REMOVAL OF LEAD FROM SOLUTION BY THE NON-VIABLE BIOMASS OF THE WATER FERN *AZOLLA FILICULOIDES*

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REMOVAL OF LEAD FROM SOLUTION BY THE NON-VIABLE BIOMASS OF THE WATER FERN *AZOLLA FILICULOIDES*

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ABSTRACT

The removal of lead from aqueous solution and lead-acid battery manufacturing waste-water by the non-viable biomass of the water fern *Azolla filiculoides* was investigated in both batch and column reactors. The maximum lead uptake by the *Azolla* biomass at a pH value of approximately 5, was found to be 100 mg lead/g biomass from aqueous solution. Lead removal varied from 30% of the initial lead concentration at pH 1.5 to approximately 95% at pH values of 3.5 and 5.6. Lead removal from aqueous solution decreased to 30% of the initial lead concentration if the lead concentration was initially over 400 mg/l. At initial lead concentrations of less than 400 mg/l, percentage lead removal was found to be over 90% of the initial lead concentration. Lead removal remained at approximately 90% between 10 °C and 50 °C. Biomass concentration (4-8 mg/l) had little effect on lead removal. The presence of iron (Fe) and lead, copper (Cu) and lead or all three metal ions in solution at varying ratios to each other did not appear to have any significant effect on lead removal. Percentage lead, copper and iron removal from aqueous solution was 80-95, 45-50 and 65-75 % respectively for the different multiple-metal solutions studied.

No break-through points were observed for lead removal from aqueous solutions in column reactors, with initial lead concentrations of less than 100 mg/l at varying flow rates of 2, 5 and 10 ml/min. This suggested that flow rate, and therefore retention time, had little effect on percentage lead removal from aqueous solution, which was more that 95 %, at low initial lead concentrations (less than 100 mg/l). At initial lead concentrations of 200 mg/l or more, an increase in flow rate, which equates to a decrease in column retention time, resulted in break-through points occurring earlier in the column run. Percentage lead removal values, from lead-acid battery effluent in column systems, of over 95 % were achieved. Desorption of approximately 30 % and 40 % of bound lead was achieved, with 0.5 M HNO_3 in a volume of 50 ml, from two lead-acid battery. Repeated adsorption and desorption

of lead by the *Azolla* biomass over 10 cycles did not result in any decrease in the percentage lead removal from effluent, which strongly suggested that the *Azolla* biomass could be re-used a number of times without deterioration in its physical integrity, or lead removal capacity. No evidence of deterioration in the *Azolla* biomass's physical integrity after 10 successive adsorption and desorption procedures was observed using scanning electron microscopy.

The *Azolla filiculoides* biomass was, therefore, found to be able to effectively remove lead from aqueous solution and lead-acid battery effluent repeatedly, with no observed reduction in it's uptake capacity or physical integrity.

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ABBREVIATIONS

AAS	Atomic absorption spectrophotometer
DWA	Department of Water Affairs
EDTA	Ethylenediaminetetraacetic acid
LIRI	Leather Industries Research Institute
LRBS	Lead-acid battery recycling site
SEM	Scanning electron microscopy

CHAPTER ONE

INTRODUCTION

1.1 General introduction

The total supply of fresh water world-wide is believed to be sufficient to meet human demand. Generally man tries to live in areas of high annual rainfall where water supply is plentiful, but has, due to population growth, been forced into areas of low rainfall. In such situations, survival depends on water conservation, ground water and springs bringing water from distant water sheds and recently from desalting sea water. The importance of potable water as a natural resource can therefore not be underestimated (Dean and Lund, 1981).

Water that is free from contamination of one form or another is fast becoming a scarce commodity, not only in South Africa but world wide. It is therefore crucial that researchers work on making waste-waters available for re-use wherever possible. Heavy metal contamination of waste-waters makes it problematic for re-use in industry, agriculture and for domestic purposes.

1.2 Water in South Africa

South Africa's location within the high pressure belt of the middle latitudes of the Southern hemisphere, means that warm dry descending air associated with high pressure systems are found over a large part of the country most of the time, which is not favourable for rain formation. However, air currents along the east and west coasts' influence tends to modify this climatic situation and give rise to precipitation. Rainfall distribution across the country is uneven, with, for example,

the average annual rainfall on the east coast being 1 070 mm compared to 58 mm at a location at the same latitude along the west coast. Severe and, at times, prolonged droughts also tend to occur, and these are usually terminated by severe floods (Department of Water Affairs, 1986).

The agriculture sector accounted for a large portion of water use in South Africa at the end of the 19th century. Most developments by the state at that time were geared toward benefiting irrigation schemes. Industrial growth, however, saw a new Water Act (Act 54, 1956) being passed, which was supposed to ensure equal distribution of water for industrial and other competing users, and at the same time create stricter control over abstraction, use, supply, distribution and pollution of water. However, the inevitable increase in development has resulted in rapid deterioration of water quality, and the scarce supplies are geographically mismatched with respect to demand. As this is a national problem, possible solutions need to be directed from State level, therefore the Department of Water Affairs (DWA) continues to refine and implement national water management strategies for the optimization of resource use and infrastructure development. This necessarily involves water allocation with appropriate controls over use and disposal, especially of polluted waste-waters. The increasing population growth, rising standards of living, industrialisation, urbanisation and agricultural activities, will strongly influence future water consumption (Department of Water Affairs, 1986).

A Commission of Enquiry into Water Matters was set up during the 1969 drought, and its recommendations were accepted by the South African government in 1970. The commission recommended, among other things, the re-cycling of water in industrial processes to reduce fresh water intake, and treatment of any effluent before discharge into natural water ways (Department of water affairs, 1986).

The National Water Bill 1998, recently (June 1998) passed by the National Assembly and signed by the President of South Africa Nelson Mandela (August 1998), is based on the principle that National Government has overall responsibility for and authority over water resource management in South Africa. The Bill serves, amongst other things, to redress the results of past racial and gender discrimination by promoting equitable access and beneficial use of water. A broad definition of the term "water use" is given in The Bill to include taking and storing water and, of interest to industry, waste discharges and disposals. The national water resource strategy provides a framework for the protection, use, development, conservation, management and control of water resources for the whole of South Africa. This framework takes into account, amongst other factors: the promotion of efficient and sustainable use of water in the public interest; the protection of aquatic and associated ecosystems and their biological diversities; and the reduction and prevention of pollution and degradation of water resources. With this in mind, responsible authorities may attach conditions to general authorisations or licences relating to: water management, including payment of charges; return flow and discharge or disposal of waste; and compensation to another person (Perkins, 1998).

A protection policy framework for water resources recognizes that water is a finite but regenerating resource, and careful, efficient utilisation of this resource is needed to ensure that it is available to all users. Sustainable management is, therefore, seen as crucial to the balancing of long and short term development and use of water resources, and this includes protection of the water resources. Four regulatory areas are used as the basis for implementing the water protection policy: resource-directed measures which define desired levels of protection for water resources by giving numerical or descriptive goals for water resource quality; source-directed controls which control the impact on the water resource through regulatory activities such as registration, permits, directives and prosecutions, and economic incentives in the form of levies and fees; managing demands on water

resources to maintain its utilisation within the limits set out for protection; monitoring of water resources throughout the country regularly, and modifying water resource management measures and impact control as and when necessary (McKay, 1998).

In order to implement a policy for raising water-use charges, clear establishment of the party responsible for such payments was important. The following were identified as responsible for payment of water uses: all registered or authorised existing lawful water users; all newly licensed water users; and all registered users of water permissible under a general authorization. Barriers to the implementation of the water pricing policy included, among other factors, existing agreements with major water uses which required negotiation (Pretorius *et al.*, 1998).

1.3 Heavy metals as pollutants

Heavy metals include about forty elements with a density of greater than five. Some of these heavy metals are required as trace elements for growth by prokaryotes and eukaryotes e.g. copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), cobalt (Co) etc, however, other metals like cadmium (Cd), mercury (Hg), lead (Pb), are not essential for cell growth, but are very toxic even at low concentrations (Trevors *et al.*, 1986). Toxic effects of heavy metals on cells is mainly due to their ability to denature proteins. An understanding of the mechanisms of microbial tolerance is important, as metals and their compounds are extensively used in fungicides and disinfectants (Gadd and Griffiths, 1978).

Concentrations of heavy metals in natural environments are generally low, and most of that present in sediments, soils and mineral deposits is biologically unavailable. Elevated metal levels can occur in some specific natural locations e.g. deep-sea vents and volcanic soils (Gadd, 1990b). Industrial

growth and increased domestic activities means the biogeochemical cycling of many elements have increased, including heavy metals. This has resulted in large amounts of metals being deposited into natural aquatic and terrestrial ecosystems (Babich and Stotzky, 1978).

Some aqueous effluent streams from energy production processes contain heavy metals which are hazardous to the environment due to their chemical or radiological properties (Shumate *et al.*, 1978). Metals like Ni, Pb, Hg, Zn, Cd, Cu, are being concentrated in ecosystems, both aquatic and terrestrial, as a result of processes like coal burning, mining, milling and smelting of ores, reprocessing and recycling industries such as battery crushing and manufacturing processes like photographic and metal plating companies (Stokes, 1983).

The change in attitude to waste-water as an important resource can be seen in the evolution of the terminology from "waste-water disposal" to "waste-water treatment or reclamation". Due to their importance as pollutants in waste-waters, heavy metals present in sewage sludge for agricultural use must be below regulation standards. Heavy metals not removed with the sludge end up in the waste-water. In areas where water re-use is practised to a large extent, the efficiency of heavy metal removal in waste-water treatment processes is important in preventing pollution of rivers. This becomes even more important where the treated water is used for potable supply (Lester and Sterritt, 1985).

