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دراسات طحلبيه تطبيقيه فى مجال المعادن الثقيله السامه ومعالجة مياه الصرف الصناعى باستخدام المرشحات الطحلبيه

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INTRODUCTION

1. Preamble.

Heavy metal pollution has resulted in many problems for human health and aquatic ecosystems as well (Rai *et al.*, 1981a and Inthorn *et al.*, 1996). Industry represents a potential source of a variety of toxic heavy metals.

According to their potential hazard, the heavy metals Pb, Hg, As and Cd were ranked by the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) as the first, second, third, and sixth highly toxic metals, respectively (Howard, 2002). However, due to the recently discovered extreme acute toxicity of Cd, this metal has now joined Pb and Hg as one of the major three heavy metals greatly hazardous to human health (Volesky, 1990).

Several conventional methods for the elimination of toxic heavy metals from aqueous solutions are well known including precipitation, electrolysis, ion exchange, filtration, evaporation, etc. (Volesky, 1994 and Schneegurt *et al*., 2001). The disadvantages of these methods are the high cost and low economic efficiency and, in some cases, the disposal of considerable amount of sludge containing heavy metals, which is a potential threat to the quality of ground water (Sandau *et al*., 1996a).

Resolving the environmental pollution problems caused by toxic heavy metal contamination resulting from the complex and diverse human activities, has for long presented a challenge. Biosorption can be a part of the solution. Biosorption is a process in which solids of natural origin are employed for binding heavy metals. It is a promising alternative method to treat industrial effluents, mainly because of its low cost and high metal binding capacity (Cossich *et al*., 2002).

Environmentally friendly processes need to be developed to clean up the environment without creating harmful waste products (Scott and Palmer, 1990 and Pappas *et al*., 1990). New biosorbents can be manipulated for better efficiency and multiple reuse to increase their economic attractiveness (Regine and Volesky**,** 2000).

Bioremoval of heavy metals from aqueous solutions is a relatively new technology applied for the treatment of industrial wastewater (Volesky, 1990). The major advantages of the bioremoval technologies are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biological materials (Volesky, 1990 and Yu *et al*., 1999).

The thesis presents researches carried out to investigate the efficiency of some algal species to remove four highly toxic heavy metals, namely, nickel, cadmium, lead and mercury from aqueous solution and from industrial wastewater as well. This kind of research is undoubtedly very important for environmental pollution control.

One of the main objectives of the present thesis was the investigation of the algal toxicity assessment and bioremoval of the metal ions, Pb, Cd, Ni and Hg by some selected algal species. Therefore, the sources, biological effects and control of these metals will be introduced in some details.

2. Sources of heavy metal ions.

Heavy metals are natural constituents of the earth's crust. They are stable and persistent chemicals, since they can not be degraded. Over decades, human activities have drastically altered the biogeochemical cycles and balance of some heavy metals. Metals and their compounds , both inorganic and organic, are released to the environment as a result of a variety and complex human activities.

Heavy industries, smelters, battery manufacturing and mining activities are among the main point sources of heavy metal pollution. The main point sources through which heavy metals enter the environment are wastewater streams originated from industrial processes such as electroplating, plastics manufacturing, mining and metallurgical processes (Yu and Kaewsarn, 1999).

The non-point or diffuse industrial sources of heavy metals are also devise and rather complicated. Combustion by-products and heavy traffic are considerable non-point sources of heavy metals (Whitton *et al*., 1981).

Atmospheric deposition of heavy metal may originate from various industrial activities such as waste incineration, heating plants and crude oil processing (Chmielewská and Medved, 2001).

Lead.

The most important source of atmospheric Pb is the combustion of fuel containing tetraalkyllead antiknock agents. Cd and Ni enter the environment via three main routes: refining and use of Cd, Cu and Ni smelting and fuel combustion (Chmielewská and Medved, 2001).

The dominant source of worldwide dispersion of Pb into the environment is the use of Pb organic compounds as antiknock motor vehicle fuel additives. Since leaded gasoline was introduced in 1923, its combustion and resulting contamination of the atmosphere has increased background levels everywhere, including the ice cap covering Northern Greenland, where there is no industry and few cars and people (US Environmental Protection Agency, 1986).

The current annual worldwide production of Pb is approximately 5.4 million tons and continues to rise (Korrick *et al.,* 1999 and Cheng *et al.,* 2001). Sixty percent of Pb is used for the manufacturing of batteries, automobile batteries, in particular, while the remainder is used for the production of pigments, glazes, solder, plastics, cable sheathing, ammunition, weights, gasoline additive, and a variety of other products (Howard, 2002). In addition, Pb is used as an industrial raw material for storage battery manufacturing, printing,

pigments, fuels, photographic materials and explosive manufacturing (Jalali *et al*., 2002).

Cadmium and Nickel.

Cadmium enters waterways through industrial discharges and galvanized pipe breakdown (Terry and Stone, 2002). Cd is widely used in a variety of industrial processes, including plastic manufacturing, electroplating and Ni-Cd battery production, as well as in pigments (Alloway, 1995 and Dudka and Adriano, 1997). These industries, as well as mining and smelting industries, release substantial amounts of Cd into the environment each year (Nriagu and Pacyna, 1988).

Airborne Cd exposure is also a risk posed by the incineration of municipal waste containing plastics and Ni-Cd batteries. Cigarette smoking constitutes an additional major source of Cd exposure (Howard, 2002).

Nickel/Cadmium battery production may deliver both Cd and Ni to ground water in untreated aqueous wastes or through uncontrolled disposal of used batteries, Cd plating introduces cyanide used as a strong complexing agent and Cd is introduced directly to the ground water from cultivated areas where Cd rich phosphate based fertilizers are used (Costa and Franca, 1998 and Zhou *et al*., 1998). Also non-ferrous metal mines represent a major source of Cd release to the aquatic environment (Nosier, 2003).

Mercury.

Mercury comes in a number of different chemical forms. Metallic mercury (Hg) is used in thermometers, dental amalgams, and some batteries. Salts of mercury are widely used in metal-processing, electrical-equipment, automotive, building industries and in medical and dental services (Howard 2002). These industries and activities are potential sources of Hg release into the environment.

Concisely, the input of different heavy metals into the environment through different and complicated routes are strongly correlated with the anthropogenic activities. It became evident that different heavy metals vary greatly in ways through which they accumulate in the environment.

3. Toxicity of heavy metal ions.

The presence of heavy metals in aquatic environments is known to cause severe damage to aquatic life. Most heavy metal salts are soluble in water and form aqueous solutions, and consequently cannot be separated by ordinary physical means of separation (Travieso *et al.*, 1999).

The presence of heavy metals in water can create serious damage to the aquatic life because they are accumulated through the food chain and produce toxic effects and teratogenic changes in plants, animals and human beings including cancer (Travieso *et al*., 2002). Moreover, high metal concentrations in wastewater can kill microorganisms or at least diminishes their activities during biological treatment of wastewater (Volesky, 1994).

Foulkes (2000) classified the heavy metal ions into four distinct classes. Class A is represented by $Fe^{2+,3+}$, which is essential for life in relatively high concentration. Class B contains metals for which no biological role has been established, and which in low concentrations exhibit little or no toxicity; they include lanthanum ,strontium, and others. A third class, C, consists of elements like Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mo^{2+} and perhaps $Cr^{4+,6+}$, all essential in trace amounts for at least some living systems. At higher concentrations, class C elements can became very toxic. Class D contains metals that are toxic even at very low levels, and for which no clear biological function has been established, e.g. Hg^{2+}, Pb^{2+}, Cd^{2+} and U^{2+} metal ions.

