4. Bioremediation of Chromium by Cyanobacteria

4.1 INTRODUCTION:

4.1.1 Chromium - toxic heavy metal:

Chromium can be acutely toxic to both plants and animals. Chromium has been considered as one of the top 16th toxic pollutants and because of its carcinogenic and teratogenic characteristics on the public, it has become a serious health concern (Torresdey et al., 2000). A sudden boost in industrial activities has contributed quantitatively to the alarming increase in the discharge of metal pollutants into environmental sink, especially the aqueous environment. Chromium sulfate is the most important ingredient used in the tanning of leather. The tanning industry which commonly utilizes ‘Chrome liquor’ in the tanning process discharges the effluents into the environment containing chrome salts in excess of the maximum permissible limit (Khasim and Hussain, 1989). Hexavalent chromium (CrVI) in the environment is almost totally derived from anthropogenic activities. The high concentrations of chromium are toxic and carcinogenic. About 80% of tanneries are engaged in the usage of chromium in hiding and skinning of tanning process. Hence there is a dire need to combat this issue of increasing chromium toxicity.

4.1.2 Dispersion of toxic chromium:

Dispersion of the metal ions in the water bodies leads to their biomagnification through the food chain and results in increased toxicity. This fact renders the removal of heavy metals from aqueous solutions indispensable. Metals discharged into water bodies are not biodegraded but undergoes chemical or microbial transformations, creating large impact on the environment and public health (Volesky, 1995). Metals and their free radicals are highly reactive attacking other cellular structures. Chromium can be acutely toxic to both plants and animals. The ability of metals to disrupt the function of essential biological molecules, such as protein, enzyme and DNA is the major cause of their toxicity. The alteration in protein can also lead to toxic consequences.

4.1.3 Removal of toxic chromium:

Even though plethora of conventional methods such as chemical precipitation, filtration, ion exchange, electrochemical treatment, and reverse osmosis are known to remove chromium ions from aqueous solutions, they often remain expensive and ineffective when
metal ions in the aqueous solution are in the range of 1–100 mg/L (Volesky, 1995). Therefore, to develop the effective remediation through biosorption and bioaccumulation must need for environment. Hence in the recent years biosorption and/or bioaccumulation have emerged as cost effective and efficient alternative method for the removal of heavy metal contamination. Biosorption is a process in which biomass are employed for binding heavy metals. Micro-organisms including cyanobacteria, bacteria, yeasts, fungi and plants can be used as biosorbents for metal removal (Wang and Chen, 2009).

4.1.4 Cyanobacteria in biosorption:

Cyanobacteria are photosynthetic prokaryotes which are present ubiquitously. In the recent past, a significant consideration is addressed towards cyanobacteria for their potent use in pollution abatement (Thajuddin and Subramanian 2005), degradation of aromatic hydrocarbons (Kumar et al. 2009), pesticide degradation (Subramanian et al., 1994; Mansy and El-Bestawy, 2002) and heavy metal biosorption (Katricioglu et al., 2008; Rajeshwari et al., 2011). Cyanobacteria are one of the biomaterials that have high potential for removing heavy metals from wastewater (Inthorn et al., 2002). The present study attempts to assess the bioaccumulation potential of the fresh water cyanobacterium Chroococcus minutus NTMS09, Oscillatoria accuminata NTMS02, Dolichospermum flos-aquae NTMS07, Calothrix fusca NTMS08, Scytonema hofmanni NTMS05, were selected to test their efficiency in removing metals at lower concentrations. In the present study, fresh water cyanobacteria are used for biosorption studies. It is already known that cyanobacteria isolated from metal-contaminated sites exhibit higher efficiency in removing metals but, in this study, freshwater cyanobacteria from agriculture field were selected to test their efficiency in removing metals at lower concentrations.
4.2 REVIEW OF LITERATURE:

Modak et al., (1996) described microorganisms are generally the first to be affected by the discharges of heavy metals into the environment. Microbial ecosystem can drastically alter the fate of the metal entering into aquatic or soil environments. Bacteria, cyanobacteria and fungi alter the form of occurrence of metal through methylation, chelation, complexation, catalysis or adsorption affecting their bioavailability and movement in the food chain. Many types of yeast, fungi, algae, bacteria and some aquatic plants have been reported to have the capacity to concentrate metals from dilute aqueous solutions and to accumulate them inside the cell structure (Kapoor and Viraghavan, 1995; Volesky and Holan, 1995). There are numerous industrial processes and related activities that result in the release of metals can be controlled before it enters in common waste streams, enormous saving in disposal costs of resulting sludge are possible. In fact, the detoxification of sludge can convert them from economies liability to a sellable resource.

Bai et al., (2001) carried out preliminary studies on biosorption reveals it to be a complex interplay of the properties of the biomolecules of the cell wall and the chemical nature of the metal ion in question. Each of the microbial group is characterized by a distinct by a distinct cell wall structure and presence of different polymers/monomers like chitin, amino acid and carboxylic acids groups provide several functional groups as binding sites for heavy metal ions due to ion exchange phenomenon. The amino and carboxyl groups, nitrogen and oxygen of the peptide bond could be available for characteristic coordination bonding with metal ions (Nourbaksh et al., 1994). Such bond formation could be accompanied by displacement of protons, dependent in part upon the extent of protonation as determined by pH.

4.2.1 Conventional treatment methods:

- Precipitation
- Ion–exchange method
- Complexation
- Electrochemical cells
- Reverse osmosis
- Biological methods

Micro precipitation is the deposition of electrically neutral material (metal or metal salt) at the surface of the biomass, and does not necessarily involve a bond between the biomass and the deposited layer. It can however be facilitated by initial binding of metal ions.
to relative sites of the biomass, which serve as nucleation sites for further precipitation. Micro precipitation is based on interactions between the solute (dissolved solid) and the solvent, and occurs when the local solubility exceeds. Ion exchange and adsorption can be the result of three different interactions, which act in combination: The main contribution for free metal ions (which are highly soluble in water) is usually the attraction of the sorbate (metal ions) to the sorbent (biomass). Additionally hydrophobic expulsion may also play a role. Complexation plays an important role in both metal-ligand and sorbate-sorbent interactions. Complexes can be neutral, positively charged or negatively charged. The number of coordinating atoms in the ligands that are directly attached to the central atom is the coordination number. The bond between the central atom and the coordination groups can be arising from principal or auxiliary valence forces. Electrodialysis is a process where the ionic components are separated through the use of a semi-permeable ion selective membrane. Application of an electrical potential between the two electrodes causes a migration of cations and anions towards respective electrodes. Because of the alternate spacing of cation and anion permeable membranes, cells of concentrated and diluted salts are formed. The disadvantage is the formation of metal hydroxides, which clog the membrane. Reverse osmosis is a process where heavy metals are separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by dissolved solids in wastewater. It is very expensive.

**Disadvantages of such physicochemical processes:**

- They are expensive
- Lack the required specificity required treating target metal against a background of competing ions.
- Unpredictable metal removal
- High reagent requirement & generation of toxic sludge, which are often difficult to dewater and require extreme caution in their disposal.
- Such approaches are not applicable to cost effective remediation of large-scale subsurface contamination in situ.