1.4 Role of microorganisms in ecosystems

Microbes play a crucial role in both aquatic and terrestrial ecosystems: they are essential components in the biogeochemical cycling of elements and are the basis for all food chains and webs, play a role in energy incorporation by chemosynthesis and photosynthesis, and are the main contributors to

waste reduction and sustaining fertility of aquatic and terrestrial ecosystems. If a specific metabolic group is destroyed e.g. the cellulose decomposers, de-nitrifiers, nitrogen fixers or chemoautotrophs, the overall ecology of the ecosystem could be adversely influenced. It is therefore crucial to understand the microbiota's response to pollutants such as heavy metals, and recognize the biotic and abiotic physiochemical factors affecting the microbial responses (Babich and Stotzky, 1978).

1.5 Effects of heavy metals on microbes

Methods of heavy metal toxicity include: the binding of various organic ligands thus causing denaturation of proteins, disruption of cell membranes, decomposition of essential metabolites and metals acting as anti-metabolites towards essential nutrients (Gadd, 1986).

Some studies have been done to determine the effects of heavy metals on microbes. Concentrations of nickel (Ni) of 4 $x10^{-4}$ M increased the lag phase of growth of the marine bacterium *Arthrobacter marinus* from three to seventy hours. Sea water with 50-25 000 mg/l of zinc (Zn) reduced initial growth of the diatom *Skeletonema costatus, Thalassiosira pseudonana* and *Phaeodactylum tricornutum*. Other metals were also shown to affect microbial growth e.g. mercury (Hg) and chromium (Cr).

Morphological changes due to metal toxicity have also been reported, with 50 mg/l Zn inhibiting trap formation by the nematode-trapping fungus, *Monacrosporium eudermatum*. Cadmium (Cd) and copper (Cu) also induced other morphological changes in the microbes studied (Babich and Stotzky, 1978).

The biochemical activities of microbes, some of which are crucial in biogeochemical cycling, have

also been shown to be affected by heavy metals. Using pure culture studies, as little as 0.005 mg/l Zn inhibited nitrogen fixation by the cyanobacterium *Anabaera spiroides*. Other metals inhibited or reduced photosynthesis by algae and nitrogen fixation in other microorganisms. In mixed population studies, 1-2.5 ppb Cu and 15 ppb Zn stopped photosynthesis of a costal marine phytoplankton community. A concentration of 1 mg/l Cd or lead (Pb) was observed to reduce photosynthesis in brackish water phytoplankton community. Reduction or inhibition of nitrogen fixation was also observed with Cu and mercury (Hg) (Babich and Stotzky, 1978).

Population dynamics studies on the effects of heavy metals on microbes have also been carried out. For example addition of Cu to fresh water to treat a bloom of *Ceratium hirundinella* resulted in a decrease in numbers of diatoms, as did the addition of Ni. The same effects were observed in marine ecosystems, where bacterial diversity was reduced by Cu or Hg. It was observed that organisms like fresh water shrimp, *Gammarus pulex* and marine oyster, *Crassostrea virginica*, had reduced viability or showed poor growth and increased death rates when fed with microbes e.g. algae or fungi contaminated with metals like Cd or Cu (Babich and Stotzky, 1978).

1.6 Metal speciation and toxicity

"Metal speciation" refers to all the possible chemical forms of a metal that can occur in different conditions. This is important since the toxicity of metal species varies and influences its availability. In hard fresh water of pH8, Cd occurs as $CdCO_3$ which is unavailable for uptake by microbes. In sea water of the same pH, Cd occurs as $CdCl_2$ totally dissolved and available for uptake. Several abiotic factors affect toxicity and speciation e.g. pH, redox potential (E_h), inorganic ionic composition, hydrous metal oxides, organics, temperature and hydrostatic pressure (Babich and Stotzky, 1978).

An increase or decrease in pH changes metal toxicity to microbes. The mechanism(s) for this effect has not been clearly defined and contradictory results have been observed. This lack of uniformity may be due to different optimum pH of media affecting observed toxic effects, e.g. an increase in pH from 5.5 to 7.5 increased the toxic effect of Zn on *Saprolegnia* and *Achyla* species, but further increases in pH appeared to reduce toxicity. Increases in pH from 4 to 8 also increased Zn's toxicity to the algae *Hormidium rivulare* (Babich and Stotzky, 1987). There has been reports of low diversity of phytoplankton as a result of surface water acidification. Trace metal toxicity has received specific attention, given the geochemical mobilisation of certain trace metals associated with surface water acidification. Increased concentrations of aluminium (Al), Mn and Zn have been noted in lakes at low pH values. Changes in pH affect trace metal uptake because of the change in the trace metal speciation in solution and hydrogen ions competition at the cell surface. Metal complexes tend to dissociate with lowering of solution pH (Schenck *et al.*,1988).

The type and number of inorganic cationic elements may reduce toxicity, more so if the metal retains its cationic state e.g. Hg^{2+} rather than $HgCl_3$. Competition of these heavy metal cations and those cations normally present in the environment determine toxicity e.g. high levels of magnesium (Mg) reduce the toxic effect of Cu on photosynthesis of *Phaeodactylum tricornutum*, and it also decreases the toxic effects of Zn on the same microorganism. Inorganic anions affect speciation and therefore toxicity, e.g. OH^- ions form coordination complexes with heavy metals, and these have different stabilities and Zn and Zn^{2+} are less toxic than $ZnCl_3^-$ (Babich and Stotzky, 1978).

Hard water is usually alkaline with considerable amounts of bicarbonate and carbonate ions rather than free carbon dioxide as in soft water. Water hardness reduces the toxicity of some heavy metals to microbes, but the toxicity of Hg is increased in hard water and that of Mn is unaffected (Babich

and Stotzky, 1978).

Hydrous metal oxides can exchange heavy metals to a lesser extent than clay, therefore reducing their uptake by microbes. At the pH of most natural ecosystems, clay minerals have negative charges which adsorb cations like K^+, Mg^{2+}, Ca^{2+}, and there is continuous exchange of cations. The concentration of competing cations or ligands and the pH affect cation exchange (Babich and Stotzky, 1978).

Soluble or particulate matter, e.g. humic acids can complex with differing amounts of metals influencing their mobility and bio-availability and therefore their toxicity. Abiotic factors eliminating or reducing the cationic valency of metals reduce their ability to complex with organic matter. Some aqueous microorganisms exude organic material which complexes with and decreases Cu toxicity, e.g. fresh water algae *Tribonema aequale* (Babich and Stotzky, 1978). Chelation has been suggested to be the single most important abiotic factor in reducing Cu toxicity in aqueous ecosystems, e.g. ethylenediaminetetra-acetic acid (EDTA).

Temperature affects the sensitivity of microorganisms to metals, but does not affect metal speciation. However there is contradictory data on metal-temperature interaction, whether microbial sensitivity increases or decreases with changes in temperature. Elimination of some toxic effects of metals at high hydrostatic pressures has been reported, while some microorganisms become more sensitive at high hydrostatic pressures and others are unaffected (Babich and Stotzky, 1978).

It is clear, therefore, that the degree of metal toxicity depends on several factors including metal concentration, metal type, oxidation state, organism involved, and other cations present in solution.

Some metals have been reported to be less toxic at higher concentrations of biomass and substrate. Mathematical models to describe metal inhibition of substrate removal generally only apply to experimental conditions and are difficult to apply to more complex systems of waste-water treatment where many other factors are involved (Tyagi, 1985).

1.7 Metal-metal interactions

Effluents seldom contain only one heavy metal pollutant, but rather multiple toxicants. The response of microbes to an individual metal may differ from their response to stress from multiple metals. Antagonistic interaction refers to the protective effects of one metal on the toxicity of a second metal. This is probably due to competition for binding sites on the cell surface with subsequent reduction in the uptake and accumulation of both metals. Synergistic interaction can be defined as the enhanced toxicity of one metal in the presence of another metal, probably due to increased permeability of the plasma membrane when stressed by several toxicants. Additive interaction is the sum effect of individual toxicities, where the concentration and sequence of exposure to different toxicants is important in metal-metal interactions (Babich & Stotzky, 1978).

1.8 Biotic factors affecting toxicity

There are some biotic factors which influence heavy metal toxicity, for example the cell size, an increase in the surface area:volume ratio results in increased sensitivity, capsulation increases resistance, extracellular polypeptide chelators produced by microbes also increase resistance. The nutritional status of the microbe is also an important factor e.g. Mg-limited *Klebsiella pneumoniae* are more sensitive to toxic metals. Glucose limited cells have in some cases also been shown to be more sensitive. Resistant strains do occur naturally in the environment, however, this is usually a physiological rather than genetic adaptation (Babich and Stotzky, 1978).

Environmental conditions can also strongly influence heavy metal toxicity. Binding of metals to organic material, precipitation, complexation and ionic interaction all require careful laboratory and field studies. Microbes obviously have varying tolerance mechanisms, mostly detoxification mechanisms, which are widespread in the microbial world and not only to microbes growing in metal contaminated environments (Gadd and Griffiths, 1987).

1.9 Mechanisms of microbial resistance

Microorganisms have developed various mechanisms of resistance to metal toxicity. Most are in the form of physiological adaptations rather than genetic evolution. One of these include hydrogen sulphide (H_2S) production, since H_2S forms insoluble sulphides with most metals. The production of H_2S by microbes usually indicates tolerance to heavy metals. This mechanism has been observed in some yeasts, e.g. copper and mercury tolerant strains of *Saccharomyces cerevisiae*. Another mechanism is the production of organic compounds. Some microorganisms produce organic substances that chelate metals, thus reducing their toxicity, e.g. metallothionein and the production of citric acid by many yeasts and fungi.