Depending on their oxidation states, heavy metals can be highly reactive and, as a consequence, toxic to most organisms. The toxic effect of heavy metals appears to be related to production of reactive oxygen species (ROS) and the resulting unbalanced cellular redox status (Pinto *et al*., 2003).

Cadmium ion is considered highly toxic and therefore its allowable concentrations in water resources is limited from 5 to 10 ppb. Recently, Cd^{2+} has become a major environmental concern due to its introduction to natural water reservoirs (Nosier, 2003).

Cd^{2+}, Hg^{2+}, Cr^{3+} and Pb^{2+} ions are non essential heavy metals and are potentially highly toxic even at very low concentrations (Gulriz, 2002). Cd^{2+}, Pb^{2+} and Hg^{2+} metal ions are the top three metals most hazardous to man and environment (Stirk and Van Staden, 2002).

3.1. Toxicity to humans:

Mercury.

The most severe incident of man-made Hg poisoning occurred during 1972 in Iraq. The first outbreaks were reported from northern Iraq where farmers had received wheat grains treated with mercurial fungicides from Mexico, and ate the grains instead of planting it (Forstner and Wittmann, 1981).

The extreme toxicity of many Hg compounds only became evident through worldwide publicized incidents of mass poisoning after the release of Hg^{2+}-containing water into Minamata Bay in Japan between 1948 and 1960 (Kaim and Schweelerski, 1994). Mothers exposed to Hg in Minamata Bay gave birth to infants with mental retardation, retention of primitive reflexes, cerebller symptoms and other abnormalities (Grandjean *et al*., 1998 and Murata *et al*., 1999).

High levels of Hg exposure that occur through, for example, inhalation of Hg vapors generated by thermal volatilization can lead to life-threatening injuries to the lungs and neurologic system. At lower chronic levels of exposure, a typical constellation of findings arises, termed *erethism* with tremor of the hands, excitability, memory loss, insomnia, timidity, and sometimes delirium that was once commonly seen in workers exposed to Hg in the felt hat industry ("mad as a hatter"). Even relatively modest levels of occupational Hg exposure, as experienced, for example, by dentists, have been associated with measurable declines in performance on neurobehavioral tests of motor speed, visual scanning, verbal and visual

memory, and visuomotor coordination (Bittner *et al*., 1998). The small amount of Hg released from dental amalgams during chewing is capable of causing significant illnesses, such as mutliple sclerosis, systemic lupus or chronic fatigue syndrome (Grandjean *et al*., 1997).

Special attention and care should be paid to methylmercury (CH_3Hg), the supertoxic and superdangerous compound that can penetrate through latex gloves, as well as skin. Exposure to only a few drops can lead to central nervous system degeneration and death (Nierenberg *et al*., 1998).

Cadmium.

Cadmium ion is a nonessential metal to living organisms and can become toxic by displacing calcium ions (Leborans and Novillo, 1996). Epidemiological studies have revealed that Cd^{2+} may contribute to some forms of cancer in humans and low exposures may result in kidney damage (Terry and Stone, 2002). Fully developed Cd^{2+} poisoning shows two main effects; renal dysfunction and emphysema. Exposure is usually by the oral route and the kidney is the most target organ (Nosier, 2003).

In animals and humans, chronic or acute exposure to Cd^{2+} can induce a variety of health disorders, including kidney damage (Buchet *et al*., 1980 and Hellstrom *et al*., 2001), osteomalacia (Nogawa and Kido, 1993 and Tsuritani *et al*., 1996), developmental defects and prostate cancer (Desi *et al*., 1998).

Acute high-dose exposures of Cd^{2+} ion can cause severe respiratory irritation. Occupational levels of Cd^{2+} exposure are a risk factor for chronic lung disease and testicular degeneration (Benoff *et al*., 2000) and are still under investigation as a risk factor for prostate cancer (Ye *et al*., 2000). Lower levels of exposure are mainly of concern with respect to toxicity to the kidney. Cd^{2+} ions damage a specific structure of the functional unit of the kidney, the proximal tubules of each nephron, in a way that is first manifested by leakage of low molecular weight proteins and essential ions, such as calcium, into urine, with progression over time to frank kidney failure (Satarug *et al*., 2000).

Considerable loss of calcium due to Cd toxicity can be severe enough to lead to weakening of the bones and extremely painful deformations of the skeleton, such symptoms have been observed on a large scale as "Itai-Itai disease"(Kaim and Schwederski, 1994). Itai-Itai disease, an epidemic of bone fractures in Japan from gross Cd contamination of rice stocks, has recently been shown to happen in more subtle fashion among a general community living in an area of relatively modest Cd contamination (Staessen *et al*., 1999). Increased Cd burden in this population was found to be predictive of an increased risk of bone fractures in women, as well as decreased bone density and height loss in both sexes (Howard, 2002).

Lead.

Lead ion exposure in children and adults can cause a wide spectrum of health problems, ranging from convulsions, coma, renal failure, and death at

extremely high level and low level of exposure affect metabolism and intelligence (Howard, 2002). However, Pb^{2+} toxicity depends largely on the exposure dose.

Low-level Pb^{2+} ion exposure in children, less than five years of age, results in deficits in intellectual development as manifested by lost intelligence quotient points (Banks *et al*., 1997).

Recent research has clearly demonstrated that maternal bone lead stores are mobilized at an accelerated rate during pregnancy and lactation (Gulson *et al*., 1997) and are associated with decrements in birth weight, growth rate, and mental development (Gonzalez-Cossio *et al*., 1997). Since bone lead stores persist for decades (Hu *et al*., 1998) it is possible that Pb can remain a threat to fetus health many years after environmental exposure had actually been curtailed.

Nickel.

Several studies have indicated that occupational inhalation exposure to Nickel aerosols can result in development of asthma specific to Ni. Davies (1986) found 3 cases of asthma among 53 Ni-plating workers without a history of asthma prior to employment. Novey *et al.* (1983) described biphasic metal-specific bronchial responses in an individual metal-plating worker exposed to Ni and Cr salts. In another case, immunological studies conducted in a 24-year old man showed Ni-specific antibodies in the serum

after several weeks of working in a Ni-plating shop using nickel sulfate (McConnell *et al.*, 1973).

Various compounds of Ni, such as nickel sulfate, nickel oxide and nickel chloride have been shown to cause lung inflammation, fibrosis, emphysema, alveolar proteinosis and cancer (Benson *et al.*, 1988; Dunnick *et al.*, 1988 and Zhang *et al.*, 2003).

Nickel ingestion has been shown to produce dermal hypersensitivity reactions in individuals with Ni sensitivity. Nickel exposure in these individuals via the inhalation, dermal, or oral route results in dermal responses characterized by eczema, erythema, and dermal eruptions (Burrows *et al.*, 1981; Gawkrodger *et al*., 1986 and Nielsen *et al*., 1990).

3.2. Toxicity to higher plants:

It became evident that soil contamination with heavy metal ions disturb plant diversity and function (Weissenhorn *et al.*, 1995). Heavy metal ions can influence various morphological, physiological and biochemical processes in plants (Wierzbicka, 1995).

Toxicity of heavy metals to higher plants, may result from the binding of metals to sulphydryl groups in proteins, leading to an inhibition of activity or disruption of structure, or from the displacing of an essential element resulting in deficiency effects (Van Assche and Clijsters, 1990). In addition, heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, perhaps resulting in oxidative stress (Dietz *et al*., 1999).