The cell wall polymers provide a multitude of chemical groups such as hydroxyl, carbonyl, carboxyl, sulphhydryl, thioether, sulphonate, amine, imine, amide, imidazole, phosphonate and phosphodiester. These chemical groups of the biopolymers in turn harbour binding sites, which provide the ligand atoms to form complexes with metal ions. In general
metal binding can be distinguished between ion exchange, sorption of electrically neutral material to specific sites and microprecipitation. These mechanisms are based on sorbate-solvent interactions, which in turn rely on some combination of covalent, electrostatic, and Vander Walls’s forces. Importance of the given group for biosorption of certain metal by certain biomass depends on various factors such as:

1) Quantity of sites in the biosorbent
2) Chemical state of the site
3) Accessibility of the site
4) Affinity between the site and the metal

Strong biosorbent behavior of certain types of microbial cells towards metallic ions is a function of the chemical makeup of microbial cells of which it consists. This aspect is particularly important when it comes to the process application, whereby new biosorbents respective ‘chemicals’ are capable of sequestering a relatively large amount of the metal. Some types of biosorbents could have broad range binding of the majority of heavy metals with no specific prosity, while others can even be specific for certain types of metals (Volesky, 1988). Bioremediation is to remove, sequester or solubilize the metals that are able to degrade compounds. This suggests that under the selective pressure of environmental pollution, a microbial capacity for the degradation of recalcitrant compounds exists that may be harnessed for pollutant removal by biotechnological process. Bioremediation using microorganisms is less intrusive, less expensive and accumulates toxic for their removal and sequesters them for large-scale removal.

4.2.2 Microbial Remediation:

Microbial research and need for new methods of water cleanup has led to great deal of expansion in the field of biological methods of industrial effluent cleanup. Microbes require heavy metals as humans’ require certain metals in their diet. The pathways by which microbes accumulate heavy metals are:

a) Binding to cell surface

b) Intracellular accumulation

c) Extra-cellular precipitation

d) Volatilization
Living biological systems are well suited for the treatment of water. The term ‘biosorption’ is used to describe the accumulation of metal ions from solutions by material of biological origin (microbe or plant). Biosorption is a process that uses inexpensive dead biomass to sequester toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents. It is a cost effective and highly specific process. This ensures reusability of biomass with short operation time especially if dead cells are used.

Bioremediation can be due to:

- **Biosorption** (by dead/live biomass)
- **Bioaccumulation** (by live biomass)
- **Enzymatic recovery** (biotransformation)

### 4.2.3 Biosorption by non-living cells:

The use of dead cells offers the following advantages over living cells:

- Non-living cells are less sensitive to metal ion concentration (toxicity effects).
- Can be operated at ambient conditions of pH and temperature.
- Low operating cost.
- Volume of chemical or biological sludge can be minimized.
- Supply of nutrients not required.
- Dead biomass can also procured from industrial sources as a waste product from the fermentation process.
- Biosorbed metal can be easily desorbed and biomass can be reused.
- Much simpler process control and biomass can be stored for long periods of time.

### 4.2.4 Mechanism of biosorption:

Prakasham et al., (1999) explained the biosorption by the microbes is attributed mainly to the ligands present in the biomolecules of their wall polymers. Biosorption includes a combination of several mechanisms such as electrostatic attraction, complexation, ion-exchange, covalent binding, Van der Waal’s forces, adsorption and microprecipitation. The kinetics of metal uptake has been suggested to take place in two stages (Gadd and White, 1993).
Biosorbents are prepared from the naturally abundant and/or waste biomass of algae, fungi or bacteria. Varieties of uptake mechanism are involved including adsorption and ion exchange. Commercial biosorbents need to fulfill a number of criteria such as:

- High biosorption capacity at equilibrium i.e. they should contain as little as possible of inert material in their binding sites.
- Favorable adsorption kinetics i.e. particles should be hydrophilic and porous in nature.
- Maintenance of smooth flow dynamics in a reactor-this prevents the use of either very small or strongly swelling particles in the column.
- Amenable to regeneration- this necessitates desorption by minimal possible volume of desorbing agent without damaging the biosorbent.
- Good mechanical strength.
- Temperature stability
- Resistance to chemicals.
- Availability of biosorbent

The need for economical, effective and safe methods for removing heavy metals from the wastewater has resulted in the search for unconventional materials that may be useful in reducing the levels or accumulation of heavy metals in the environment. The newly discovered metal sequestering properties of certain types of microbial biomass of fungi, bacteria and algae offers considerable promise (Volesky and Kuyucak, 1988) and offer an alternative to the existing methods for metal detoxification and their recovery. The present investigation envisages use of dead or non-living biomass available in large quantities for removal of heavy metals from aqueous solution.
4.2.5 Heavy metal:

Nies (1999) defined heavy metals are with a specific weight usually more than 5.0 g/cm$^3$, which is five times higher than water. The toxicity of heavy metals occurs even in low concentrations of about 1.0-10 mg/L. Of the 90 naturally occurring elements, 21 are non-metals, 16 are light-metals and the remaining 53 (with as included) are heavy metals. Most heavy metals are transition elements with incompletely filled d orbital’s. These d orbital’s provide heavy-metal cations with the ability to form complex compounds which may or may not be redox-active. Thus, heavy-metal cations play an important role as trace elements in sophisticated biochemical reactions.

Florence (1986) described that trace element is considered essential if it meets the following criteria: it is present in all healthy tissues of living things; its concentration from one animal to the next animal is fairly constant; its withdrawal from the body induces, reproducibly the same physiological and structural abnormalities regardless of the species studied; its addition either reverses or prevents these abnormalities; the abnormalities induced by deficiency are always accompanied by pertinent, significant biochemical changes and these biochemical changes can be prevented or cured when the deficiency is corrected. A total of 30 elements are now believed to be essential to life. They can be divided into the 6 structural elements, 5 macro minerals and 19 trace elements.

Young (1996) experimented on the metals whether essential or inessential can exhibit toxicity above certain threshold concentrations which for highly toxic metal species may be extremely low. The toxicity caused by heavy-metals is generally a result of strong coordinating abilities (Gadd, 1992). Certain metals have been known to be toxic for centuries. For example, Theophrastus of Erebus (370-287 B.C.) and Pliny the Elder (23-79) both described poisonings that resulted from Arsenic and Mercury. Other heavy-metals, such as cadmium were not recognized as poisonous until the early nineteenth century. Based on the physiological effect and toxicity, heavy metals are classified as follows

<table>
<thead>
<tr>
<th>Table 4: Classification of heavy metals based on toxicity (Thakur, 2006).</th>
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<tbody>
<tr>
<td>Fe, Mo, Mn</td>
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<tr>
<td>Zn, Ni, Cu, V, Co, W, Cr</td>
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<tr>
<td>As, Ag, Sb, Cd, Hg, Pb, U</td>
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4.2.6 Toxicity of Chromium (VI):

Congeevaram (2007) explained that hexavalent Chromium is known to cause various health effects, respiratory problems, weakened immune systems, kindney and liver damage, alteration of genetic material, lung cancer and death (Ramachandra TV). According to the Indian standards, the permissible limit of Cr (VI) is 0.05 and 0.1 mg/L for potable and industrial discharge water respectively.