Metal uptake and accumulation is used by some microorganism as a means of reducing metal concentrations in their environments. There appears to be two primary mechanisms of metal uptake in microbes. The first involves non-specific binding of metals to ligands on cell surfaces, slime layers and extracellular matrices. The second is a metabolism-dependent intracellular uptake which in most microbes accounts for significantly higher uptake compared to the first.

Biological transformation of some heavy metals is carried out by many microbes. This usually results in changes in metal valency and/or a change into organometallic compounds. Volatilization and

hence removal of the metal may occur e.g. mercury has been shown to undergo both volatilization and methylation by some bacteria. Some methylated metals like mercury are more toxic than the metal ions, but they are released into the atmosphere by volatilization.

Some plasmids controlling antibiotic resistance have also been shown to control bacterial resistance to metals, e.g. *Staphylococcus aureus* has genes for resistance to certain metals as well as for penicillin resistance. Mercury plasmid-mediated resistance is the best studied of these mechanisms (Gadd and Griffiths, 1978).

1.10 Treatment of metal-contaminated water

Increased awareness of the effects of toxic metals in ecology and their accumulation throughout the food chains, has resulted in the need for purification of industrial waste-waters prior to discharge into water courses. Metal toxicity and commercial value warrants metal recovery from aqueous solutions. Conventional methods of heavy metal removal from aqueous streams include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment and evaporative recovery. These processes, however, can be ineffective or very expensive when trying to reduce heavy metal concentration from 10-100 mg/l down to less than 1 mg/l (Shumate *et al.*,1978). Consequently, application for new biosorbent materials have been considered for detoxification of contaminated waters, decontamination of radioactive waste-waters; recovery of metals from ore processing solutions and concentration and recovery of rare metals from sea water (Volesky, 1987).

1.11 Conventional methods for treatment of metal-contaminated effluents

Most industrial effluents are treated to some extent before discharge into natural water bodies. In

countries where there are fast flowing rivers with large volumes of water, dilution is used as a treatment method for lead contaminated waste-water. In Britain for example, the extent of control depends on the dilution capacity of the receiving water and the impact of the discharge on the water body in relation to its designated use. The impact assessment is based on both immediate and potential effects on aquatic life and the pathways leading to human exposure via food or water (Laxen and Harrison, 1983).

Legislation on water pollution in most countries requires heavy metal removal to acceptable levels not only before industrial waste-waters are discharged into natural water ways, but also before ocean or land disposal (Dean *et al.*, 1972) Heavy metals mostly occur in the form of sulphides, oxides, carbonates and silicates, and these are usually insoluble in water. Therefore, very small metal ion concentrations can be expected in neutral solutions, due to the insolubility of these compounds. However, when in solution, the following are some of the commonly used methods for metal removal from solution (Dean *et al.*, 1972).

1.11.1 Chemical precipitation

Lime treatment is the preferred chemical precipitation method for metal removal from solution in cases where complex chemicals are not involved, and economic recovery is not a factor. Lime precipitation is relatively simple and inexpensive, and with metals like zinc , copper, iron and nickel, almost complete precipitation of the hydroxide metals can be achieved with no special modification required. Lead, mercury, and cadmium often give incomplete precipitation and modified methods, e.g. a modified flow sheet using soda ash for lead, are required. Chlorination is sometimes required to break down complex organic metallic compounds before chemical precipitation (Kapoor and Viraraghavan, 1995).

1.11.2 Electrodeposition

Electrodeposition involves the use of insoluble anodes to recover metals in waste solutions. For example, spent solutions resulting from sulphuric acid cleaning of copper may be saturated with copper sulphate. In such cases, high-quality cathode copper can be deposited and free sulphuric acid is regenerated (Kapoor and Viraraghavan, 1995).

1.11.3 Cementation

The cementation method is used in systems where metal recovery is desirable. Mixing a metal-bearing solution with the correct metal powder or scrap will precipitate certain metals as a metallic 'sponge'. Iron, in the form of shredded de-tinned cans, is a commonly used cementation metal. It can be used for copper-containing waste, with the recovery of the copper as a marketable by-product called cement copper. Since the method operates in an acid solution, there is dissolution of iron which needs to be removed later by lime precipitation, along with any residual copper (Kapoor and Viraraghavan, 1995).

1.11.4 Solvent extraction

This method makes use of preferential interaction of a given metal with a given organic solvent. This allows the metal to dissolve in the solvent, and an acid-treatment of the organic fraction releases the metal in a concentrated water-soluble form. This method has been used successfully in the treatment of wastes with uranium and copper (Kapoor and Viraraghavan, 1995).

1.11.5 Ultrafiltration

Ultrafiltration or reverse osmosis uses semi-permeable membranes that act as 'molecular sieves' which allow soluble molecules of a given size range to pass through their pores. The synthetic membranes are made of laminated organic material. They are placed in pressurised ducts which allow continuous flow, with the filtrate passing through parallel ducts. The method gave high-purity effluents when used on tertiary stage biological waste (Kapoor and Viraraghavan, 1995).

1.11.6 Ion exchange

The destructive effect of some industrial waste materials on ion exchange resins has meant that the initial response to ion exchange resins was not very positive. In addition to this, there have been problems of limited loading capacity, high costs of resin production and system operation. However, the use of ion exchange resins in pre-treated dilute streams may give more successful results (Kapoor and Viraraghavan, 1995).

1.11.7 Activated carbon adsorption

Activated carbon is used as a sorbent instead of synthetic resins. This method has a number of advantages over ion exchange; it generally has higher loading capacities, is cheaper and is not as susceptible to adverse operation conditions. It has been used successfully in the removal of gold from cyanide solutions. Periodical re-activation of the carbon after elution of the metal ions with acid is achieved by heating in a small rotary kiln (Dean *et al.*, 1972). Adsorption onto activated carbon is a recognized method for metal removal from waste-water, but its high cost limits its application in waste-water bioremediation (Kapoor and Viraraghavan, 1995).

1.11.8 Evaporative recovery

Evaporative methods are usually time-intensive and require large open areas for implementation, and are therefore not extensively employed in industry. In general, with high metal concentrations in the range of 1-100 mg/l, most conventional methods are expensive, inefficient and have limited application (Kapoor and Viraraghavan, 1995).

1.12 Microbes in metal uptake

Despite the toxic effects of many metals as described, many microorganisms and plants can accumulate heavy metals from contaminated environments, and this ability is important in detoxification and environmental clean-up in natural aquatic ecosystems and in biological waste-

water treatment systems. Adsorption allows metal ions to bind to organic ligands found on cell walls, slime, capsule layers and membranes of cells. The cation binding capacity of several algae, bacteria, fungi, mosses and leaf litter has been demonstrated. Heavy metals accumulation by algae growing in waste-water treatment systems has been closely studied. Other studies have been carried out to identify microorganisms in different stages of waste-water treatment processes e.g. blooms of *Chlorella* and single-cell algae in primary ponds; *Cladophora, Spirogyra* and others in subsequent ponds. These organisms appear to be very effective in removing dissolved and particulate heavy metals, such as Zn, Cu, Pb, and Cd uptake and some common features can be seen (Gale, 1986).

Metal uptake by activated sludge in waste-water treatment processes is an important part of the process. Researchers have looked at the influence of the nature of the sludge on adsorption, and tried to define optimum conditions for efficient metal removal. With respect to zinc adsorption, thickened anaerobic and de-watered sludge appeared to be the best (Artola and Rigola, 1992).

Some studies have examined the selective accumulation of heavy metal ions by various microorganisms. Uranyl, mercury and lead ions were readily accumulated by most microorganisms tested. Special attention was given to the recovery of uranium by immobilized *Streptomyces albus* cells which have a very high uranium uptake. Almost all the uranium adsorbed by *S. albus* was desorbed by 0.1 M Na_2CO_3. There are several advantages to uranium uptake by microbes from aqueous systems, in that the microbial cells have a high uranium-absorbing ability and can absorb it selectively. Microbial biomass can also be produced relatively cheaply and disposal is very simple. Inter-ionic competition appears to play an important role in metal uptake by microorganisms (Nakajima and Sakaguchi, 1986).

Heat inactivated (killed) *Penicillium digitatum* mycelium have been shown to accumulate several metals which include iron, nickel, zinc, copper, cadmium, lead. It seems that heat, alkali and other chemical treatments are able to expose latent binding sites, presumably by denaturative changes in the protein (Galun *et al.*,1987).

Heat pre-treated *Penicillium* biomass (100°C for 5 minutes) was found to have increased initial metal uptake rate ($g.g^{-1}.min^{-1}$) by up to 91 % of the rate in untreated biomass at an initial metal concentration of 50 mg/l. However, in the case of lead, the total amount of metal bound did not increase. (Galun *et al.*, 1987)

Studies with fungal and yeast biomass have shown that they may be more efficient biosorbents for certain heavy metals and radionuclides e.g uranium, than activated carbon and other cation-exchange resins (de Rome and Gadd, 1991). Yakubu and Dudeney (1986) demonstrated that fungal pellets of *Aspergillus niger* were able to adsorb uranium 14 times more efficiently than adsorption onto a commercial ion-exchange resin. Activated carbon is an effective metal adsorbent, but it is relatively expensive to produce (Quek *et al.*, 1998).