Cadmium.

Plants readily take up Cd from the soil. However, exposure to high levels of Cd results in reduced rates of photosynthesis, chlorosis, growth inhibition, browning of root tips (Kahle, 1993), decreases in water and nutrient uptake, and finally death (Toppi and Gabbrielli, 1999).

Cadmium has been shown to reduce the ATPase activity of the plasma membrane of wheat and sunflower roots (Fodor *et al*., 1995). Some studies have indicated the negative effects of Cd on electron energy transfer reactions in plant mitochondria (Miller and Koeppe, 1970 and Bittell and Koeppe, 1973). However, Cd stimulated, at low concentrations (10 to 50µM) and inhibited, at higher concentrations (>100µM), the oxidation of NADP by isolated corn mitochondria (Koeppe and Miller, 1970).

Nickel.

Abnormal cell divisions occur in roots of the broad bean (*Vicia faba*) during exposure to various inorganic nickel salts at Ni concentrations from 0.1 to 10 mgl^{-1}. All Ni salts tested produced more abnormal cell divisions than did controls (USPHS, 1993). In beans, nickel nitrate was the most effective inorganic Ni compound tested in producing deformed cells, abnormal arrangement of chromatin, extra micronuclei, and evidence of cell nucleus disturbances; however, Ni salts showed only weak mutagenic action on rootlets of peas, *Pisum* sp. (USPHS, 1993). Nickel sulfate induced

chromosomal abnormalities in root tip cells of onions, *Allium* sp. (Donghua and Wusheng, 1997 and Abdel-Migid *et al.*, 2004).

Lead.

Lead in particular is known to strongly inhibit plant growth, root elongation, seed germination, seedling development, cell division, photosynthesis, transpiration, chlorophyll production, etioplast development and/or lamellar organisation in chloroplasts (Rucinska *et al*., 2004).

3.3. Toxicity to algae:

Deleterious effects of heavy metals to microalgae depend on two major variables: sensitivity of the target organisms and environmental conditions of exposure. The latter includes medium composition, which is important since it directly affects the speciation form of the chemical and therefore its bioavailability and toxicity. In fact, pH, nutrients of the medium, as well as chelators are factors that have been shown to affect the response of algal cells to toxic metals (Sunda and Lewis,1978; Babich and Stotzky, 1983; Kuwabara, 1985 and Vasseur *et al*., 1986).

Cadmium.

Bartlett *et al*. (1974) studied the algistatic effects of Cd on *Selenastrum capricornutum.* The metal growth inhibition started at $50\mu gl^{-1}$, with complete inhibition at $80\mu gl^{-1}$ after four days. Blaise *et al*. (1986) reported that the EC_{50} (minimum metal concentration inhabiting algal growth by 50% relative for control) of this metal varies between 30 and $55\mu gl^{-1}$.

Delmotte (1980) has shown that cells of *Anabaena cylindrica* are damaged at Cd concentrations of 2 mgl^{-1}. This concentrations had also significant effect on nitrogen fixation metabolism. Stratton and Corke (1979) reported that the growth of *Anabaena inaequalis* was already inhibited significantly at Cd concentrations greater than 0.03 mgl^{-1}, and completely inhibited at 0.06 mgl^{-1} after 12 days. In the case of Hg, Stratton *et al*. (1979) found that the growth of the same alga was inhibited at concentrations of the metal as low as 2μgl^{-1}.

Mercury.

Mercury affects membrane permeability in a number of algal species including, *Chlorella sp, Coelastrum microporum* and *Dunaliella tertiolecta* (Parry and Hayward, 1973; Holderness *et al*., 1975; De Filippis and Pallaghy, 1976 and Kayser, 1976). Mercury causes potassium loss (Courchene and Chapman,1975) and changes in cation exchanges capabilities (Fujita *et al*.,1978).

Lead, Cupper and Zinc.

Takamura *et al*. (1989) and De Filippis and Ziegler (1993) stated that, cyanophyceae are sensitive to Cu, Cd and Zn metals than other algae tested for photosynthetic activity, through the inhibition of photosystem II and/or reduction in activities of enzymes involved in the fixation of CO_2.

Higher concentrations of Zn decreased cell division, movement, total chlorophyll content, ATP (De Filippis *et al*., 1981), carotenoids/chlorophyll ratio (Rai *et al*., 1981b) and ATPase activity (Labyntseva *et al*., 1998).

It has been found that, the toxicity of Pb is relatively low to algae (Kirchman and Bonott, 1971; Malanchuk and Gruendling, 1973; Hessler, 1974 and Zavodnik, 1977).

3.4. Mechanism of metal toxicity:

The toxic effect of heavy metals appears to be related to production of reactive oxygen species (ROS) and the resulting unbalanced cellular redox status (Emani *et al*., 2003). Heavy metals can promote oxidative damage both by directly increasing the cellular concentration of reactive oxygen species (Winterbourn, 1982) and by reducing the cellular antioxidant capacity (Sies, 1999).

Cadmium.

Cadmium toxicity is associated with the formation and disruption of sulfhydryl and metal thiolate bonds, alterations in protein secondary structure, changes in the redox status of the cell, and interference with essential metal uptake, transport and metabolism (Brennan and Schiestl, 1996; Chaoui *et al*., 1997; Ouariti *et al*., 1997; Nies, 1999 and Sandalio *et al*., 2001). In addition, Cd poisoning of metalloproteins involved in redox or electron transfer processes may result in increased free radical production,

leading to nonspecific damage to proteins, lipids, and other biological molecules (Stohs *et al*., 2000 and Schützendübel *et al*., 2001).

Mercury.

The inorganic Hg salts are toxic, but the toxicity of methylmercury (CH_3Hg) is much more severe due to the facts: i) the organic moiety gives it a lipophilic character so that it can easily pass through the biological lipid membrane and ii) the Hg is tightly bound to carbon so that the molecule is not easily degraded and it may maintain its toxic action for a long time while the harms caused by Hg are invariably reversible (Das, 1990). The deleterious effects of CH_3Hg depend on two natural process, biomethylation and bioamplification. Biomethylation is a process through which inorganic Hg ion is converted into the more toxic agent, CH_3Hg and it is the most critical step in the environment in its toxicology. The methylation may occurs either enzymatically or non-enzymatically. Bioamplification is a process by which the poison can be concentrated along the food chain.

Nickel.

Singh *et al*. (1989) reported that the addition of Ni, Hg and Cd inhibited the growth, oxygen evolution and oxygen uptake in the cells of *Cylindrospermum* sp. Nickel toxicity reduces photosynthesis, growth, and nitrogenase activity of freshwater and marine algae at 30-125 μgl^{-1} (Outridge and Scheuhammer, 1993 & Lee and Lustigman, 1996).

4. Tolerance of algae to heavy metals.

Tolerance means that, the ability of some algae to grow in abnormally high concentrations of heavy metals, which are considered environmentally hazardous.

Euglena gracilis was found to be more resistant, with no death occurring after 96 hrs in 1.0 mgl^{-1} Cd (Fennikof *et al*., 1978). Mang and Broda (1976) indicated that, the growth of *Chlorella pyrenoidosa* to be reduced at 1.1 mgl^{-1} Cd, but not totally inhibited at 10.0 mgl^{-1} Cd.

