biomass of metal for copper in the highest concentration of metal taken for biosorption studies. The pH selected for this observation was 5. A similar biphasic metal uptake pattern as in *Aulosira fertilissima* was reported in yeast. (Norris *et al* 1977) and in unicellular algae (Khummongkol *et al* 1982)

Metabolic energy dependent uptake of copper was proposed as mechanism of uptake of metal in a study conducted on *Nostoc calcicola* (Verma and Singh 1990). It is also noted from our studies that metabolic residual energy derived from glycogen in cyanobacteria could drive slow uptake of metal ions in the cyanobacteria *Aulosira fertilissima*. This conclusion was arrived at by keeping the culture taken for biosorption studies in the dark. However exposure to longer duration of time resulted in depigmentation and autolysis of the cells of *Aulosira fertilissima*. Decrease in uptake capacity was noticed due to dark incubation. *Mastigocladus laminosus* on dark treatment and incubation under anaerobic condition withstood lysis. Metal uptake pattern was not much affected in dark treatment. It was equal to a low amount of 22.5 mg/gram dry weight of biomass in thermophilic *Mastigocladus*. The adsorption of metal ions were maximum at pH 5. pH influenced the surface metal binding sites.
We have also unravelled the biotechnological potential of *Mastigocladus laminosus* as a biofertiliser in heavy metal contaminated sites by studying its nitrogen fixation ability under heavy metal stressed condition. The results of that study are discussed in the nitrogen fixation studies on chapter 4 of section D.

The inability of the thermophilic organism *Mastigocladus laminosus* to uptake heavy metals at a low rate could be explained in terms of characteristic cell wall composition with chemical moieties that could not chelate metal ions predominating cell wall and capsular polysaccharide secreted by the organism. Capsular polysaccharide produced by *Mastigocladus* was characterized by uronic acid as well as the presence of pentoses and these features are rarely found in other prokaryotic groups (Gloguen *et al* 1999).

For further detailed kinetic characterization a graph was plotted by taking reciprocal of the fraction of the metal bound to biomass per unit time along the ordinate against reciprocal of the adsorbed concentration of metals on abcissa. A graph of the pattern as shown in figure 4.11-4.13. The pattern obtained was typical of Michaelis Menton type kinetics and so Lineweaver Burk plot was applicable. From the Lineweaver Burk plot the maximal velocity (V$_{max}$) and metal concentration at which half maximal velocity (K$_m$) were calculated for different metals with the biomasses of *Aulosira fertilissima* and *Mastigocladus laminosus*. Details are given in table of Km and V$_{max}$ (Table 4.1).
Table 4.1: Km and V max of different metals in cyanobacteria

<table>
<thead>
<tr>
<th>Metal Type</th>
<th>Km</th>
<th>V max</th>
</tr>
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<tbody>
<tr>
<td>M. laminosus (Cu)</td>
<td>80 ppm</td>
<td>2 mg/L</td>
</tr>
<tr>
<td>M. laminosus (Cr)</td>
<td>100 ppm</td>
<td>0.121 mg/L</td>
</tr>
<tr>
<td>A. fertilissima (Cr)</td>
<td>200 ppm</td>
<td>3.3 mg/L</td>
</tr>
</tbody>
</table>

Fig. 4.11: Kinetics of chromium uptake in Aulosira fertilissima
Fig. 4.12: Kinetics of copper uptake in *Mastigocladus laminosus*

Fig. 4.13: Kinetics of chromium uptake in *Mastigocladus laminosus*
High affinity was noted for chromium despite high Km value in *Aulosira fertilissima*. A low uptake of heavy metal was shown by comparatively low Km value observed with *Mastigocladus laminosus*.

Kinetic studies also revealed that heavy metal accumulation in cyanobacteria *Aulosira fertilissima* recorded was consisting of two phases, a rapid phase of metabolism independent binding to the cell wall followed by a slower phase due to simultaneous effect of growth, surface adsorption, active uptake etc. In all cases initial adsorption was independent of light. The initial adsorption phase accounted for 83.33% uptake of chromium and 94% uptake of copper at an initial concentration of 100ppm leaving just 16.67% residual chromium 6% residual copper in the case of *Aulosira fertilissima* at pH 5 where as thermophilic *Mastigocladus* showed 95.85% residual concentration of Chromium giving an uptake percentage of 4.15%. With copper as adsorbate *Mastigocladus laminosus* proved to be a poor performer with 92%of copper leaving behind as residual fraction.

Copper is apparently essential for normal growth of *Aulosira fertilissima* and *Mastigoocladus laminosus* as copper is a micronutrient incorporated into the culture media of the organism where as chromium was nonessential. The higher metal uptake of copper compared to chromium was mainly because of copper ion channel in the cyanobacterium and the possible absence of specific chromate ion transporters on the membrane.

Once inside the cell, the metal ion can bind to any oppositely charged entities in the cell leading to the initiation of toxic effects either directly or through free radicals. But the operation of development of
tolerance suggests metal sequestering mechanism inside the cell. Thioredoxin mediated defence is described in chapter 4 of section C. However an additional mechanism based on polyphosphate bodies is possible. A mechanism based on polyphosphate bodies in response to heavy metal toxicity was described in Plectonema boryanum (Torres et al 1998).

*Mastigoclados laminosus* is resistant to anerobiosis and dark treatment for a considerable period of time five days. But the organism when subjected to pilot scale experimentation with 50ppm of chromium in the fermenter culture media it could induce bloom with no appreciable amount of chromium uptake. However when the organism reached the stationary phase of growth, under the said conditions, the cells began to autolysse indicating poor tolerance development among stationary phase cells. The results point to the fact that young cells of *Mastigoclados* could tolerate heavy metal toxicity concentration below LC₅₀ dose successfully. Our experimentation of *Mastigoclados* in control pilot scale experimentation in fermenter, the organism remained viable by giving the same Chlorophyll a concentration of 50μg/ml for a period of more than one year under light regime as described earlier. The inability of the resistant cells to colonise in the fermenter, in which 50ppm of chromium was added, after the stationary phase of growth is due to the limiting concentration of biomass of the resistant cells. In laboratory scale experimentation resistant cells could be observed after 30 days of treatment and colonized on subsequent cycle of growth. Various approaches are available to improve the biosorption capacity of alga with various treatment procedures.
Metal adsorbed on algal biosorbate occurring in natural condition can be desorbed after harvesting the algal biomass by water with a slight shift in pH towards the acidic range depending on the type of metal ion and the algal biomass.