Both viable and non-viable cells as well as products derived from or produced by microorganisms can be effective metal bisorbents, and there is evidence to suggest that some biomass-based bioremediation processes are economically viable. However, many aspects of the metal-microbe interaction still require extensive research if they are to be applied effectively to bioremediation (Gadd, 1990a).

Biosorbent materials, therefore, can offer alternatives to conventional processes for metal recovery

from industrial waste-water. Some algal biomass, e.g. *Sargassum natans* and *Ascophyllum nodosum* has been demonstrated to be more efficient than ion exchange resins in binding gold and cobalt from solution. Non-viable biomass of *Saccharomyces cerevisiae* and *Rhizopus arrhizus* was able to remove more copper, zinc, cadmium and uranium from solution compared to viable biomass (Kuyucak and Volesky, 1988). Brady and Duncan (1994a) described complete removal of cobalt, copper and cadmium from aqueous solution by immobilized *Saccharomyces cerevisiae* cells.

1.13 Process of Metal Uptake

The process of heavy metal uptake in viable microorganisms appears to be bi-phasic. The first phase is metabolism-independent while the second phase is metabolism-dependent (Gadd, 1986). The initial heavy metal binding process is rapid but is affected by pH, ionic strength and competing ions. Binding ability also seems to be influenced by age of the cells or the stage of development in some cases. A slower energy-dependent uptake of heavy metals across the cytoplasmic membrane follows (Gale, 1986). This energy-dependent phase can be inhibited by decreases in temperature, glucose analogues and metabolic inhibitors and uncouplers. Phosphate is also required within the cell. Electroneutrality is possibly maintained by potassium (K^+) efflux, and high metal influx rates can depend on prior loading of cell with K^+ (Gadd, 1986). The accumulated metal ions often become associated with small molecular weight proteins, which can be very specific. In algae the heavy metals may also associate with membrane vesicles, some of which can be lysosomes, mitochondria, chloroplasts and sometimes nuclei (Gale, 1986). Studies carried out by Brady and Duncan (1994b) also reported rapid loss of K^+ ions and slower release of cellular Mg^{2+} with copper accumulation by *Saccharomyces cerevisiae*.

Dead or non-viable fungal and yeast cells also bind metals. Their binding capacity depends on the

extent of cell wall disruption due to the killing process. Most heavy metals taken up by *S. cerevisiae* and *Saccharomyces carlsbengensis* are located in vacuoles and bound to low molecular weight polyphosphates. In yeasts lacking vacuoles, accumulation occurs in cytoplasmic granules and possibly polyphosphates (Brady *et al.*, 1994b). Where yeasts are concerned, a drop in external pH generally results in decreased surface and intracellular metal binding and influx, with pH 6.5 appearing to be the optimal for Zn uptake. The relationship between metal uptake and toxicity varies, with some yeasts showing high uptake with high toxicity levels e.g. Cu in *S. cerevisiae*. The presence of other metal ions in solution appear to be able to reduce the toxicity of others (Gadd, 1986).

Zn toxicity to fungi and yeasts is generally low eg. *Candida utilis* accumulated high levels of intracellular Zn without reduction of growth rate or viability. *S. cerevisiaes*' K efflux with cation uptake maybe a physiological mechanism for maintaining ionic balance or a symptom of membrane disruption and cell death. The ability of resistant strains to accumulate metals is generally less than that of sensitive strains.

S. cerevisiae and *Pseudomonas aeroginosa* have been used to examine the rate of uranium (Ur) uptake by microorganisms. Uranium was found to associate with the microbial cells, and several mechanisms have been suggested. The uranium may react to form a precipitate which discretely settles with the cells or may settle as a fine colloid entrapped by extracellular polymers. Uranium might also be transported into the cell and react to form a stored product. Uranium uptake was very temperature dependent, being greater at 40 °C compared to 25 °C, at an optimum pH of 3 (Shumate *et al.*,1978).

1.14 Biosorption and bioaccumulation

Biosorption is generally defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material (Artola and Rigola, 1992). "Adsorption" is generally used to refer to a metabolism-independent uptake or binding of heavy metals to biomass surfaces. "Biosorption" is sometimes used to describe the non-directed binding that occurs between metals and cellular components, whether it be physical or chemical (de Rome and Gadd, 1991). When referring to the metal removal processes by the biomass, which may include both energy-dependent (active) and energy-independent activities, the term "bioaccumulation" is usually used as is the term "uptake". Volesky (1987) defined 'bioaccumulation' as the active uptake or concentration of metals by living organisms.

There are, however, some types of microbial biomass that can passively bind and accumulate metals, even when metabolically inactive or dead. This microbial biomass can chemically attract and bind metallic species from the aqueous environment. Biosorption depends on the chemical composition of the cell wall and also on external physicochemical factors and the metal species in solution. One or more mechanisms can be involved in biosorption e.g. complexation, ion exchange, coordination, adsorption, chelation or microprecipitation (Volesky, 1987).

This ability of microorganisms to accumulate heavy metals from the external environment is used in waste-water treatment. Metal-microbe interaction, resulting in accumulation and removal of metals from the external environment, is of current biotechnological interest for both recovery of valuable metals and detoxification of polluted effluents (Gadd, 1990a).

Some microorganisms are known to have the ability to directly remove heavy metals from solutions

containing high concentrations of metals. Metal removal is by retention of metal ions onto the surface of the cell, or by metabolism dependent intracellular uptake (Norris & Kelly, 1979). Biosorption and/or complexation of dissolved metal species by microorganisms is receiving more attention due to its potential application in the treatment of waste-water contaminated with metals, including radioactive metals as suggested by Ruchhoft in1949, after observation of radionuclides removal in activated sludge processes (Shumate *et al.*,1978).

1.15 Mechanism of metal biosorption

A wide varied of ligands may be involved in biosorption of metals, and these include, carboxyl, hydroxyl, sulphydryl, amine and phosphate groups. Due to the complex solution chemistry of many metals, it is difficult to determine the metal species involved, and the relative importance and involvement of each ligand in biosorption. The affinity of any ligand to a metal ion may vary between metal species (de Rome and Gadd, 1991).

It appears that sorption of metals by fungal biomass and other biosorbents is a relatively non-specific process, with competition taking place for any given binding site between metal ions in solution. The amount of metal ions of one kind that are bound is affected by their concentration and chemical properties, the nature of the ligand, and the external physicochemical factors (de Rome and Gadd, 1991).

The difference in cell wall composition is probably responsible for the some of the observed differences in surface binding capacities, e.g. the cell wall of *S. cerevisiae* consists of glucan and mannan with traces of chitin, and the cell wall of *S. roseus* mainly of chitin and mannan with some glucan and traces of gamma-aminobutyric acid (Mowll and Gadd, 1983). *Rhizopus arrhizus*, a

Mucorale filamentous fungus, can bind lead up to 10 % of its own dry mass. A high content of sequestering groups like amino, amide, hydroxyl, caroxyl, sulphydryl and phosphate groups on their cellular wall make them effective biosorbents (Fourest *et al.*, 1994).

1.16 Biosorption of metals by plant biomass

The use of abundant natural materials, especially of cellulosic nature, have been suggested as potential bisorbents for heavy metals, there has not been much work done in that respect. Most studies on metal biosorption have concentrated on the use of fungal, algal bacterial and yeast biomasses. Early attempts at biosorption of metals by plant biomass was described in 1935 by Adams and Holmes. They described the removal of calcium and magnesium ions by black wattle bark (*Acacia mollissma*). While other researchers (Aval, 1991; Bryant *et al.*, 1992; Chan *et al.*, 1992) have investigated the metal biosorption properties of sawdust. Some metal removal studies with seaweed carried out by Kuyucak and Volesky (1990) gave impressive biosorption results (Volesky and Holan, 1995). Inexpensive agricultural by products such as bagasse, flour waste, paddy husk, paddy straw, onion skin and garlic skin were used in metal uptake studies. The results observed indicated that pre-treated onion skin was the most efficient, removing up to 100 % metal ions from solution (Kumar and Dara, 1980). Cut and dried leaves of the typha plant, commonly known as Cattails, which grows easily and profusely in Ontario, Canada, were investigated for the efficiency in removing the toxic metals arsenic, mercury and cadmium from solution. The cattails were able to remove mercury and cadmium with efficiencies of up to 55 % and 48 % respectively (Krishnan *et al.*, 1987). Most of the experiments done using plant material to remove metals from solution looks at cheap and readily available biomass.

1.17 *Azolla* biomass as a biosorbent

Azolla was the biomass of choice in this study. *Azolla* is a genus of small water ferns that are widely distributed. A symbiotic association with a heterocysous blue-green alga, *Anabaena azollae*, is found to allow the plant to sustain growth in nitrogen-free media. The blue-green alga is able to fix atmospheric nitrogen. Interest in *Azolla* has grown, not only as a botanical curiosity, but due to its importance in nature as a weed and as a fertilizer (source of nitrogen). *Azolla* can easily colonize water bodies which are deficient in combined nitrogen, and unsuitable for other aquatic plants. It forms dense mats over water surfaces, which at times is able to support terrestrial plant growth. This usually reduces the area of open water and therefore limits water usage (Ashton and Walmsley, 1976).

Azolla plants are very fragile and susceptible to fragmentation if physically disturbed. Growth on areas of open water exposed to high winds and wave action is poor, and *Azolla* mat formation is generally not possible.

Azolla filiculoides is native to South America and appears to have been introduced into South Africa approximately 50 years ago. It has since found its way into several water systems and dams, where its prolific growth has become a cause for much concern (Ashton and Walmsley, 1976).