4.2.7 Biosorption and Bioaccumulation:

Malik (2004) defined bioaccumulation is the phenomenon of living cells; whereas, biosorption mechanisms are based on the use of dead biomass. To be precise, bioaccumulation can be defined as the uptake of toxicants by living cells. The toxicant can transport into the cell, accumulate intracellularly, across the cell membrane and through the cell metabolic cycle. Conversely, biosorption can be defined as the passive uptake of toxicants by dead/inactive biological materials or by materials. Metal-sequestering properties of non-viable biomass provide a basis for a new approach to remove heavy metals when they occur at low concentrations (Volesky, 1990). Biosorption possesses certain inherent advantages over bioaccumulation processes, which are shown in the below.

Table 5: Comparison of the features of Biosorption and Bioaccumulation.

<table>
<thead>
<tr>
<th>Features</th>
<th>Biosorption</th>
<th>Bioaccumulation</th>
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<tbody>
<tr>
<td>Cost</td>
<td>Usually low. Most biosorbents used were industrial, agricultural and other type of waste biomass. Cost involves mainly transportation and other simple processing charges.</td>
<td>Usually high. The process involves living cells and; hence, cell maintenance is cost prone.</td>
</tr>
<tr>
<td>pH</td>
<td>The solution pH strongly influences the uptake capacity of biomass. However, the process can be operated under a wide range of pH conditions.</td>
<td>In addition to uptake, the living cells themselves are strongly affected under extreme pH conditions.</td>
</tr>
<tr>
<td>Temperature</td>
<td>Since the biomass is inactive, temperature does not influence the process. In fact, several investigators reported uptake enhancement with temperature rise.</td>
<td>Temperature severely affects the process.</td>
</tr>
<tr>
<td>Maintenance/storage</td>
<td>Easy to store and use</td>
<td>External metabolic energy is needed for maintenance of the culture</td>
</tr>
<tr>
<td>Selectivity</td>
<td>Poor. However, selectivity can be improved by modification/processing of Biomass</td>
<td>Better than biosorption</td>
</tr>
<tr>
<td>Versatility</td>
<td>Reasonably good. The binding sites can accommodate a variety of ions</td>
<td>Not very flexible. Prone to be affected by high metal/salt</td>
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</table>
4.2.8 Cyanobacterial biosorption:

Veglio and Beolchini (1997) tested on the capability of some microorganisms to accumulate metallic elements. Numerous research reports have been published from toxicological points of view, but these were concerned with the accumulation due to the active metabolism of living cells, the effects of metal on the metabolic activities of the microbial cell and the consequences of accumulation on the food chain (Volesky, 1988). However, further research has revealed that inactive/dead microbial biomass can passively bind metal ions via various physicochemical mechanisms. With this new finding, research on biosorption became active, with numerous biosorbents of different origins being proposed for the removal of metals. Researchers have understood and explained that biosorption depends not only on the type or chemical composition of the biomass, but also on the external physicochemical factors and solution chemistry. Many investigators have been able to explain the mechanisms responsible for biosorption, which may be one or combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and micro precipitation.

The uptake of heavy metals by microorganisms generally comprises two phases: binding of fractions to the negatively-charged groups on the cell surface (passive) and the subsequent metabolism-dependent, intracellular uptake (active). The passive process is very rapid and occurs in a short time after the microorganisms come into contact with the metal;
Gadd (1990) experimented the uptake rate of a heavy metal is dependent on its speciation which is a key factor in determining its toxicity. Environmental variables such as pH, redox potential, salinity, alkalinity, temperature, available nutrients, metal concentration, cell density, extracellular metabolites and organic acids can affect metal toxicity (Gad and Griffiths, 1978; Rai et al., 1981).

Fogg and Westlake (1955) stated some information is available regarding the ability of some cyanobacteria to produce extracellular secretions to protect themselves from toxic metals. The ability of extracellular organic material to reduce the toxicity of heavy metals showing that cultures of Anabaena cylindrical produced polypeptides which complexed cupric, zinc and ferric ions. Living or non-living microbial cells can reversibly bind significant quantities of metal ions from aqueous solutions, and various functional groups such as carboxyl, amino, phosphoryl, sulfhydryl and hydroxyl, which are found on cell wall components, and proteins and lipids are implicated (Siegel and Siegel, 1973, Christ et al., 1981, Greene et al., 1986, Singh et al., 1989, Greene and Darnall, 1990).

Olson and Brinckman (1987) described the use of non-living microbial cells for biosorption has been suggested to be advantageous for selective removal and recovery of metal contaminants from water since dead organisms are not affected by conditions that would normally be detrimental to living organisms. However, it is noted that non-living algal cells do not possess the metabolic activities of living systems, e.g. the ability to volatalize, precipitate and accumulate metals intracellularly. Studies have shown that cyanobacteria can survive and reproduce in metal-contaminated habitats. The genera Oscillatoria, Phormidium, Plectonema and Schizothrix were dominant in zinc-enriched water (Say and Whitton, 1980; Whitton, 1980), and isolates from such sites are often resistant to zinc.

Whitton et al. (1982) reported a range of Cyanobacteria, i.e. Anabaena, Nostoc, Oscillatoria, Phormidium and Scytonema, has been recovered from copper-rich soils and Plectonema is a frequent inhabitant near mine tailings containing high levels of zinc, cobalt, nickel and lead. The capacity to biosorb Cu(II), Fe(II), Ni(II) and Zn(II) by non-viable biomass of the cyanobacterium Phormidium laminosum entrapped in polysulfone and epoxy resin beads was investigated. The biosorption process depended on the wetting of biomass beads, the rate of metal biosorption decreasing when dry biomass beads were used. The
amount of metal biosorbed increased with the biomass and the amount of metal available. (Blanco et al., 1999).

Awasthi and Rai (2004) reported on the alginate immobilized Cyanobacteria (Anacystis nidulans) and two green algae (Chlorella vulgaris, Scenedesmus quadricauda) were compared for their ability to accumulate nickel, zinc and cadmium on their cell surfaces. Cadmium and nickel were more efficiently adsorbed by Anacystis nidulans while Chlorella vulgaris showed better adsorption of zinc. Combined toxicity of nickel, zinc and cadmium showed different response in all the organisms studied.

The process of biosorption of heavy metal ions Cr$^{3+}$, Cd$^{2+}$, Cu$^{2+}$ by blue-green algae Spirulina sp. was studied. Spirulina sp. was found to be a very efficient biosorbent (Chojnacka et al., 2005).

Anjana et al., (2007) studied the biosorption of Cr (VI) using native strains of cyanobacteria from metal contaminated soil in the textile mill has been reported. The biomass of Chroococcus sp. HH-11 was found to be more suitable for the development of an efficient biosorbent for the removal of Cr(VI) from wastewater, as it showed higher values of $q_m$ and $K_f$, the Langmuir and Freundlich isotherm parameters.

Raungsomboon et al., (2008) reported on Pb$^{2+}$ removal ability of the viable-freshwaer cyanobacterium Gloeocapsa sp. was studied in batch experiments. These results showed that Pb$^{2+}$ concentration in the range of 0-20mgL$^{-1}$ was not inhibitory to Gloeocapsa sp. growth but reduced its Pb$^{2+}$ removal efficiency by 4.5% when Pb$^{2+}$ concentration increased from 2.5 to 20 mg L$^{-1}$. Pb$^{2+}$ removal characteristics followed the Langmuir adsorption isotherm with the maximum removal capacity (qmax) of 232.56 mgg$^{-1}$.