The effects of different concentrations of Hg^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+} and Cr^{3+} were investigated on the growth of the diatoms *Achnanthes hauckiana*, *Amphora coffeaetormis, Cyclotella meneghiniana, Fragilaria pinnata, Navicula confervaceae, Nitzschia obtuse, N. palae, Skeltonema costatum, Syndera tabulate, Thalassiosira weisflogii and Triceratium dubium.* Generally, marine diatoms were more tolerant to the metals than freshwater diatoms (Rao *et al*., 1994).

Mechanisms underlying metal tolerance in algae are not fully recognized and may rely on several mechanisms operating simultaneously. Silverberg (1975) suggested that intracellular compartmentalization of Pb can be a mechanism of detoxification of the metal excess in *Stigeoclonium tenue*. It was also estimated that in the giant cells of *Chara corralina*, most of the intracellular Zn^{2+} was stored in vacuoles (Reid *et al*., 1996).

Terry and Stone (2002) reported that tolerance of algae to heavy metals differed among different algal species. In addition, the algae isolated from polluted water were more tolerant to metal toxicity.

Achnanthes minutissima isolated from River Kosaka and *Ulothrix variabilis* from River Takatori have maintained their tolerance when cultured in normal medium without Cu^{2+} and Cd^{2+} or elevated Zn^{2+} for 4 years suggesting that tolerance to these metals may be a genetically stable character (Takamura *et al*., 1989).

Two unicellular green algae, *Scendesmus bijugatus* and *S. dimorphus* and the filamentous *Stigeoclonium tenue* could adapt to very high concentrations of heavy metal ions and the algae retained the tolerance even after the metal stress was removed, while the diatoms, *Syndera tabulate, Nitzschia obtuse* and *N. palae* lost their tolerance immediately after the metal stress was removed (Rao *et al*., 1994 and Skowronska, 2003).

4.1. Mechanism of tolerance to heavy metals:

It has been concluded that metal tolerance varies greatly among different algal species and largely dependent on metal and the prevailing conditions.

One mechanism by which many plants and algae respond to and apparently detoxify toxic heavy metals is the production of the amino acid proline (Delauney and Verma, 1993; Schat *et al*., 1997; Shah and Dubey, 1998; Mehta and Gaur, 1999 and Verma, 1999). The accumulation of proline in

stressed plants is associated with reduced damage to membranes and proteins (Alia *et al*., 1997; Shah and Dubey, 1998 and Verma, 1999).

Algae can also respond to heavy metals by induction of several antioxidants , including diverse enzymes such as superoxide dismutase, catalase, glutathione peroxidase, ascorbate peroxidase, and the synthesis of low molecular weight compounds such as carotenoids and glutathione (Emani *et al*., 2003 and Pinto *et al*., 2003).

A variety of mechanisms for heavy metal tolerance were also reported for algae such as chelation of metals by organic acids, amino acids, peptides or proteins (metallothionein and phytochelatin conjugates) or their compartmentalization away from metabolic processes by transport into the vacuoles (Howe and Merchant, 1992; Kaplan *et al*., 1995; Lee *et al*., 1996; Cohen *et al*., 1998; Guerinot, 2000, Hu *et al*., 2001 and Hall, 2002).

Algae detoxify Cd by forming Phytochelatin-Cd complexes which play an important role in heavy metal tolerance -mainly Cd- by decreasing their free concentrations (Gulriz, 2002).

Mallich and Rai (1998) found that cyanobacterium, *Anabaena dolilolum*, synthesized low molecular weight Cd-binding protein in response to Cd and they concluded that, this protein may play a role in metal tolerance. In the cyanbacterium *Anacystis nidulans*, protection against Cd^{2+} toxicity results from binding of Cd ions by a protein of low molecular weight analogous to the mammalian metallothionein (Maclean *et al*., 1972).

The tolerance of cyanobacterial mats to high metal concentrations may be due to their ability to precipitate insoluble metal salts outside the mat cells as either sulfides, oxides or hydroxides. Thick amorphous metal precipitates have been found on the surfaces of mats exposed to Pb, Ni and Cd ions. The precipitation of these metals in mats is likely to be controlled by a complex of mechanisms including the zonation of oxygen and redox, pH management and the availability of anionic pools such as sulfide and hydroxide. In certain cases, several mat mechanisms, at the cellular and community levels, become coupled in a complex precipitation process (Bender *et al*., 1995).

Albertano (1989) examined 45 species and strains of *Chlorella* and found that *Chlorella emersonii* was much more tolerant than all other species of *Chlorella*; and that within each species of *Chlorella*, strains tested exhibited different resistance to mercury. He stated that the tolerance of *Chlorella emersonii* was due to the peculiar structure of the cell wall which is double layered, with the outer trilaminar layer containing sporopollenin (a naturally occurring carotene like polymer which is able to bind the toxic material and then removes it from the cell). All other *Chlorella* species have a monolayered cell wall and are unable to produce sporopollenin. It is noteworthy that even in *Scendesmus* sp. A double-layered cell wall has been recognized (Pickett-Heaps and Staehelin, 1975).

Bartless *et al*. (1977) reported that *Chlorella* produces glucose dehydrogenase which reduced Hg ions to volatile metallic Hg at outer cell surface. De Filippis and Pallaghy (1976) and De Filippis (1978) found that

resistance of *Chlorella* to Hg was due to its cells has high capacity to volatilize Hg into the atmosphere. Apparently, the mechanisms of metal resistance depend largely on both the species of algae and the metal involved (Bariaud *et al*., 1985).

5. Heavy metal ions removal.

5.1. Conventional methods:

The existing conventional technologies used in heavy metal remediation involve precipitation, filtration, ion exchange, electrolysis, membrane processes and evaporative recovery. These are very expensive and have operational problems such as sensitivity to acids and salts and fouling (Aderhold *et al*., 1996 and Noraho and Gaur, 1996).

In addition, there are some constraints in the application of these technologies, such as poor selectivity, the production of solid residuals (10 times higher than the weight of the metal itself), outflow of dangerous ionic metals from the treatment facilities, organic compounds frequently inhibiting the process, and low efficiency of removal at metal concentrations lower than 10 mgl^{-1} or in large effluent volumes (Eccles, 1999 and Chojnackaa *et al*., 2004). One major disadvantage of conventional treatment techniques is the production of metal laden sludge, which represents a big solid disposal problem (Volesky, 1990 and Gupta *et al*., 2000).

Conventional methods for removing heavy metals from industrial effluents are often ineffective and costly particularly when heavy metals are present in

the wastewater at low concentrations or when very low concentrations of heavy metals in treated water are required (Sandau *et al*., 1996a; Jalali *et al*., 2002 and Travieso *et al*., 2002). The adsorption process with activated carbon attracted the attention of many scientists because of the effectiveness for the removal of heavy metal ion at trace quantities, but the process has not been used extensively due to its high cost (Shukla and Sakhardande, 1991; Benjamin *et al*.,1996 and Navarro *et al*.,1996).

Other metals clean up technologies including sedimentation, flocculation, adsorption with granular activated carbon (GAC), co-precipitation, cation and anion exchange, complexation, precipitation and oxidation/reduction are either inadequate or too expensive to some Countries. Therefore, research efforts has been directed towards wetlands as an alternative low cost means of removing heavy metals from domestic, commercial, mining and industrial wastewaters (Matagi *et al*., 1998; Holl, 2001 and Neyens *et al*., 2001).

There is a great need for an inexpensive, rapid and easy process for the removal of heavy metal ions from environmental samples such as industrial effluents (Stirk and Van Staden, 2002).