Attempts have been made over the past few years to use viable *Azolla* biomass for the removal of metals from contaminated waters. Mishra *et al.*, (1987) investigated the accumulation of mercury by the *Azolla* biomass, and the effect on the toxic heavy metal on the water fern's growth. *Azolla* was found to remove up to approximately 50 % of mercury in contaminated samples. However, the removal of mercury was found to be related to the decrease in growth of the *Azolla* plant. Jain *et*

al., (1989) showed that viable *Azolla* biomass was able to remove copper and iron from solution at low concentrations (1-8 mg/l), but the metal ions were toxic at higher concentrations. Uptake of cadmium, copper and uranium from solution by *Azolla* resulted in the loss of potassium, chloride and magnesium from *Azolla* roots. Therefore accumulation of heavy metals by *Azolla* was correlated with damage caused by the loss of essential nutrients (Sela *et al.*, 1988). Several studies have been carried out on the uptake of lead from solution by viable *Azolla* biomass. de Wet *et al.*, (1990) investigated the removal of metals (including lead) from mine and industrial waste-water discharged into the Blesbok Spruit river in South Africa. Bioaccumulation of lead was found to be relatively good with an uptake capacity of approximately 109 μg/g. Sarkar and Jana (1987) demonstrated that lead had an adverse effect on the Hill activity, and therefore growth of the water fern, *Azolla pinnata*. Priel recently reported (1995) on the remediation work at the Hebrew University involving both viable and non-viable *Azolla* biomass. The studies at the Hebrew University appear to be the first reported studies on the use of non-viable *Azolla* biomass for purification of industrial waste-water and the patenting of *Azolla*-based technology. Their results showed that the *Azolla* biomass, in special installations, removed 99.9 % of lead from industrial waste containing 1 g Pb/l (Priel, 1995). Zhao and Duncan (1997a and b) have also reported on the use of non-viable *Azolla* biomass in the remediation of metals from aqueous solution and electroplating effluent.

1.18 Lead in solution and lead uptake by microorganisms

Lead, the metal investigated in this study, has been classified by the European Council Directive on Dangerous Substances as a List II material due to its toxicity. Lead is conserved and accumulates in the environment. Although most lead in the environment results from atmospheric and particulate sources, some industries, e.g. lead-acid battery industry, smelting and paper mills, generate effluents containing significant amounts of lead (Ho *et al.*, 1996).

The toxicology of lead has probably been studied more than for any other metal. Lead poisoning has been linked to the fall of the Roman Empire. High levels of lead found in the bones from the Roman era supports the hypothesis that the use of lead containers for wine and other liquids and the use of lead water pipes etc, may have contributed to the destruction of the ruling class who could afford the lead containers (Volesky, 1990).

Inorganic lead (Pb^{2+}) is a known metabolic poison and enzyme inhibitor (as are many other metals), with the organic form of lead, tetraethyl lead or tetramethyl lead being even more poisonous. Physical symptoms of lead poisoning include excitement, depression and irritability. Lead in children has been found to cause mental retardation and semi-permanent brain damage. Inorganic lead has also been found to replace calcium in bones, where it accumulates as a reservoir for long-term release after the initial uptake. A figure of 0.2 mg/l lead in the blood appears to be the generally accepted limit. However, natural levels in the human blood are so close to this limit that there seems to be little margin left to allow for any exposure to lead (Volesky, 1990).

Although there are contradictory reports on the accumulative characteristic of lead, there is evidence to show a progressive increase in lead content in the ancient snow deposits in northern Greenland. Of the approximately 3 million tons of the annual lead consumed, 40 % is used in the production of electrical accumulators and batteries, 20 % in gasoline as alkyl additives, 12 % in building construction, 6 % in cable coatings, 5 % in ammunition and the other 17 % for other uses (Volesky, 1990).

The occurrence of lead in industrial waste is mainly in the form of the bivalent Pb(II) ion as a hydrolysis product, $PbOH^+$ and/or organic complexes, such as lead tetraethyl. A large amount of lead

is discharged annually into the atmosphere in the exhaust gases of internal-combustion engines fuelled with leaded petroleum. This atmospheric lead, in the form of oxides and salts, is washed back down to the earths' surface by rain. In industrial effluents, lead concentrations can be up to 200-250 mg/l, while water quality standards for lead are 0.1-0.05 mg/l (Sağ *et al.*, 1995).

A considerable amount of literature is available on the toxicity of lead in humans, but there does not appear to be much literature on lead toxicity to plants and microorganisms. This may be due to the fact that lead is considered relatively immobile in soils. However, dissolved lead in ground water in areas of lead-contaminated soil has been found to be significantly high. The main lead minerals are sulphides and carbonates. The Pb^{2+} forms slightly stable complexes with nitrate, chloride and cyanide. Sparingly soluble salts of divalent lead are chloride, bromide, iodide, fluoride, sulphate and carbonate. Lead concentration in unpolluted waters ranges from 0.05 to 10 mg/l, but the amount of dissolved lead does not exceed 0.01 mg/l (Galvin, 1996).

The need to develop media to culture marine phytoplankton of individual species in the laboratory led to studies on the effect of different metals on the growth of marine phytoplankton. Some trace metals, in particular iron, were found to necessary to promote growth of healthy phytoplankton cultures. However, other metals e.g. copper, mercury and lead were found to be toxic to the marine phytoplankton. Lead at concentrations of 1 µM was found to cause significant reduction in chlorophyll production (Davies, 1983).

In terms of the potential for biosorbents to remove lead from solution, *Scenedesmus, Selenastrum* and *Chlorella* algae were found to accumulate Pb(II) ions from solutions of 100 mg/l with an efficiency of up to 97 % (Brady *et al.*, 1994a). After observing the ability of *Pseudomonas*

aeruginosa PU21 to selectively adsorb Hg^{2+} with a maximum capacity of approximately 400 mg/g, Chang *et al.*, (1997) investigated the potential of this organism in the uptake of lead, copper and cadmium. Values of up to 98 % adsorption were obtained with maximum lead capacity value of 110 mg/g. Thompson and Watling (1987) demonstrated that heterotrophic bacteria isolated from marine sediments, such as *Klebsiella oxycota, Escherichia coli, Bacillus sp.* and others, were able to remove an average of 22 % lead from solution. D'Avila *et al.*, (1992) on the other hand showed that activated carbon could remove 98 % of lead from solution with an initial lead concentration of 50 mg/l within 5 minutes of contact in batch reactors.

1.19 Research Aims

The primary aim of this research project was to evaluate the capacity and efficiency of the non-viable biomass of the water fern, *Azolla filiculoides*, for the removal of lead from aqueous solution and from lead-acid battery manufacturing effluent. Preliminary studies examined the effect of parameters such as pH, temperature, biomass and lead concentrations on lead removal from aqueous solution in batch reactors. Sorption isotherms that were generated were used to determine the maximum lead binding capacity of the *Azolla* biomass. Competition studies using multiple-metal solutions in batch reactors were used to determine the effect of the presence of other metal ions in solution to mimic real effluent situations.

The potential re-usability of the *Azolla* biomass was investigated by recovery of biomass-bound lead by elution with dilute mineral acids and reconditioning the biomass with a dilute base. Repeated adsorption- desorption studies were carried out to determine the efficiency of lead removal/recovery over ten cycles in fixed bed column reactors. The effect of different flow rates and initial lead concentrations in fixed bed column reactors were also investigated.

The effectiveness of the *Azolla* biomass in the remediation of lead from lead-acid battery manufacturing effluent from two different lead-acid battery producing companies subsequently was investigated. An attempt was also made to study the effect of lead removal/recovery and biomass regeneration on the physical integrity of the *Azolla* biomass using scanning electron microscopy.

CHAPTER TWO

LEAD REMOVAL FROM AQUEOUS SOLUTION IN BATCH SYSTEMS BY *AZOLLA FILICULOIDES*

2.1 INTRODUCTION

The ability of plant material, both aquatic and terrestrial, to remove toxic metals from solution has been reported by several researchers (Mishra *et al.*, 1987; Sela *et al.*, 1988; Jain *et al.*, 1989; de Wet, 1990; Holan & Volesky, 1994). In order to determine the capacity of a given biomass in metal adsorption from solution, it is important to evaluate several factors that may affect adsorption and these include the nature and availability of the biomass, its stage in development, any pre-treatment requirements and the composition of the waste-water of interest (Jain, Vasudevan & Jha, 1990).

Aquatic plants are known to be able to take up heavy metals from water. The aquatic bryophyte *Fontinalis antipyretica* has been shown to accumulate dissolved copper ions to 30 000 - 40 000 times the concentration in solution. Widespread occurrence of aquatic bryophytes has led to their being used successfully as bioindicators of freshwater contamination by heavy metals (Gonçalves and Boaventura, 1998).

Non-viable biomass of the water fern *Azolla filiculoides* was chosen for this study because it provides a possible alternative means of control of this aquatic weed, and it provides a cheap and readily available biomass for possible application in waste-water bioremediation. *Azolla* offers a natural biosorbent material which may be more effective and cheaper than the well established commercially

available ion exchange sorbents.

Lead was chosen as the pollutant for study due to its high toxicity even at relatively low concentrations and two South African lead-acid battery manufacturers have reported high levels of lead in their effluents. Expensive precipitation methods are being used to treat the battery manufacturing effluents. Excess levels of lead have been reported to cause the following in human beings; anaemia, kidney and liver diseases, paralysis, brain damage, convulsions and at times death. Low levels of lead may result in hyperactivity, learning disabilities in children, night blindness and the suppression of the body's immune system. Lead is therefore a potent environmental pollutant and health hazard (Jain *et al.*, 1990). *Azolla* has been shown to have potential for removal of lead from industrial waste (Priel, 1995).