Arunakumara et al., (2008) described the growth of S. platensis was adversely affected by Pb$^{2+}$ at high concentrations (30, 50 and 100 µg/ml). However, at low concentrations (5µgL$^{-1}$), Pb$^{2+}$ could stimulate its growth slightly. The pigment contents were decreased in a dose-dependent manner. The highest reductions were observed in 100 µgL$^{-1}$ treatment group. Effects of heavy metals (Pb$^{2+}$ and Cd$^{2+}$) on the ultrastructure, growth and pigment contents of the unicellular cyanobacterium Synechocystis sp. PCC 6803 was studied. Alterations in the ultrasturcture of the Synechocystis sp. PCC 6803 cells became evident with the increased (>4 mg/L Pb$^{2+}$) metal concentration. The photosynthetic apparatus (thylakoid membranes) were found to be the worst affected. Deterioted or completely destroyed thylakoid membranes have made large empty spaces in the cell interior. In addition, at the highest concentration
(8mg/L), the polyphosphate granules became more prominent both in size and number. Despite the initial slight stimulations, both metals inhibited the growth in a dose-dependent manner as incubation progressed. Pigment contents were also decreased with increasing metal concentration.

Katriciaglu et al. 2008 isolated Oscillatoria sp. H1 from Mogan Lake was used for the removal of cadmium ions from aqueous solutions as its dry biomass, alive and heat-inactivated immobilized form on Ca-alginate. Particularly, the effect of physicochemical parameters like pH, initial concentration and contact time were investigated. The sorption of Cd(II) ions on the sorbent used was examined for the cadmium concentrations within the range of 25-250 mgL⁻¹. Maximum biosorption capacities for plain alginate beads, dry biomass, immobilized live Oscillatoria sp. H1 and immobilized heat-inactivated Oscillatoria sp. H1 were 21.2, 30.1, 32.2, and 27.5 mgg⁻¹ respectively.

4.2.9 Requirement of metals:

Blencowe & Morby (2003) experimented that cyanobacteria depend upon a variety of metal cations to maintain the cellular metabolism. During photosynthesis Mn is required to generate the holo-form of the water-splitting complex (Kehres & Maguire, 2003) and is a co-factor of several enzymes. Two important proteins in the thylakoid (plastocyanin and a c-type cytochrome oxidase) are Cu-requiring proteins. Moreover, the necessity of delivering this element to the thylakoid membranes imposes an extra Cu pathway in cyanobacteria that is absent from other prokaryots (Cavet et al., 2003) and the metalloprotein cytochrome c6 is thought to participate in this trafficking, delivering Cu to the soluble domain of cytochrome oxidase (Paumann et al., 2004). The molecule of chlorophyll needs Mg as the central atom of its porphyrin ring (Reid & Hunter, 2004) while the enzyme RuBisCo (Ribulose 1,5 bisphosphate carboxylase/oxygenase) depends indirectly upon Zn in order to act as carboxylase (Smith & Ferry, 2000). It is important to note that Zn is a ubiquitous (micro) nutrient, that serves as a co-factor in several classes of enzymes and plays an important role in numerous physiological processes, namely maintaining the protein structure.

Thiel et al., (2002) proved many cyanobacteria have the ability to fixate atmospheric N₂ conferred by the enzymatic multiprotein complex, the nitrogenase, which consists of two proteins: the dinitrogenase and the dinitrogenase reductase. This first requires a Fe-Mo co-factor while the second requires a Fe co-factor (Thiel et al., 1995). Once inside the cell, nitrate is reduced to ammonium by two sequential reactions catalyzed by nitrate reductase
and nitrite reductase, respectively. The enzyme nitrate reductase requires a Mo co-factor (Rubio et al., 1999).

Schroder et al., (2003) investigated iron is essential to virtually all organisms, but poses problems of toxicity and poor solubility, particularly Fe(III). As stated before, cyanobacteria have evolved in an environment that propitiated their Fe-dependence. Siderophore (extracellular ferric chelator) production has been found in the freshwater cyanobacteria Anabaena cylindrical (Itou et al., 2004; Itou et al., 2001) and Synechocystis sp. PCC 6803 (Katoh et al., 2001). However, siderophore production has been more investigated in coastal and marine cyanobacteria (Barbeau et al., 2001) since in spite of being the fourth most abundant element in the Earth’s crust Fe concentration in the oceans is extremely low (Mills et al., 2004). Some marine cyanobacteria such as the nitrogen-fixing Trichodesmium sp. have high cellular Fe requirements and others as Synechococcus sp. are often present in regions where the Fe concentrations are very low and suspected to be limiting (Tortell et al., 1999). Evidence has suggested that cyanobacteria may modify Fe chemistry in the sea through the production of ligands, thereby controlling the availability of dissolved Fe (Tortell et al., 1999). Intracellular reserves of Fe can be found within proteins (ferritin, bacterioferritin) for use when external supplies are short (Keren et al., 2004). In many environments the insolubility of Fe(III) iron has to be overcome by the employment of assimilatory ferric reductases. Most bacterial assimilatory ferric reductases are flavin reductases, that play an essential role to generate the more soluble Fe(II) which is incorporated into the cell, and these have been found in many gram-negative bacteria, although their occurrence and mechanism of action in cyanobacteria have not yet been fully investigated.

Currently only nine Ni-dependent enzymes are known in microorganisms, clearly indicating the specificity of this element (Mulrooney & Hausinger, 2003). Cyanobacteria are amongst these since they require a Ni-Fe co-factor for the hydrogenases. These enzymes catalyze the H₂ production and three different types are present in cyanobacteria: a Ni-Fe-dependent, a Fe-dependent, and metal-free hydrogenase (Tamagnini et al., 2002).

4.2.10 Metal Uptake:

Self et al., (2001) described the uptake of heavy metals by microorganisms generally comprises two phases: binding of cations to the negatively-charged groups on the cell surface (passive) and the subsequent metabolism-dependent, intracellular uptake (active). The
passive process is very rapid and occurs in a short time after the microorganisms come into contact with the metal; the active process is slow (Khummongkol et al., 1982, Les & Walker, 1984, Campbell & Smith, 1986). Most cells have two types of import systems: those that are nonspecific and are able to import more than one species across the cytoplasmic membrane and those with high substrate specificity. The first are faster and driven by the chemiosmotic gradient across the cytoplasmic membrane. The second often uses ATP hydrolysis as the energy source and its transporters usually are ABC-type or P-type ATPases (Nies, 2003). The ABC transporters translocate a variety of biological molecules across cell membranes, such as peptides and amino acids, sugars and ions in general (Mikkat & Hagemann, 2000; Holland & Blight, 1999). Microbial genome analysis has shown putative ABC transporters in *Synechocystis* sp. PCC 6803 as one of the most conspicuous superfamilies of membrane carriers (Paulsen et al., 1998). Concretely it has been shown that, in this species, a high affinity ABC-type Mn transport system is involved in regulating the uptake of this element (Rukhman et al., 2005). Based on genome sequence information and similarity to *Escherichia coli* genes, an ABC-type Mo transporter is assumed to exist in *Synechocystis* sp.