5.2. Bioremoval of heavy metal ions:

The use of biological materials for removing and recovering of heavy metals from contaminated industrial effluents has emerged as a potential alternative method to conventional techniques (Kratochvil *et al*., 1997). Bioremoval

processes are generally rapid and are in theory suitable for the extraction of metal ions from large volumes of water (Brady *et al*., 1999).

Recently, the efficiency of certain microorganisms and higher plants have been employed to remove heavy metals from large-scale processes such as the industrial effluents and mine water to much smaller operations involving the recovery of precious metals from process streams or waste water from the electroprocessing and jewelry industries (Madrid *et al*., 1998).

Compared to conventional methods, bioremoval technologies maintain several potential advantages including; (i) use of naturally abundant renewable biomaterials that can be cheaply produced, (ii) ability to treat large volumes of wastewater due to rapid kinetics, (iii) high selectivity in terms of removal and recovery of specific heavy metals and (iv) ability to handle multiple heavy metals and mixed waste (Wilde and Benemann, 1993).

Moreover, the bioremoval technologies are less expensive, can overcome many operational problems such as available space and has been proved to be efficient under a wide range of physicochemical conditions including temperature, pH, hardness and the presence of other ions, including Ca and Mg (Cossich *et al*., 2002).

5.3. Removal of heavy metal ions by algae.

Methods based on using biological systems have recently been developed, since certain microorganisms have a great capacity to accumulate heavy metal ions (Wong and Kwok, 1992; Singh *et al*., 1992 and McHale and

McHale, 1994). Microalgae have been found the most effective organisms used to remediate metal (Maeda and Sakaguchi, 1990; Burdin and Bird, 1994 and Gonzalez-Davila *et al*., 1995). Compared to other microorganisms, microalgae are able to take up, accumulate and concentrate heavy metals in significant amounts from aqueous solutions (Xue *et al*., 1988; Wilde and Benemann, 1993 and Sandau *et al*., 1996b). Algae are gaining increasing attention, due to the fact that they are relatively cheap to process and able to accumulate high metal content (Sandau *et al*., 1996b). Due to their ubiquitous occurrence in nature, the microalgae have been used to remove metals from aqueous solutions and diverse environmental samples including industrial wastewater as well (Bender *et al*., 1995 and Gekeler *et al.*, 1998).

Algae have been found to be potential suitable biosorbents because of their cheap availability, relatively high surface area and high binding affinity (Fehrmann and Pohl, 1993; Roy *et al*., 1993 and Bakkaloglu *et al*., 1998).

Microalgae have been used for the removal of heavy metals without the dangers imposed on the environment as it happens in the traditional physical–chemical purification processes (Canizares-Villanueva and Travieso, 1990, 1991and 1992). Moreover, the use of microalgae for metal removal has the potential to achieve greater performance at a lower cost than conventional wastewater treatment technologies. This is consistent with the recent trend for growing interest in biosorbent technology for removal of trace amounts of toxic metals from dilute aqueous waste (Wilde and Benemann, 1993).

Algae have been widely used for wastewater treatment and water quality improvement. Algae use solar energy while absorbing nutrients and other chemicals including heavy metals from wastewater to fix carbon substance and produce massive biomass (Wehrheim and Wettern, 1994 and Disyawongs, 2002). Microalgal biomass has been successfully used as sorbing agent to remove metal, because microalgae use light as an energy source, facilitating the maintenance of metabolism in the absence of organic carbon sources and electron acceptors required by bacteria or fungi (Holan and Volesky, 1994; El-Enany and Issa, 2000 and Azab *et al*., 2004). The microalgal based wastewater treatment system, predominated mainly by *Scenedesmus* and *Chlorella* was able to remove heavy metals with the ranges between 52.3% to 100% in a batch system and from 64.2% to 100% in case of the continuous system (Hammouda *et al*., 1995). The small scale cultivation tests have shown that over a long period heavy metal adapted cells of *Chlorella vulgaris* are able to bioaccumulate heavy metal cations in low concentrations and to remove them from the cultivation suspention (Sandau *et al*., 1996b).

Perez-Rama *et al.* (2002) found that living cells of the marine microalga *Tetraselmis suecica* removed 98.1% of added Cd. *Cladophora parriaudii* removed 80-94% of Cd introduced from a synthetic wastewater (Sternberg and Dorn 2002). Inthorn *et al.* (1996) reported that the filamentous cyanobacterium, *Tolypothrix tenuis*, exhibited a high level of Cd tolerance and had the highest Cd^{2+} removal ability among other 17 cyanobacterial

strains tested. The maximum removal of Cd^{2+} was achieved within 30 min. Inthorn *et al.* (2002) tested the efficiency of forty-six strains of green and blue green microalgae to remove Hg^{2+}, Cd^{2+} and Pb^{2+} from aqueous solutions within the relatively short time of 10-20 minutes. They found that the highest Hg removal was achieved by *Scenedesmus* sp. (97%) and the lowest by the cyanobacterium *Fischerella* (92%). However, the highest Cd removal was achieved by *Lyngbya heironymusii* (97%) and the lowest by *Scenedesmus acutus* (88%). Pb^{2+} removal was the highest with *Nostoc punciforme* (98%) and the lowest with *Chlorella vulgaris* (84%).

Sobhan and Sternberg (1999) studied the ability of *Cladophora* to remove Cd from a synthetic wastewater. They found that Cd^{2+} removal varied from 86% to 96%, with high degrees of removal observed in the first 48 hrs. *Chlorella minutissima*, adsorbed greater than 90% of the initial Pb^{2+} and greater than 98% of the initial Co^{2+} concentrations (Roy *et al*., 1993).

The accumulation of Cd^{2+} by *Scenedesmus* showed that the saturation was obtained within two hrs (Venkatarman *et al*., 1992). In a similar study with Cd^{2+} and Pb^{2+}, *Scenedesmus* could attain 80% accumulation of heavy metal within first 12 hrs after incubation in Cd^{2+} and Pb^{2+} containing test solutions. Uptake of Pb^{2+} and Cd^{2+} by *Scenedesmus* and *Spirulina* during dark phase was similar to the uptake during light (Venkatarman and Becker, 1986).

It may be relevant to mention that Wang *et al.* (1998) determined the specific adsorption capacity of *Phormidium* sp. to be 13.6 mg/kg for Pb^{2+} and 10.1 mg/kg for Cu^{2+}.

Shieh and Barber (1973) have demonstrated that immobilized *Chlorella* rapidly accumulate Hg^{2+}, and that uptake is dependant on inoculum density. *Chlorella* can accumulate Hg^{2+} rapidly and reach saturation within 1 hr at room temperature (Becker and Venkatarman, 1982).

Ibrahim *et al*.(2000) studied the efficiency of free and Ca-alginate immobilized seven algal strains in bioremoval of ten heavy metals from laboratory prepared test solutions and six industrial effluents. They found that the highest bioremoval efficiency for most of the ten metals was that of *Chlorella ellipsoida* followed by *Scenedesmus quadricauda var. longispina* then *Nitzschia palea*. They designed immobilized algal batteries at different flow rates for biological treatment of industrial effluents using the highest bioremoval efficiency species individually and in a mixture. All batteries were successful to remove heavy metals from the industrial effluents. Slow rate (1L/hr) was the best one in bioremoval of ten metals.

It is evident that the bioremoval of heavy metals by algae is largely dependent on algal species and on metal removed.