This study investigated the capacity of dried *Azolla* biomass in the biosorption of lead from solutions made up in the laboratory. The effects of pH, temperature, biomass concentration, initial lead concentration and other metal ions in solution were investigated. The study also allowed the comparison of the lead uptake capacity of *Azolla* biomass with that of other biosorbents reported in literature.

2.2 MATERIALS AND METHODS

2.2.1 Biomass

Azollla filiculoides biomass was obtained locally from a farm dam between the towns of Grahamstown and Port Alfred in the Eastern Cape, South Africa. The biomass was dried in a constant environment room at 37°C for 72 hours, after which time it was ground by hand to a

consistent size, determined by screening to exclude particles over 2 mm in size.

2.2.2 Solutions

All experimental work was done using de-ionised water to reduce the possibility of metal contamination. All reagents were of an analytical grade and purchased from Saarchem, South Africa. Borosilicate glassware, washed prior to use with 25 % nitric acid and rinsed with de-ionised water, was used for all experiments due to its negligible metal-binding capacity.

Metal solutions were made by dissolving the appropriate metal salt ($PbNO_3$, $Cu(NO_3)_2$ and $Fe(NO_3)_2$) in de-ionised water to give stock solutions containing 1000 mg/l (unless otherwise stated) of the metal ion. These were then diluted as required and used in subsequent experiments.

2.2.3 pH profiles

All pH adjustments were done using 1 M stock sodium hydroxide (NaOH) and 1 M hydrochloric acid (HCl) solutions as required. Subsequent metal removal equilibrium experiments were as described below.

2.2.4 Metal removal experiments

Experiments were performed in duplicate. *Azolla* biomass (5 g biomass / l solution unless otherwise stated) was added to 100 ml volumes of the metal solution, at the desired concentration and pH, in 300ml Erlenmeyer flasks. The pH was adjusted to the desired value using NaOH and HCl. A 2 ml sample was immediately taken and the flasks were placed in a shaking incubator at 25 °C (or the required temperature) at 170 revolutions per minute (rpm). Samples of 2 ml volumes were taken every 10 minutes for the first hour, every 20 minutes for the second hour, every 30 minutes for the

third hour, after a further hour and lastly after a further 2 hours. Each 2 ml sample was filtered using a millipore filter system with a 25 mm diameter, 0.45 μm pore size cellulose acetate filter. The filtrate was analysed for the metal of interest using an atomic absorption spectrophotometer. Control experiments of metal solutions with no biomass present were carried out to determine the effect of lead uptake by the cellulose acetate membrane filters and borosilicate glassware. This was found to be negligible.

2.2.5 Metal analysis

Analysis of metal in solution was done using a GBC 909 atomic absorption spectrophotometer (AAS). Atomic absorption standard solutions were purchased from Saarchem, South Africa and appropriate concentration made by dilution with de-ionised water. The operation parameters were as set out in table 2.1 below.

Table 2.1: Atomic absorption spectrophotometer operating conditions

Element	Flame	Wavelength (nm)	Lamp current (mA)	Slit width (nm)	Working Range (mg/l)	Sensitivity (mg/l)
Copper	A-A*	327.4	3.0	0.5	2.5 - 10	0.050
		217.9	3.0	0.2	7.5 - 30	0.16
Iron	A-A	248.3	7.0	0.2	2 - 9	0.05
		372.0	7.0	0.2	20 - 80	0.45
Lead	A-A	217.0	5.0	1.0	2.5 - 20	0.06
		283.3	5.0	0.5	7.0 - 50	0.16

* **A-A = Air - Acetylene**

(Rothery, 1980)

2.3 RESULTS AND DISCUSSION

2.3.1 Rate of lead removal

The first batch experiment was used to get an indication of the *Azolla* biomass' rate of lead removal from aqueous solution (figure 2.1). The initial lead (as $PbNO_3$) concentration of 37 mg/l was chosen because it was within the range (10-95 mg/l) of that found in industrial battery effluent. The biomass concentrations of 4 g *Azolla* / l of metal solution was similar to reported optimum concentration (Zhao and Duncan, 1997b). Figure 2.1 shows the rate of lead removal from aqueous solution and the pH profile of the system over 4 hours. Rapid lead removal from aqueous solution is observed in the first 25 to 30 minutes of the experiment. The pH also reaches an equilibrium pH value of approximately 6.3 in 25 to 30 minutes. Very little lead removal takes place subsequent to the initial rapid phase, and this agrees with other published literature (Larsen and Schierup, 1981; Gadd, 1988; Ho *et al.*, 1996) involving studies of metal ion adsorption by non-viable biomass.

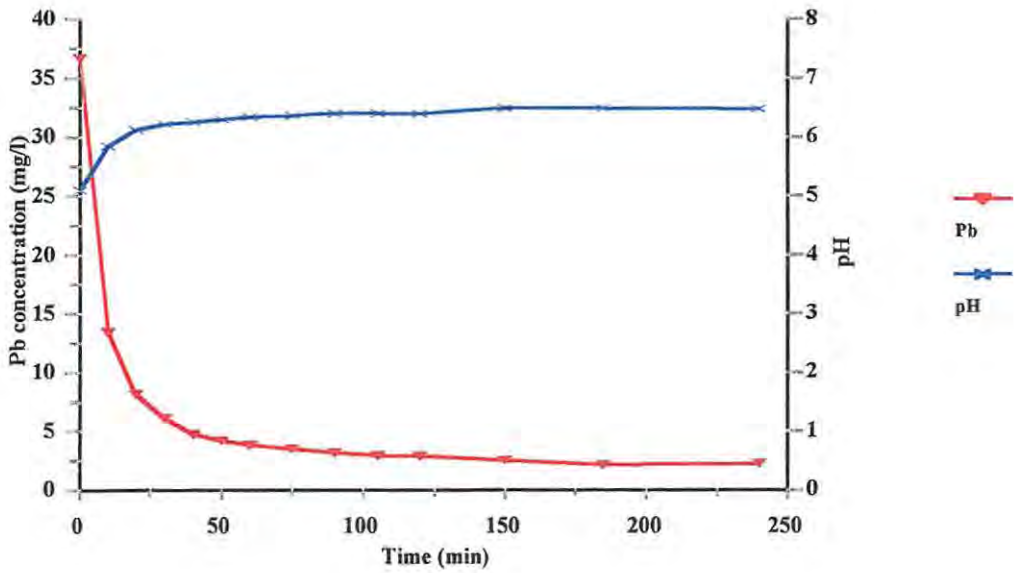

Figure 2.1: Rate of lead removal from aqueous solution and pH profile. Initial Pb concentration, 37 mg/l; biomass concentration, 4 g *Azolla* / l solution; initial pH, 5.1; temperature, 25 °C; shaking rate, 170 rpm.

This profile differs from viable biomass absorption systems in which biphasic metal uptake is observed, an initial rapid energy independent phase associated with adsorption to the biomass' surface, followed by a gradual energy dependent active uptake phase which may involve the organism's transport systems (Gadd, 1988; Mowll and Gadd, 1983; Tsezos, 1985; Kasan and Baecker, 1989).

2.3.2 pH profile for lead nitrate precipitation

A lead nitrate ($PbNO_3$) precipitation profile with respect to pH was generated (figure 2.2), in order to determine the behaviour of $PbNO_3$ at different pH values. Adjusting the pH of the $PbNO_3$ solution using NaOH from pH 5 to pH 7 resulted in rapid precipitation (approximately 75 %) of the lead out of solution. Precipitation studies at pH values between 5 and 7 would have been useful as there are contradictory values in literature for lead precipitation, Forster and Wase (1997) reported precipitation for $Pb(OH)_2$ at a pH value of 6.3. The precipitation profile gave an indication of how much precipitation contributed to the lead removal by the *Azolla* biomass at a given pH value.

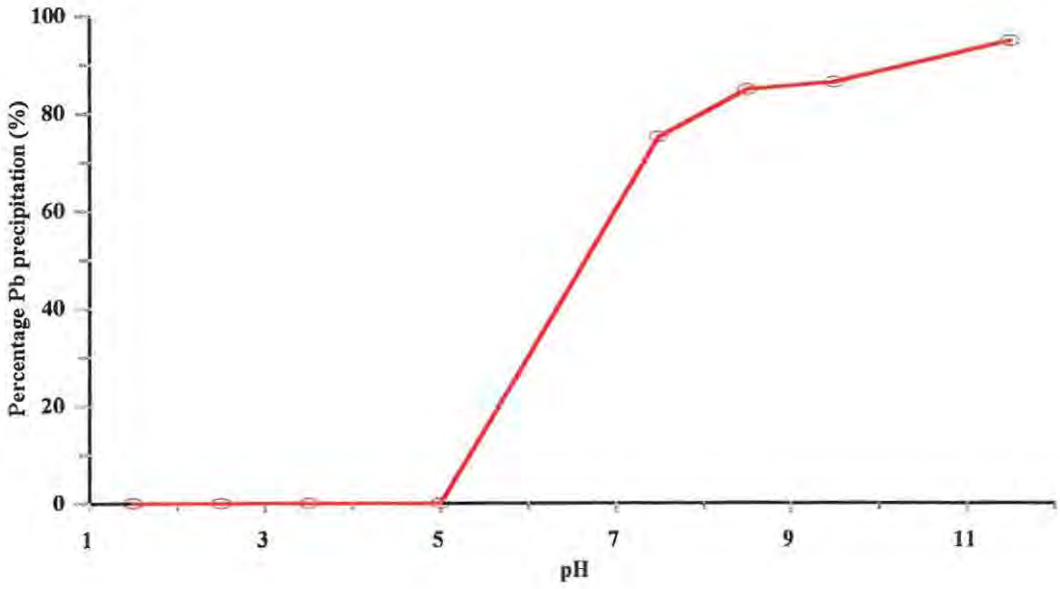

Figure 2.2: Percentage lead precipitation with varying pH values for $Pb(NO_3)_2$. Initial Pb concentration 83 mg/l; temperature, room temperature (~20 °C); NaOH & HNO_3 to adjust pH.