Mulrooney & Hausinger (2003) stated an ABC-type transporter specific for Zn is thought to occur in *Synechocystis* sp. PCC 6803 (Cavet et al., 2003). A P-type ATPase is a ubiquitous membrane transporter that carries metal ions, being a mechanism for the control of cytoplasmic metals (Arnesano et al., 2002). Besides the proteins that transport metals through the membrane, there are also smaller soluble proteins that deliver metals to specific target proteins. Among these are the soluble metal receptor proteins, known as metallochaperones, which deliver the metal ion to its proper destination by ensuring that adventitious reactions and binding to sites other than the appropriate ones do not take place (O’Halloran & Cullota, 2000). The lack of intracellular compartments in prokaryotes accounted for the prediction that no such chaperones would occur in these organisms. However, they were found in the gram-positive *Enterococcus hirae*—a Cu chaperone-like protein (Odermatt & Solioz, 1995)—and since then related proteins have been found in other bacteria groups including the cyanobacterium *Synechocystis* sp. PCC 6803 (Banci et al., 2004). Evidence that Ni metallochaperones assist in the delivery of ions to target proteins, or target compartments, has been growing.

Singh & Yadava (1985) reported the uptake rate of a heavy metal is dependent on its speciation which is a key factor in determining its toxicity. Environmental variables such as pH, redox potential, salinity, alkalinity, temperature, available nutrients, metal concentration,
cell density, extracellular metabolites and organic acids can affect metal toxicity (Gadd & Griffiths, 1978, Rai et al., 1981, Reed & Gadd, 1990). One important influence on the physicochemical state of a metal is pH. An increase in toxicity under acidic conditions has been reported for Cd$^{2+}$ in *Nostoc calcicola* and *Anacystis nidulans*, Cu$^{2+}$ in *Anacystis nidulans* and *Nostoc muscorum*, Pb$^{2+}$ in *Nostoc muscorum* and Al$^{3+}$ in *Anabaena cylindrical*, and was suggested to be due to the increase in free metal ions available to the cyanobacteria (Horikoshi et al., 1979, Singh & Pandey, 1981, Pettersson et al., 1985b, Schecher & Driscoll, 1985, Singh, 1985). Under acidic conditions metals tend to exist in the free ionic form, whereas under alkaline conditions they may precipitate as insoluble complexes or in a hydroxylated form which might have an altered activity (Gadd & Griffiths, 1978, Babich & Stotzky, 1983). However, acidic conditions can result in competition between free metals ions and H$^+$ for the same uptake sites, leading to a decrease in cellular heavy metal uptake and toxicity (Peterson et al., 1985). Maximal accumulation of Cs$^+$ in *Synechocystis* PCC6803 was reported to occur at pH 10 and was attributed to hyperpolarization of the membrane (Avery et al., 1991). Also, the optimal growth pH of 8.5 for *Anacystis nidulans* was favorable for maximum Cd$^{2+}$ uptake (Singh & Yadava, 1985).

The presence of other cations can also affect heavy metal uptake and toxicity. A decrease in toxicity of several heavy metals has been described as a result of direct competition between different cations for the same uptake/binding site. Calcium and magnesium salts also form complexes with toxic metals in hard and eutrophic waters which reduce their toxicity (Whitton, 1970, Rai et al., 1981). Reduction of metal toxicity in cyanobacteria has been attributed to the phosphate concentration in cells. The toxicity of aluminum and copper in *Anabaena cylindrical* and *Nostoc calcicola*, respectively, was suggested to be ameliorated by phosphorus-rich cells where both metals were accumulated in polyphosphate granules as a detoxifying mechanism (Pettersson et al., 1988, Verma et al., 1991, 1993).

Schecher & Driscoll (1985) described the number of cyanobacterial cells can affect metal uptake. Increases in cyanobacterial cell densities did not increase the amount of cadmium absorbed per cell in *Anacystis nidulans*, uranium in *Synechococcus elongatus*, and copper and lead in *Nostoc muscorum* (Horikoshi et al., 1979, Schecher & Driscoll, 1985, Singh & Yadava, 1985). Decreases in uptake/toxicity of cadmium in dense cultures was attributed to lower amounts available per cell than in low cell density cultures where the increased distance between cells also contributed to more adsorption of cadmium (Singh &
Yadava, 1985). In *Nostoc muscorum*, high numbers of cells was found to form aggregates with the extracellular sheath. This decreased the total cell surface area exposed to solution and, as a consequence, metal uptake was moderate.