5.4. Mechanism of metal ions removal.

Three mechanisms by which microorganisms can remove metals from solutions are well recognized: (i) extracellular accumulation/precipitation; (ii) cell-surface sorption or complexation; (iii) intracellular accumulation (Muraleedharan *et al*., 1991). The first mechanism facilitates the second mechanism and both can occur with alive or dead microorganisms while, the

third mechanism requires specific physiological activities (Cossich *et al*., 2002). Binding or accumulation of heavy metal ions by biologically active algal molecules may occur by several mechanisms, such as surface binding or precipitation, or by intracellular transport and chelation (Wood and Wang, 1983).

Muramoto and Ohi (1983), Wilde and Benemann (1993) and Omar (2002) suggested that bioremoval is usually a two-stage process; an initial fast reversible metal binding phase biosorption followed by slow irreversible, ion bioaccumulation phase. Such process can occur via complexation, chelation and ion exchange.

Heavy metal ions uptake by cells of *Anacystis nidulans* can be attributed to two principal mechanisms: an initial passive uptake on cell wall surfaces and a slower active intracellular accumulation (Khattar *et al*., 1999). Bioaccumulation, generally, occurs in two stages, biosorption where metal ions bind to the cell wall via an ion exchange mechanism and metal ion transportation into the cellular interior (Chojnacka, 2003).

Nourbakhsh *et al*. (1994) stated that the kinetics of metal ions uptake took place in two stages. The first stage, thought to be physical adsorption or ion exchange at the cell surface, it occurs very rapidly within short time after the alga comes into contact with the metal. The second stage, is mainly related to metabolic activities, and occurs at relatively slower rates. Considerable amounts of metal ions may be bound to the cell wall, or to an intracellular

insoluble fraction (Garnham *et al*., 1992), or to polyphosphates (Jensen *et al*., 1982), or removed via phytochelation (Robinson, 1988).

Some studies revealed that, algae can remove toxic metals through possible three ways; intracellular chelation by biological polymers; precipitation of the heavy metals on the cell wall surface; or adsorptive surface binding to various cell wall chemical functional groups (Greene and Bedell, 1990). Several chemical functional groups have been proposed for the sequestering of metal ions. These have included amino, thioether, sulfhydryl, carboxyl, carbonyl, imidazole, phosphate, phenolic, hydroxyl, and amide groups (Crist *et al*., 1981; Greene *et al*., 1986 and Xia and Rayson, 2002). Carboxyl groups on algal cell biomass are responsible for binding of various metal ions (Gardea-Torresdey *et al*., 1990). Parsons *et al.* (2005) reported that gadolinium (III) and neodymium (III) ions were bound to alfalfa biomass through oxygen or nitrogen legands coordinated to carbon atoms.

The cell surface of many microorganisms, including cyanobacteria, consists of polysaccharides, proteins, and lipids, which act as a basic binding site of heavy metals. These functional groups within the wall provide the amino, carboxylic, sulfydryl, phosphate, and thiol groups that can bind metals (Ting *et al*., 1991). All the metal ions before gaining access to the plasma membrane and cell cytoplasm come across the cell wall. The cell wall consists of a variety of polysaccharides and proteins and hence offers a number of active sites capable of binding metal ions. Difference in cell wall composition among different groups of micro-organisms cause significant

differences in the type and amount of metal ion binding to them. Among the photoautotrophs, eukaryotic algal cell walls are mainly cellulosic. The potential metal binding groups in this class of microbes are carboxylate, amine, imidazole, phosphate, sulfhydryl, sulfate and hydroxyl. Of these, amine and imidazoles are positively charged when protonated and may build negatively charged metal complexes (Gupta *et al*., 2000).

Charged groups such as carboxylate and hydroxyl present in the biopolymers of algal biomass cell walls are believed to be responsible for the sequestration of metal ions (Hashim and Chu, 2004). Certain functional groups, such as amines and imidazoles are positively charged when protonated and may electrostatically bind negatively charged metal complexes (Ehrlich and Brierley, 1990).

Gardea-Torresdey *et al*. (1990) found that structural and functional groups of proteins and polysaccharides on algal cell walls of several algal species such as *Chlorella vulgaris*, and *Spirulina platensis* played a significant role in the heavy metal ions adsorption. *Spirulina platensis* cell wall is rich in protein (54-67%) and contains relatively small amount of polysaccharides (10-18%), other eukaryotic algal species have higher cell wall carbohydrate contents, for example, the brown marine alga *Eisenia bicyclis* cell wall is 60% carbohydrates (Gardea-Torresdey, 1988).

The role of algae in waste water treatment and their affinity for heavy metal cations, based on high negative surface charge, has also been recognized (Chmielewská and Medved, 2001). Although adsorption on the cell surface

is the dominant mechanism, both surface adsorption and internal diffusion are involved in the uptake of metals by algae (Kuyucak and Volesky, 1989b). Metallothioneins (polypeptides) which bind metal ions like Cd^{2+}, Hg^{2+}, and Pb^{2+} in metal-thiolate clusters. These polypeptides are abundant in cysteine residues and often possess a characteristic pattern of sulfur containing amino acids (Turner and Robinson, 1995).

Algae are able to bind high amounts of heavy metals from the surrounding water (Revis *et al*., 1989) and they respond to the intracellularly accumulated metal by synthesizing phytochelatins of different chain-length (Ahner and Morel, 1995; Gekeler *et al*., 1998; Skowronska *et al*., 1998 and Skowronska, 2000). Skowronska (2002) found that exposure of *Stichococcus bacillaris* to Pb^{2+}, induced rapid production of thiol peptides as a consequence of intracellular Pb^{2+} accumulation.

Ion-exchange is an important concept in biosorption, because it explains many of the observations made during heavy metal uptake experiments (Davis *et al*., 2003). Hörcsik and Balogh (2002) tested the intracellular distribution of Cr^{3+} in *Chlorella pyrenoidosa* and found that 70% of accumulated Cr was localized in the cell wall while the cell membrane and soluble fraction contains a smaller part of Cr^{3+}, also they found that high concentration of chromium ions in the algal cells causes a higher concentration of calcium and a lower concentration of magnesium ions and iron ions. The reason of this phenomena can be an ion exchange. Schneider *et al*. (2001) reported that the sorption of heavy metal ions onto algae,

bacteria and higher plants is through a specific ion exchange mechanism. The sorption is thought to involve the replacement of protons or other cations by the heavy metal ions.

The dominant chemical moiety involved in binding Cd was found to be carboxylates. Kropfl *et al.* (2003) found that algal biofilms can accumulate various metal ions (e.g. Cd^{2+}, Cu^{2+}, Cr^{2+}, Pb^{2+} etc.) from the aquatic environment. The accumulation of metals by algal biofilms can be attributed to the complex- or chelat-forming capacity of the extracellular polymeric substances produced by the biofilms and consists of high number of carboxyl groups (Geesey and Jang, 1989), hydroxyl and acetyl groups (Sutherland, 1990).

Algal mucilage consists mainly of polysaccharides with smaller amounts of protein (Decho and Herndl, 1995 and Lee, 1997) may contain relatively large amounts of functional groups for metal binding. From the investigations of Amemiya and Nakayama (1984) and Weckesser *et al*. (1988), isolated mucilaginous sheaths were able to bind large amounts of metals and contained more metals than the surrounding medium. Algal surfaces (cell wall/mucilages) have been found containing different functional groups such as carboxyl, hydroxyl, sulphate and other charged groups which are created by their carbohydrate, protein and lipid components and that mucilage may be able to explain the binding mechanisms (Tien, 2002).