2.3.3 Effect of initial pH on lead removal

The initial pH value of the metal solutions appeared to have the most significant effect on lead removal from aqueous solution by the *Azolla* biomass (figures 2.3 and 2.4). After a rapid decrease, a slight increase in the amount of lead in solution was observed at the pH value 1.5 after 100 minutes (figure 2.3) and this closely agrees with other literature. Acids are known to be efficient desorbing agents for metal ions (de Rome and Gadd 1991), which would explain the observed trend of lead ions being released back into solution at a pH of 1.5. At pH values of 1.5, 8.0 and 9.5, lead removal from aqueous solution due to adsorption reached percentage removal equilbria values of less than 40 % (figure 2.4). The effect of precipitation at pH 8.0 and 9.5 was accounted for by subtracting the percentage lead that was found to precipitate out of solution on adjusting the pH of the lead solution in the absence of the biomass, from the total percentage lead removal observed at the same pH in the presence of the biomass. Maximum percentage removal of approximately 96 % was observed at initial pH values between 3.5 and 5.7.

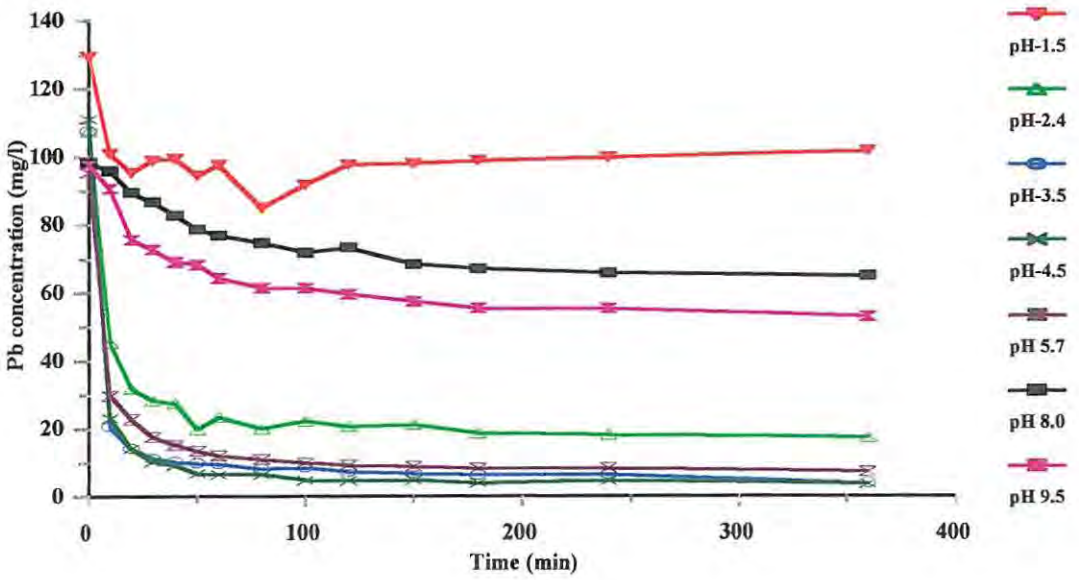

Figure 2.3: The rate of lead removal from aqueous solution with varying pH values. Initial Pb concentration 110 (± 10) mg/l; biomass concentration, 5 g *Azolla* / l solution; temperature, 25 °C; shaking rate, 170rpm.

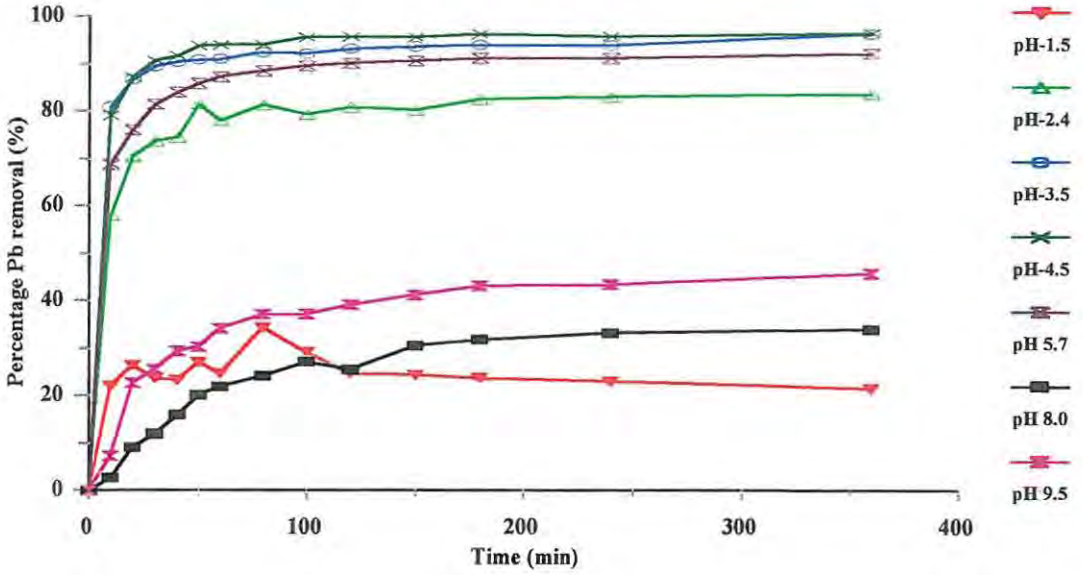

Figure 2.4: Percentage lead removal with time, from aqueous solution with varying pH values. Initial Pb concentration, 110 (± 10) mg/l; biomass concentration, 5 g *Azolla* / l solution; temperature, 25 °C; shaking rate, 170 rpm.

Figure 2.5 shows the pH profiles throughout the experiments. The general trend observed was that for aqueous solutions with initial pH values less than 6 there was an initial rapid increase in the pH of the system until an equilibrium pH value was reached. There was an initial rapid decrease in the pH of the system when the initial pH of the aqueous solution was great than 6, until an equilibrium pH value was reached.

The significant effect that pH had on metal uptake by the *Azolla* biomass was probably due to its effect on the chemistry of both the sequestering groups on the surface of the biomass, and the lead ions in solution. Protons (H^+) available at lower pHs are likely to compete with the metal ions for available binding sites. However, Galun *et al.* (1987) found that at a pH value of 2, lead appeared to be adsorbed effectively by *Penicillium* biomass with no apparent competition from H^+ ions. Kuyucak and Volesky (1988) reported similar effects of pH on the solution chemistry of the metals,

the activity of functional groups on the biomass and the competition of metallic ions for binding sites. At pH 4-5, lead is probably ionized as a cation species and sequestering groups will largely be dissociated to give negatively charged sites.

The change in pH by 0.5 to 2 units from the initial pH observed in this experiment (figure 2.5) is difficult to explain. It may be due to the sequestering groups on the biomass surface contributing to the over-all chemistry of the system. In effluents the contribution of precipitation to the removal of metal ions from solution would, in most cases, be an added advantage, unless recovery of the metal is required.

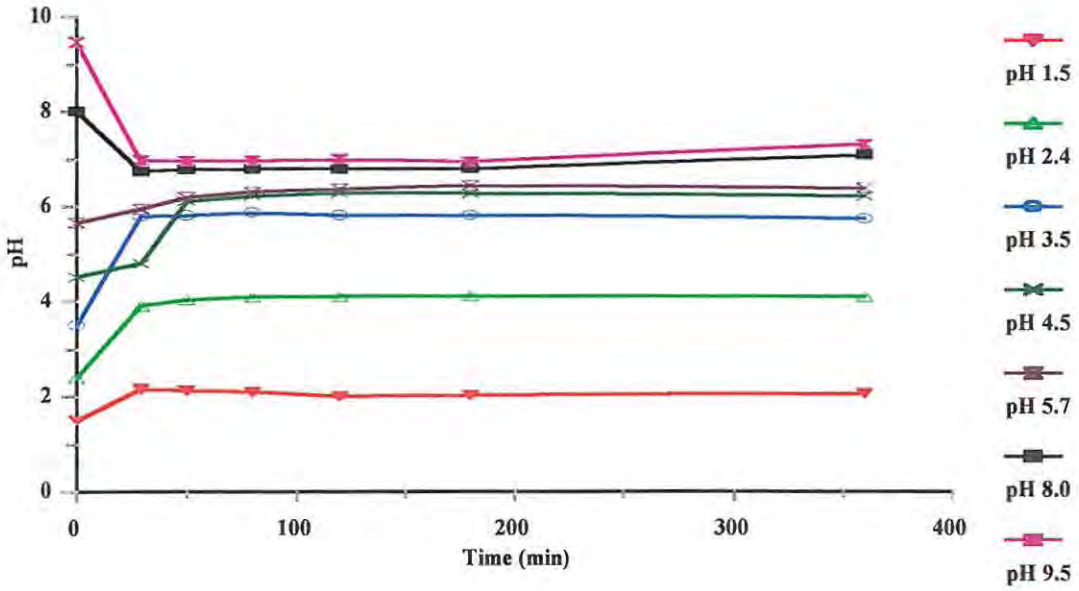

Figure 2.5: pH profiles for lead removal from aqueous solution with varying initial pH values. Initial Pb concentration, 110 (±10) mg/l; biomass concentration, 5 g *Azolla* / l solution; temperature, 25 °C; shaking rate, 170rpm.