**Table 6**: Some examples of metal removal by cyanobacteria immobilized cells; CPS Capsular polysaccharides.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Element</th>
<th>Uptake</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Synechococcus</em> sp. PCC7942&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cr</td>
<td>0.8 ±1 mg g&lt;sup&gt;-1&lt;/sup&gt; biomass</td>
<td>Gardea-Torresdey et al., 1998</td>
</tr>
<tr>
<td><em>Synechocystis</em> sp. PCC6803</td>
<td>Cd</td>
<td>199.83 mg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Arunakumara et al., 2007</td>
</tr>
<tr>
<td><em>Synechococcus</em> sp. PCC7942&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cd</td>
<td>7.2 ± 2.1mg g&lt;sup&gt;-1&lt;/sup&gt; biomass</td>
<td>Gardea-Torresdey et al., 1998</td>
</tr>
<tr>
<td><em>Oscillatoria</em> sp.</td>
<td>Cd</td>
<td>0.98 µg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Ray &amp; White, 1976</td>
</tr>
<tr>
<td><em>Gloethece magna</em></td>
<td>Cd</td>
<td>115-425 µg mg&lt;sup&gt;-1&lt;/sup&gt; CPS</td>
<td>Mohamed, 2001</td>
</tr>
<tr>
<td><em>Chroococcus paris</em></td>
<td>Cd</td>
<td>53 mg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Les &amp; Walker, 1984</td>
</tr>
<tr>
<td><em>Anacystis nidulans</em></td>
<td>Cd</td>
<td>13.2±0.04 µmol mg&lt;sup&gt;-1&lt;/sup&gt; protein</td>
<td>Awasthi et al., 2004</td>
</tr>
<tr>
<td><em>Anacystis nidulans</em></td>
<td>Cd</td>
<td>3.7 nmol µg&lt;sup&gt;-1&lt;/sup&gt; protein</td>
<td>Singh &amp; Yadava, 1985</td>
</tr>
<tr>
<td><em>Anabaena</em>7120</td>
<td>Cd</td>
<td>70 µg mg&lt;sup&gt;-1&lt;/sup&gt; cells</td>
<td>Massalski et al., 1981</td>
</tr>
<tr>
<td><em>Anabaena</em> cylindrica</td>
<td>Al</td>
<td>33.1 mg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Pettersson et al., 1985b</td>
</tr>
<tr>
<td><em>Cyanospira capsulata</em></td>
<td>Cu</td>
<td>115 ± 5.1 mg g&lt;sup&gt;-1&lt;/sup&gt; protein</td>
<td>De-Philippis et al., 2007</td>
</tr>
<tr>
<td><em>Chroococcus</em> paris</td>
<td>Cu</td>
<td>120 mg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Les &amp; Walker, 1984</td>
</tr>
<tr>
<td><em>Anabaena</em>7120</td>
<td>Cu</td>
<td>7 µg mg&lt;sup&gt;-1&lt;/sup&gt; cells</td>
<td>Massalski et al., 1981</td>
</tr>
<tr>
<td><em>Synechococcus</em> sp. PCC7942&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cu</td>
<td>11.3 ±0.8 mg g&lt;sup&gt;-1&lt;/sup&gt; biomass</td>
<td>Gardea-Torresdey et al., 1998</td>
</tr>
<tr>
<td><em>Oscillatoria</em> sp.</td>
<td>Cu</td>
<td>2.35 µg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Ray &amp; White, 1976</td>
</tr>
<tr>
<td><em>Oscillatoria</em> planetonica</td>
<td>Cu</td>
<td>23.31 mg g&lt;sup&gt;-1&lt;/sup&gt; pretreated biomass</td>
<td>Peng et al., 2009</td>
</tr>
<tr>
<td><em>Oscillatoria</em> planetonica</td>
<td>Cu</td>
<td>22.22 mg g&lt;sup&gt;-1&lt;/sup&gt; intact biomass</td>
<td>Peng et al., 2009</td>
</tr>
<tr>
<td><em>Nostoc</em> calcicola&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cu</td>
<td>242.15 nmol mg&lt;sup&gt;-1&lt;/sup&gt; protein</td>
<td>Singh et al., 1989b</td>
</tr>
<tr>
<td><em>Nostoc</em> calcicola</td>
<td>Cu</td>
<td>96.69 nmol mg&lt;sup&gt;-1&lt;/sup&gt; protein</td>
<td>Verma &amp; Singh, 1990</td>
</tr>
<tr>
<td><em>Nostoc</em> PCC7936</td>
<td>Cu</td>
<td>85 ± 3.2 mg g&lt;sup&gt;-1&lt;/sup&gt; protein</td>
<td>De-Philippis et al., 2007</td>
</tr>
<tr>
<td><em>Oscillatoria</em> planetonica</td>
<td>Zn</td>
<td>19.76 mg g&lt;sup&gt;-1&lt;/sup&gt; pretreated biomass</td>
<td>Peng et al., 2009</td>
</tr>
<tr>
<td><em>Oscillatoria</em> planetonica</td>
<td>Zn</td>
<td>18.59 mg g&lt;sup&gt;-1&lt;/sup&gt; intact biomass</td>
<td>Peng et al., 2009</td>
</tr>
<tr>
<td><em>Chroococcus</em> paris</td>
<td>Zn</td>
<td>65 mg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Les &amp; Walker, 1984</td>
</tr>
<tr>
<td><em>Anacystis nidulans</em></td>
<td>Zn</td>
<td>12.4±0.03 µmol mg&lt;sup&gt;-1&lt;/sup&gt; protein</td>
<td>Awasthi et al., 2004</td>
</tr>
<tr>
<td><em>Synechococcus</em> sp.</td>
<td>U</td>
<td>1764 µg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Sakaguchi et al., 1978</td>
</tr>
<tr>
<td><em>Synechococcus</em> elongatus</td>
<td>U</td>
<td>158 µg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Horikoshi et al., 1979</td>
</tr>
<tr>
<td><em>Synechocystis</em> sp. PCC6803</td>
<td>Pb</td>
<td>156.63 mg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Arunakumara et al., 2007</td>
</tr>
<tr>
<td><em>Synechococcus</em> sp. PCC7942&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Pb</td>
<td>30.04 ± 2.2 mg g&lt;sup&gt;-1&lt;/sup&gt; biomass</td>
<td>Gardea-Torresdey et al., 1998</td>
</tr>
<tr>
<td><em>Spirulina</em> platensis</td>
<td>Pb</td>
<td>188mg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>Arunakumara et al., 2008</td>
</tr>
</tbody>
</table>
### 4.2.11 Biosorption Mechanism in cyanobacteria:

Cyanobacteria can survive and reproduce in metal-contaminated habitats. The genera *Oscillatoria, Phormidium, Plectonema* and *Schizothrix* sp were dominant in zinc-enriched water (Say & Whitton, 1980, Whitton, 1980), and isolates from such sites are often resistant to zinc (Shehata & Whitton, 1981). A range of cyanobacteria, i.e. *Anabaena, Nostoc, Oscillatoria, Phormidium* and *Scytonema*, has been recovered from copper-rich soils (Whitton & Shehata, 1982), and *Plectonema* is a frequent inhabitant near mine railings containing high levels of zinc, cobalt, nickel and lead (Whitton et al., 1981). Heavy metal tolerances by microorganisms revealed that they may occur by several mechanisms, such as extracellular binding or precipitation, impermeability and exclusion, internal detoxification and metal transformations (Reed & Gadd, 1990). The first three mechanisms have been demonstrated in cyanobacteria and are discussed below.

### 4.2.12 Extracellular or surface binding

Yee et al., 2004 tested on the cyanobacterial cell surface proton-active carboxyl, phosphoryl, hydroxyl, and amine functional groups have been shown to deprotonate and bind metal ions to form metal-ligand surface complexes (Yee et al., 2004; Phoenix et al., 2002). However, the cell surface in cyanobacteria is not homogenous. In mucilage-producing cyanobacteria it has been suggested that the binding of metals should take place through the polysaccharides. Studies with *Gloeothecae magna*, in which the extracellular polysaccharides (EPS) and envelope polysaccharides were extracted, have shown the ability of these polymers to bind Cd(II) and Mg(II) (Mohamed 2001). The EPS produced by *Anabaena spiroides* have affinity to bind Mn(II), Cu(II), Pb(II) and Hg(II) (Freire-Nordi et al., 2005). Analysis of
Anabaena flos-aquae fractions of peptidoglycan has indicated that the Cu(II) is coordinated by amine and carboxyl ligands, while in whole-cell samples is coordinated by phosphate, carboxyl and amine ligands (Kretschmer et al., 2004). The authors concluded that the carboxyl groups on the cyanobacterial cell wall are the dominant reactive sites and represent the most important sink for metal ions at near neutral pH.

4.2.13 Metal impermeability and exclusion:

Decreased metal transport, impermeability or metal efflux systems have been observed in some cyanobacteria. The active transport of Ni\(^{2+}\) in Anabaena cylindrica is dependent on the membrane potential, is decreased in the dark, and is inhibited by metabolic uncouplers and electron transport inhibitors (Campbel & Smith, 1986). Active Cd\(^{2+}\) uptake has also been reported in Anacystis nidulans but was competitively inhibited by Ca\(^{2+}\) and Zn\(^{2+}\) (Singh & Yadava, 1985). In Nostoc calcicola, an energy dependent Cu\(^{2+}\) efflux system was present in a resistant mutant which resulted in a net reduction in Cu\(^{2+}\) (Verma & Singh, 1991). An exclusion mechanism was suggested to explain reduced Cu\(^{2+}\) uptake by a tolerant strain of Anabaena doliolum (Rai et al., 1991). In a tolerant strain, isolated by repeatedly subculturing into medium containing increasingly higher levels of copper, only 32 and 40\% Cu\(^{2+}\) was taken up compared with the wild-type. The acquired tolerance was attributed to a change in the cytoplasmic membrane permeability caused by increased lipid production.