Another type of mechanism is described as molecular mimicry, whereby metal ions either compete for binding to multivalent ion carriers (such as Ca^{2+} channels) or, after binding to low molecular weight thiols (such as cysteine) (Pinto *et al*., 2003).

It seems that the mechanisms by which algae remove metal ions are diverse and ranged from a simple physical phenomenon to highly complicated physiological and biochemical processes.

5.5. Time required for bioremoval:

Sakaguchi *et al*. (1979) and Khummongkol *et al.* (1982) reported that Cd^{2+} adsorptions by *Chlorella vulgaris* and *Chlorella regularis* were rapid and completely achieved within 10 to 30 minutes, respectively. Singh and Prasad (2000) studied the sorption of Cd^{2+} in *Spirogyra* sp. immobilized on modified silica gel. They reported high adsorption rates, in which adsorption was achieved in about 20 min. Hashim and Chu (2004) examined seven species of brown, green, and red seaweeds for their abilities to remove Cd ions from aqueous solutions, they found that Cd^{2+} uptake was fast as 90% or more of the uptake occurring within 30–40 min of contact time. Yin *et al.* (2001) investigated biosorption and desorption of Cd^{2+} from aqueous solutions by the biomass of marine alga *Laminaria japonica*. They found that more than 90% of the adsorption occurred within 20 minutes and the adsorbed Cd^{2+} cannot be desorbed by distilled water, but it can be effectively recovered by using acidic or EDTA solutions.

Kaewsarn and Yu **(**2001) investigated the removal of Cd^{2+} by marine alga *Padina* sp. from aqueous solutions, and they found that 90% of removal took place within 35 min. Horikoshi *et al*. (1979) reported that *Chlorella regularis* rapidly uptook uranium during the first 6.0 min. Les and Walker (1984) and Disyawongs (2002) reported that Cu^{2+}, Pb^{2+}, Hg^{2+} and Cd^{2+} ions were removed rapidly by *Chroococcus paris* and approximately 90% of the total amount of added metal was removed within 1.0 minute.

Zhao *et al.* (2001) found that biomass of red tide alga *Prorocentrum micans* has a high uptake capacities for six heavy metal ions (Cu^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Ag^{2+} and Cd^{2+}) and about 90% of the biosorption occurred within 10.0 min.

The Cr^{3+} ions biosorption by biomass of the marine alga *Sargassum* sp. was fast, reaching 60% of the total biosorption capacity in 10.0 minutes (Cossich *et al*., 2002). Wang *et al*. (1998) determined the specific adsorption capacity of *Phormidium* for Pb^{2+} and Cu^{2+} ions. They found that, the alga could reduce metal ions concentration from 1.0 mgl^{-1} to very low levels of 0.01 mgl^{-1}within 60 min. Nuhoglu *et al*.(2002) found that the rate of removal of Cu^{2+} from aqueous solutions by *Ulothrix zonata* was extremely rapid in the first 20 min. Carrilho *et al*.(2003) investigated the ability of *Chlorella vulgaris* to accumulate heavy metals in solutions. This alga was able to bind Cr^{3+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, and Zn^{2+} ions at pH 8.0 in 15 min of contact time. *Oscillatoria anguistissima* rapidly adsorbs appreciable amounts of Co^{2+} ions from the aqueous solutions within 15 min of initial contact with the metal solution (Ahuja *et al.*, 1999).

5.6. Optimum pH range of metal ion bioremoval:

Rangsayatorn *et al.* (2002) examined the efficiency of alginate immobilized cells of *Spirulina platensis* to remove low concentrations of Cd^{2+} (less than 10 mgl^{-1}) from wastewater. They found that the removal was pH dependent and the Cd^{2+} removal was significantly increased from 72.64% at pH 4.0 to 91.86% at pH 6.0. The maximum uptake was 95.93% at pH 7.0.

The uptake of heavy metal ions (Pb^{2+}, Cu^{2+} and Cd^{2+}) by algal biomass reached a high plateau at around pH 4.0-5.0 or slightly higher (Matheickal and Yu, 1996 and Matheickal *et al*., 1997 and 1998). Hashim and Chu (2004) examined seven species of brown, green, and red seaweeds for their abilities to sequester Cd^{2+} ions from aqueous solution and found that Cd^{2+} uptakes was similar within the pH 3.0–5.0 range but decreased significantly when the solution pH was reduced to pH 2.0. Zhou *et al*. (1998) found that the uptake of Cu^{2+} and Cd^{2+} ions by *Laminaria japonica* and *Sargassum kjellmanianum* increased with increasing pH from 3.0 to 5.0, but further increases above pH 5.0 led to a reduction in sorptive capacity.

The cyanobacterium *Lyngbya taylorii* exhibited high uptake capacities towards four metals namely cadmium, lead, nickel, and zinc from aqueous solution. The optimum pH for the uptake was between pH 3.0 and 7.0 for Pb^{2+}, Cd^{2+}, and Zn^{2+} ions and between pH 4.0 and 7.0 for Ni^{2+} ions (Klimmek *et al.,* 2001**)**. Sanchez *et al*. (1999) investigated the adsorption of two metal ions Cu^{2+} and Zn^{2+} ions in a single-component system by the brown alga *Cymodocea nodosa* at different pH values. They found that pH

significantly affected the uptake rate, with maximum reached at a pH value of 4.5. The cyanobacteria *Oscillatoria anguistissima* rapidly adsorbs Cu^{2+} from aqueous solution with maximum removal achieved at pH 5.0 (Ahuja *et al.*, 1997).

Gardea-Torresdey *et al.* (1996a) studied the effect of pH on uptake of Cu^{2+}, Pb^{2+} and Ni^{2+} ions to the *Synechococcus* biomass. High affinity for all metal ions was noticed as the pH increased from 2.0 to 6.0 with optimum binding occurring at pH 5.0.

6. Efficiency of living and nonliving algal cells to remove metal ions: A comparative outlook.

Biosorption is an inexpensive and efficient method to remove metal ions from solutions and has an application in controlling industrial effluents. It happens in two ways; passive and active. The sorbent may be living, and uptake would include both passive and active methods, or non-living where only passive uptake would occur (Stirk and van Staden , 2002). Many studies have been performed on metal ions uptake by both living and nonliving cells of microalgae. Although dead algae have been utilized successfully in heavy metal adsorption experiments (Leusch *et al*., 1995 and Holan *et al*., 1998), living algae remove significantly more metal ions than nonliving algae at all metal concentrations examined, probably due to metabolic uptake and continuous growth (Maeda and Sakaguchi, 1990 and Terry and Stone, 2002).

Lucido and Iwasaki (1991) investigated Cu^{2+} ions adsorption using heat-dried *Cyanidium caldarium* and they found that no Cu^{2+} ions was adsorped from a 50 ppm Cu^{2+} solution. They concluded that the heat-drying destroyed the surface adsorption properties.

Garrido *et al*.(1998) studied the accumulation of Cu^{2+} ions by live and dead cells of the marine microalga *Nannocbloropsis gaditana* and found that the amount of adsorbed Cu^{2+} (69%) is higher in the alive cells than in the dead cells (61%). The difference in Cu^{2+} uptake was mainly attributed to the existence of an active defense mechanism of the living cells when exposed to the metal such as metal pumping (complexation or precipitation of metal on the cell surface) and the lack of photosynthetic processes which are possessed by living cell.