4.2.14 Internal metal detoxification:

Rachlin et al., (1984) stated that cyanobacteria can accumulate metals internally, with localization involving binding or precipitation at specific sites. Polyphosphate bodies in Anabaena cylindrica and Anacystis nidulans also accumulated aluminum and titanium, respectively (Crang & Jensen, 1975, Pettersson et al., 1985a). In Anabaena flos-aquae, cadmium was incorporated into both the cellular cytoplasm and polyphosphate bodies (Rachlin et al., 1984). These studies suggest that polyphosphate bodies are a means of binding cations in a non-toxic state within cells, and may serve as storage sites for metals and for detoxification if metals are present at toxic levels. Other examples include Synechococcus elongatus, where uranium uptake leads to formation of dense, internal deposits (Horikoshi et al., 1979). A Synechococcus sp. synthesized large quantities of an intracellular polymer that could bind nickel, the cell interior appearing highly granular (Wood & Wang, 1983). A cellular detoxification mechanism was also suggested to explain the presence of extra
intracellular membrane whorls in *Plectonema boryanum* exposed to nickel, cobalt, zinc, mercury, copper and cadmium.

Mallick & Rai (1998) dealt another aspect of internal compartmentalization is the synthesis of metal-binding components which may function in detoxification. A metal-binding metallothionein protein has been isolated from a *Synechococcus* sp. (Olafson *et al.*, 1979). The term metallothionein has been applied to low-molecular-weight proteins or polypeptides, with a high metal and cysteine content, no aromatic amino acids or histidine, fixed distribution for cysteine residues, which bind metal ions in metal-thiolate clusters and whose synthesis increases in response to elevated concentrations of certain metals. The first bacterial metallothionein to be characterized has been a Cd metallothionein found in *Synechococcus* sp. and, since then, their biochemistry and molecular genetics have been characterized for other cyanobacteria (Turner & Robinson 1995). A Zn metallothionein found in *Synechococcus* sp. PCC 7942 is highly synthesized in response to elevated concentrations of Zn primarily, but also to Cd and Cu. A Zn metallothionein-like sequence has been shown in the genome of other cyanobacterium (*Anabaena* sp. PCC 7120 and *Synechocystis* sp. PCC 6803) (Blindauer *et al.*, 2002; Robinson *et al.*, 2001). *Nostoc linckia* has seemed to be tolerant to Zn and Cd because, among other mechanisms, these metals are sequestered via metal binding proteins (El-Enany & Issa, 2000). A low molecular weight Cd-induced protein of *Anabaena doliolum* confers co tolerance to metals and also provides multiple-tolerance to environmental stresses such as heat and cold shocks.

Bioremediation application is facing the great challenge; there are two trends for the development of the biosorption process for metal removal. One trend is to use hybrid technology for pollutants removal, especially using living cells. Another trend is to develop the commercial biosorbents using immobilization technology, and to improve the biosorption process including regeneration/reuse, making the biosorbents just like a kind of ion exchange resin, as well as to exploit the market with great endeavor (Wang and Chen, 2009).
4.3 MATERIALS AND METHODS:

4.3.1 Sample collection and maintenance:

The samples were collected from agricultural field Mathur region, Tiruchirappalli, Tamilnadu, India (Lat. 10°41’N / Lon. 78°44’15” E). Pure culture of the *Chroococcus minutus* NTMS09, *Oscillatoria acuminate* NTMS02, *Dolichospermum flos-aquae* NTMS07, *Calothrix fusca* NTMS08, *Scytonema hofmanni* NTMS05 were grown on BG 11 medium and composed as following: NaNO₃ (1.75 g), K₂HPO₄ (0.02 g), MgSO₄·7H₂O (0.075 g), CaCl₂·2H₂O (0.036 g), Na₂CO₃ (0.02 g), Citric acid (0.006 g), ferric ammonium citrate (0.006 g), EDTA (0.001 g) and trace elements solution (1 mL) in 1,000 mL distilled water. Trace elements solution containing H₃BO₃ (2.86 g), MnCl₂·4H₂O (0.22 g), Na₂MoO₄·2H₂O (0.39 g), CuSO₄·5H₂O (0.079 g), Co (NO₃)₂·6H₂O (0.0494 g) in 1,000 mL distilled water. The pH of the medium was adjusted to 7.0 ± 1. The cells were grown in sterile flasks containing 100 mL of BG 11 medium. Experimental cultures were incubated at 25 ± 2°C, 14/10 hours light/dark cycle, with illumination of 27 μEm⁻²s⁻¹ under cool white fluorescent lamps. The cultures were gently shaken by hand on alternate days.

4.3.2 Preparation of Cr (VI) solution:

Synthetic stock solution of Cr (VI) was prepared by dissolving a calculated quantity of K₂Cr₂O₇ (AR Grade) in double distilled water and working standards were obtained by further dilutions.

4.3.3 Experimental setup:

Metal removal capacities of the test organisms were studied. Cyanobacterial cells were inoculated in 100 ml of fresh BG11 medium at under varying pH 6, 7, 8 supplemented with various initial metal concentrations (2.5, 5, 7.5, 10 mg/L). The cyanobacterial cells without Cr (VI) in the medium served as the control. The cultures were gently shaken by hand on alternate days.

4.3.4 Determination of concentrations of Cr (VI) ion:

Chromium (VI) was determined spectrophotometrically using diphenyl carbazide method. A 0.25% w/v solution of diphenyl carbazide was prepared in 50% acetone. 1 ml each of the sample solutions, containing various concentrations of Cr (VI) were taken into test tubes. To this 0.7ml of 6N H₂SO₄ was added followed by 0.2 ml of diphenyl carbazide and the total volume was made upto 10 ml using deionised, double distilled. Chromium
concentration estimated by the intensity of the colour complex formed was measured using a UV-visible spectrophotometer. The absorbance was measured against a reagent blank at 540-nm wavelength maximum. A linear plot was obtained indicating adherence to the Beer Lambert's law in the concentration range studied (Clesceri et al., 1996).

4.3.5 Adsorption isotherms:

The relationship between the adsorbed amount of metal and its equilibrium concentration in solution has been tested by adsorption isotherms. The Langmuir and Freundlich adsorption isotherms have been investigated in this study. The Langmuir equation suggests the monolayer sorption on to a surface containing finite number of identical sites (Ergene et al., 2006).

\[
\frac{C_e}{q_e} = \frac{1}{Q_{\text{max}}} b + \frac{C_e}{Q_{\text{max}}}
\]

Freundlich equation based on sorption on a heterogeneous surface is given by the following equation (Morell and Hering, 1993).

\[
q_e = K_f C_e^{1/n}
\]

Based on the fitting of the straight line in the isotherms, the best metal removal was calculated.
4.4 RESULTS AND DISCUSSION:

4.4.1 Metal removal:

4.4.2 Effect of pH:

The pH of the aqueous solution is clearly an important parameter that controlled the adsorption process. Different values of pH 6, 7, 8 were studied at various initial metal concentrations, standard contact time of 2 days and temperature of 26°C. The experimental results of this stage are presented in Plate 25. As it is shown, the optimum pH of solution was observed at pH 7, but pH 6 and 8 does not show the major influence on Cr (VI) removal. It was revealed that the maximum sorption in percent was achieved at pH 7. Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients. Biodegradation of petroleum hydrocarbons is optimal at a pH 7 (US-EPA, 2006) similar pattern of results have been obtain in this experiment. The sorption percentage was increased in pH 7 in all the organisms studied; beyond it followed a decrease in sorption with other pH. The maximum removal of 99.6 % was observed in Chroococcus minutus NTMS09. Similar result was suggested by Rama Krishna in (2005) who reported Cr (VI) reduction above 80% could be achieved in the bioreactor with an initial concentration of Cr (VI) 5 mg/L at a hydraulic retention time of 8 h. Turick et al., (1996) documented that Acinetobacter sp. isolated from polluted site which is removed 85% of chromate Cr (VI) in pH7 under aerobic conditions, comparing to the other microbes cyanobacteria as Oscillatoria accuminata NTMS02. (93.2%), Dolichospermum flos-aquae NTMS07 (91.2%), Calothrix fusca NTMS08 (76.6%) and Scytonema hofmanni NTMS05 (62.6%) showed better removal efficiency. At low pH negligible removal of chromium (VI) may be due to the competition between hydrogen and metal ions adsorbed on the surface of the organism and at alkaline pH, formation of metal hydroxides inhibit the metal removal.

4.4.3 Effect of contact time:

The adsorption experiment of chromium (VI) was carried out for different contact times at pH 7. The results were depicted in Plate 26. Different contact time of 1 to 5 days was studied in the interval of 24 hrs at various initial metal ion concentration, standard pH and temperature of 26°C. The sorption percentage of metal increased in contact time of 2nd day. The maximum percent removal was observed at 2nd day for all the organisms tested. The maximum removal of 99.6 % was observed in Chroococcus minutus NTMS09
comparing to the other organisms as *Oscillatoria accuminata* NTMS02. (93.2%), *Dolichospermum flos-aquae* NTMS07 (91.2%), *Calothrix fusca* NTMS08 (76.6%) and *Scytonema hofmanni* NTMS05 (62.6%). After 2nd day, the percent removal was decreased this may be due to desorption of small amount of metals adsorbed by organism as in Chromium. This may be due to the accumulation of metals which is slow process followed by adsorption. Physical adsorption on to the surface of the organism is fast but accumulation process includes transport of metal across the membrane which requires long exposure time. After 2nd day of incubation, the concentration of metal increased in the medium which denotes the decreased percent removal. This may be due to the desorption of metal which is adsorbed on the surface of the organism.

### 4.4.5 Effect of metal concentration:

Effect of the initial concentration of chromium (VI) ion uptake by *Chroococcus minutus* NTMS09, *Oscillatoria accuminata* NTMS02, *Dolichospermum flos-aquae* NTMS07, *Calothrix fusca* NTMS08, *Scytonema hofmanni* NTMS05, at various days of contact time, pH 7 and temperature 26ºC is illustrated in Fig. 26. At the 2nd day of incubation, *Chroococcus minutus* NTMS09, *Oscillatoria accuminata* NTMS04, *Dolichospermum flos-aquae* NTMS07, *Calothrix fusca*. NTMS08, *Scytonema hofmanni* NTMS05, removal was observed respectively 99%, 93%, 91%, 76%, 62% removal was observed at 2.5 mg/L of initial chromium concentration further the removal was decreased with increasing concentration Plate 26. This may be due to the less available sites in higher concentration of metal. The ability of cyanobacteria to bind metals may be attributed to the presence of effective groups on the surface of biomass such as hydroxyl phosphate, amino and carboxyl that can capture metals (Khummongkol *et al.*, 1982, Xue and Sigg, 1990). The cyanobacterial envelope consists mainly of polysaccharides that are negatively charged and rich in uronic acids thus they exhibit high metal binding capacity (Manzini *et al.*, 1984; Volesky, 1995; Subramanian and Uma, 1996; Kumar *et al* 2011). The test organism was found to adsorb and accumulate the chromium metal and showed maximum percent removal at 2.5mg/L. Rehman *et al.*, 2008 assessed the ability of *Bacillus* sp to reduce hexavalent chromium into its trivalent form. Immobilized *Oscillatoria* sp. and *Phormidium* sp. found to be a good sorbent at 2 mg/L (Rajeshwari *et al.*, 2011). The test organism showed decreased percent removal at 5, 7.5, 10 mg/L. This result corresponds to the work of Kumar *et al.*, (2009) which described the same phenomenon in cyanobacteria and the condition was attributed to cellular degradation or adaptability of cyanobacteria to the stress environment. The metal removal
was at its maximum at 1\textsuperscript{st} and 2\textsuperscript{nd} day of incubation. After 2\textsuperscript{nd} day of incubation, the concentration of metal increased in the medium which denotes the decreased percent removal. This may be due to desorption of metal which is adsorbed on to the surface of the organism.

4.4.6 Adsorption isotherms:

Several isotherm models are available to describe this equilibrium sorption distribution. Langmuir and Freundlich sorption isotherms have been investigated in this study. Langmuir model assumes monolayer biosorption onto a surface with a finite number of identical sites and the model is described by the following linear equation (Crist \textit{et al.}, 1981):

$$
\frac{C_e}{q_e} = \frac{1}{q_m}C_e + \frac{k_d}{q_m}
$$

Where $C_e$ is the equilibrium chromium concentration (mg/L), $q_e$ the metal adsorbed on the adsorbent (mg/g dry wt.), $q_m$ the maximal biosorption capacity, $K_d$ is the Langmuir constant of the system. In this model, $C_e/q_e$ is linearly related to $q_e$. However, the chances that a molecule adsorbed onto the surface may make it more or less difficult for another molecule to get attached to a neighbouring site on the biosorbent and this might lead to a deviation from the Langmuir biosorption equation. Under such a situation, Freundlich isotherm may be more suitable, which can be expressed by the linear equation in logarithmic form as:

$$
\log q_e = \log K_f + \frac{1}{n} \log C_e
$$

Where $K_f$ is Freundlich constant indicating adsorbent capacity (mg/g dry wt.) and $n$ is the Freundlich exponent known as adsorbent intensity (Freundlich \textit{et al.}, 1939). This model shows that $\log q_e$ is linearly related to $C_e$.

On applying the constant parameters of pH 7, contact time of 2 days, metal concentration of 2.5 mg/ml and temperature 26˚C. The results of Freundlich and Langmuir models for \textit{Chroococcus minutus} NTMS09 are shown in Table 7, Figs 8 &9. The Langmuir adsorption isotherm had a higher correlation coefficient than that of the Freundlich isotherm in all the cyanobacteria tested. The mechanisms involved in Cr (VI) removal by cyanobacteria are discussed based on the Langmuir isotherm parameters. Other organisms were \textit{Oscillatoria accuminata} NTMS02, \textit{Dolichospermum flos-aquae} NTMS07, \textit{Calothrix fusca} NTMS08 and \textit{Scytonema hofmanni} NTMS05. Data used for this isotherms represented in Tables 8 to 11 based on the Log values and percentage removal, the isotherm described in
the term of Langmuir and Freundlich isotherms Figs 10 to 17 Langmuir isotherm $R^2$ values are respectively 0.993, 0.997, 0.998, 0.986 data's are represented table 7. The test organisms of Cr (VI) adsorption isotherms for Freundlich ($R^2$) and Langmuir ($R^2$) values are given in Table 12. Langmuir isotherms strongly support the surface of the adsorbent is uniform, that is, all the adsorption sites are equivalent, adsorbed molecules do not interact, all adsorption occurs through the same mechanism.