Synechococcus sp. was found to possess a Cu^{2+}-transporting P-type ATPase in the thylakoid membrane (Bonilla *et al*., 1995).

Moreover, living algae possess intracellular polyphosphates which participate in metal sequestration, as well as algal extracellular polysaccharides that serve to chelate or bind metal ions (Van Eykelenburg, 1978; Kaplan *et al*., 1987; Zhang and Majidi, 1994 and Gardea-Torresdey *et al*., 1996a).

7. Superiority of immobilized algae to remove metal ions.

In order to retain the ability of microbial biomass to sorb metal ions during the continuous industrial process, it is important to utilize an appropriate

immobilization technique. The free cells can provide valuable information in laboratory experimentation but are not suited for column packing in industrial applications (Ross and Townsley, 1986). The free cells generally have low mechanical strength and small particle size and excessive hydrostatic pressures are required to generate suitable flow rates. High pressures can cause disintegration of free biomass. These problems can be avoided by the use of immobilized cell systems (Cotoras *et al.,* 1993 and Leusch *et al.,* 1995). Immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems (Holan and Volesky, 1994).

Cell immobilization, which is applied to various biotechnological processes, is one approach that can avoid the biomass–liquid separation requirement. It can prevent the independent movement of cells during the aqueous phase of the system. Immobilization appears to be one of the best techniques to separate physically micro-algal cells from their culture medium for the purpose of algal tertiary wastewater treatment. Moreover, by using immobilization on screens, removal of nutrients from wastewater was higher than with conventional biological tertiary wastewater treatments (Kaya *et al.,* 1994).

The ease in harvesting and potential for repeated use makes the immobilized cells good tools for scavenging heavy metals from metal contaminated environments (Rai and Mallick, 1992).

In a number of studies, the biomass has been immobilized using inert solid supports as biofilms. These inert matrices include polyvinyl chloride, glass, metal sheets, plastics, uneven surfaces e.g. wood shavings, clay, sand, crushed rocks and porous materials like foams and sponges (Gupta *et al*., 2000). For cells of *Spirulina platensis* immobilized in both alginate and silica gels, Cd adsorption was higher than 95% for both immobilized cells (Rangsayatorn *et al*., 2004). Park *et al*. (1997) found that both alginate and silica immobilized algal cells have an advantage, over free cells when applied to treatment of the most heavy metal contaminated wastewater.

Alginate immobilized and free cells of *Anabaena doliolum* and *Chlorella vulgaris* were compared for their use in the removal of Cu^{2+} and Fe^{3+} ions. Compared with free cells, the immobilized cells showed greater efficiency for metal removal, even over 3 repeated cycles (Rai and Mallick, 1992).

The accumulation and volatilization of Hg by non-immobilized and immobilized *Chlorella emersonii* have been studied in batch culture systems. Reduction in the Hg^{2+} concentration in the growth medium by non-immobilized cells was highly dependent on inoculum density, whilst reduction in Hg^{2+} concentration by immobilized cells was rapid at all inoculum densities. Hg^{2+} accumulation by immobilized cell biomass was significantly greater than by non-immobilized cells (Wilkinson *et al*., 1990).

Kropfl *et al*.(2003) found that algal biofilms can accumulate various metal ions (e.g. Cd^{2+}, Cu^{2+}, Cr^{2+}, Pb^{2+} etc.) from the aquatic environment . This

accumulation has been exploited to reduce metal contamination of rivers and lakes.

8. Elution of heavy metal ions removed by algal biomass.

Ehrlich and Brierley (1990) found that gold ion binding to algae is reversible. A column containing silica-immobilized *Chlorella pyrenoidosa* was prepared to bind gold ions. An acidic thiourea solution was then introduced into the column, to recover bound gold ions. Gold ions loading and stripping cycles were repeated more than 50 times with no loss of column performance.

Nakajima (2003) screened thirty species of microorganisms (8 bacteria, 9 actinomycetes, 8 fungi and 5 yeasts) for maximal gold accumulation. Most of the actinomycetes, fungi and yeasts had lower ability to accumulate gold ions than bacteria. The gold ions adsorbed on the cells were easily desorbed with 0.1 M thiourea solution. All immobilized cells could be used repeatedly in the adsorption–desorption cycle using 0.1M acidic thiourea solution as a desorbent agent.

Lau *et al*. (2003) screened three seaweed species collected from Hong Kong waters for their adsorption abilities for Cu^{2+}, Ni^{2+} and Zn^{2+} ions. *Ulva lactuca* having the highest metal ion removal capacity. They found that 0.1M thiourea efficiently recovered close to 100% adsorbed metal ions from *Ulva lactuca*. In three successive adsorption-desorption cycles, reduction in metal ion removal capacity was found in the second and third cycles, but

almost all adsorbed metal ion could be recovered. Dziwulska *et al.* (2004) demonstrated the ability of immobilized green alga *Chlorella vulgaris* for selective binding of Platinum and Palladium ions from acidic solutions (at pH range 1.5–1.8). They found that the use of immobilized algae packed into a column in a flow mode provides good efficiency and reproducibility of the biosorption process ($95.2 \pm 0.4\%$ for Pt^{2+} and $98.3 \pm 0.9\%$ for Pd^{2+}). The best efficiency of elution for both metals was obtained with 0.3 M acidic thiourea (in 1.0 M HCl) used as a stripping reagent.

Gardea-Torresdey *et al.* (1998) using biorecovery system from *Medicago sativa* (alfalfa) biomass for the recovery of gold ions from aqueous solutions in an environmentally friendly manner. They found that, immobilized alfalfa binds gold quickly (within five minutes). Treatment of the column containing immobilized alfalfa with 0.2M acidic thiourea (in 0.2M HCl) recovered up to 99.1% of the bound gold metal.

9. Removal-elution cycles.

The reusability of the immobilized *Spirulina platensis* cells was tested in five cycles of Cd^{2+} adsorption and desorption. After the first cycle, Cd^{2+} uptake by alginate and silica immobilized cells was reduced from 94.07% and 92.67% to 70.79% and 66.99%, respectively. The adsorption efficiency of both alginate and silica immobilized cells was still high and ranged from 68.47% to 63.21% with five successive cycles (Rangsayatorn *et al*., 2004).

Jalali *et al*.(2002) found that *Sargassum hystrix* biosorbed Pb^{2+} rapidly, in less than 30 min of contact. Removal of Pb from *Sargassum* biomass was successfully achieved by eluting with 0.1M HNO_3 for 15 min. Pb^{2+} released to this dilute mineral acid with 95% elution efficiency. In repeated use of biomass experiment, the Pb^{2+} uptake capacity of *Sargassum* biomass was constantly retained (98%) and no significant biomass damage took place after 10 sorption-desorption cycles. Stirk and Van Staden (2002) found that the brown alga *Ecklonia maxima* was promising with no deficiency in its metal binding capacity being recorded after 4 desorption cycles. Similarly, both *Sargassum fluitans* and *Ascophyllum nodosum* can be used for a number of adsorption desorption cycles (Aldor *et al*., 1995).

The fern *Azolla filiculoides* could be regenerated for up to 6 cycles with adsorption being only slightly lower than for Cycle 1 (Zhao *et al*., 1999). Schneider *et al*., (1999) observed that, although there was some loss of Cu^{2+} sorptive capacity of freshwater macrophyte *potomogenten lucens* biomass after several loading and elution cycles, the sorptive capacity remained essentially unchanged for up to 100 loading and elution cycles.