2.3.4 Effect of biomass concentration on lead removal

Figure 2.6 shows the concentration of lead remaining in solution with varying biomass concentrations. Figure 2.7 shows the corresponding percentage lead removal curves with respect to

time. The initial rapid lead removal from solution is evident in all the curves. The 1 and 2 g/l samples reached a percentage lead removal equilibrium of approximately 70 and 80 % respectively. Increasing the *Azolla* biomass concentration from 2 g/l to 4 g/l resulted in an increase in the percentage removal equilibrium to approximately 95 % in 20 to 25 minutes. This may be because at lower biomass concentrations, there may be more metal ions in solution compared to the number of available sequestering groups on the biomass surface. No significant increase in the percentage lead removal from solution was observed on increasing the *Azolla* biomass from 4 g/l to 6g/l and then 8 g/l. This suggested an optimum biomass concentration of between 4 and 6 mg/l, for the system, and a biomass concentration of 5 mg/l was chosen as the optimum concentration for further studies. Lack of further lead removal on increasing the biomass concentration may be due to the system reaching its equilibrium or saturation point. Exceeding the optimum biomass concentration in a system results in biomass which does not contribute to the efficiency of the system, and is therefore wasteful.

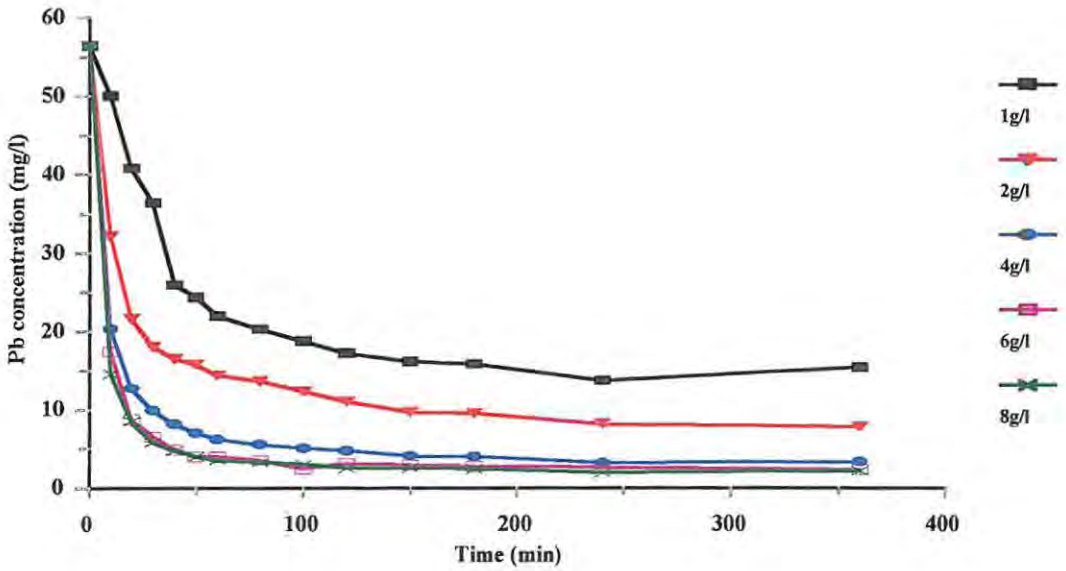

Figure 2.6: Rate of lead removal from aqueous solution with varying concentrations of *Azolla* biomass. pH, 5.7; initial Pb concentration, 56.5 mg/l; temperature, 25 °C; shaking rate, 170 rpm.

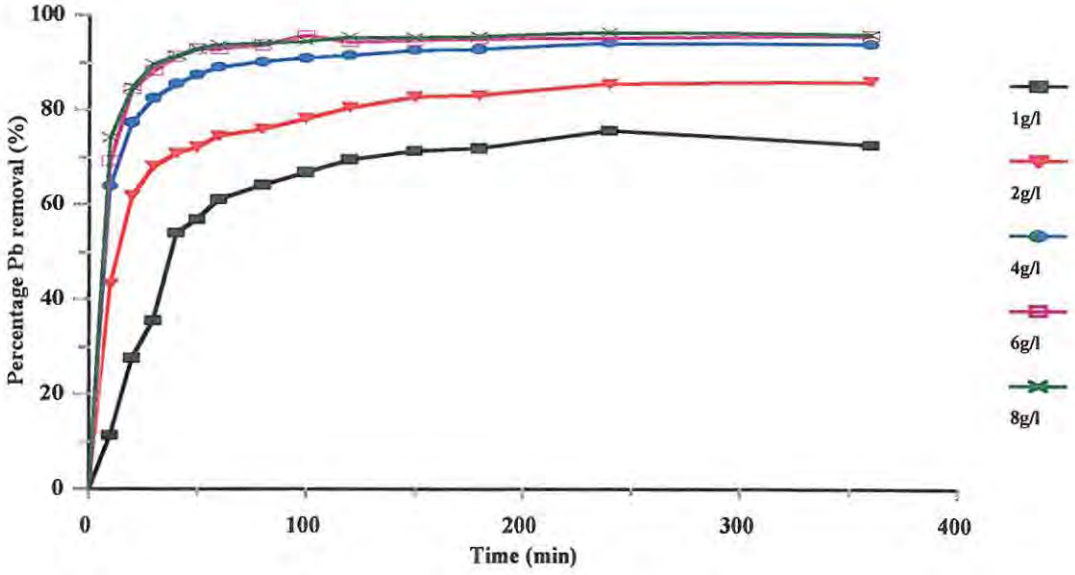

Figure 2.7: Percentage lead removal with time, from aqueous solution with varying concentrations of *Azolla* biomass. pH, 5.7; initial Pb concentration, 56.5 mg/l; temperature, 25 °C; shaking rate, 170 rpm.

2.3.5 Effect of initial lead concentration on lead removal

The percentage lead removal from solution was not affected to any great extent for a range of initial lead concentrations of 10 mg/l to 400 mg/l (figures 2.8 and 2.9). Figure 2.8 shows that with initial lead concentrations above 400 mg/l (in this case 780 and 830 mg/l), the lead concentration remaining in solution reaches an equilibrium of more than 300 mg/l. In figure 2.9, percentage lead removal equilibrium of more than 85 % was attained within approximately 25 minutes for samples with initial lead concentrations of 400 mg/l or less. The percentage lead removal after about 25 minutes for the 780 and 830 mg/l samples were 45 % and 50 % respectively, which was significantly lower than the solutions at lower concentrations. It is possible that at high lead concentrations, the physicochemical environment of the system is altered to some degree, resulting in reduced lead removal. The large number of lead ions in solution may also be saturating the system, reducing the amount of adsorption that can take place. Therefore, a lead concentration of 95 mg/l was chosen for further experiments.

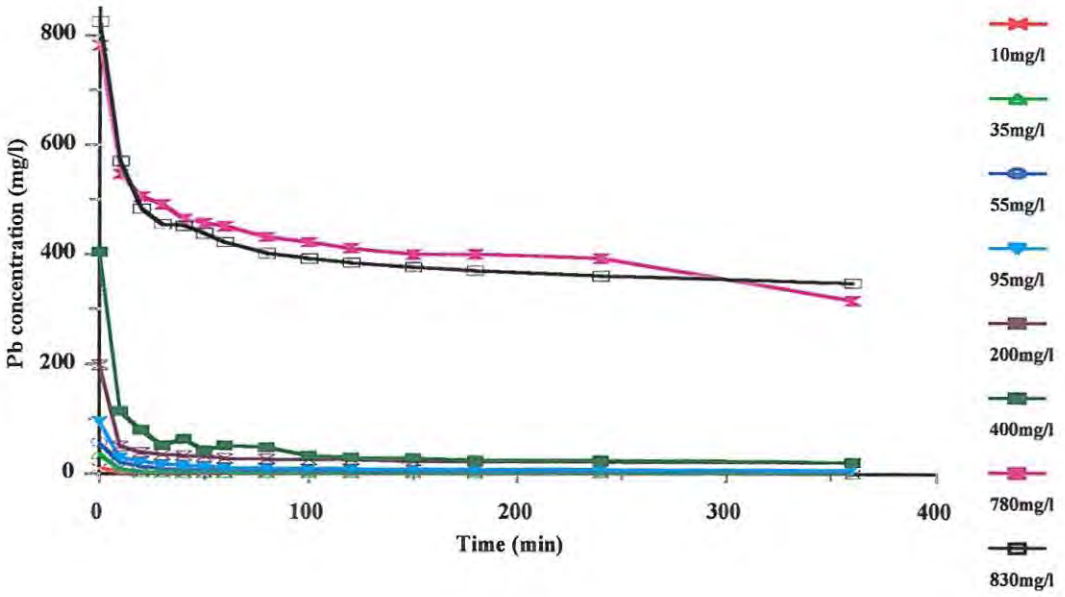

Figure 2.8: Rate of lead removal from aqueous solution with varying initial lead concentrations. pH, 5.7; biomass concentration, 5 g *Azolla* / l solution, temperature, 25 °C; shaking rate, 170 rpm.

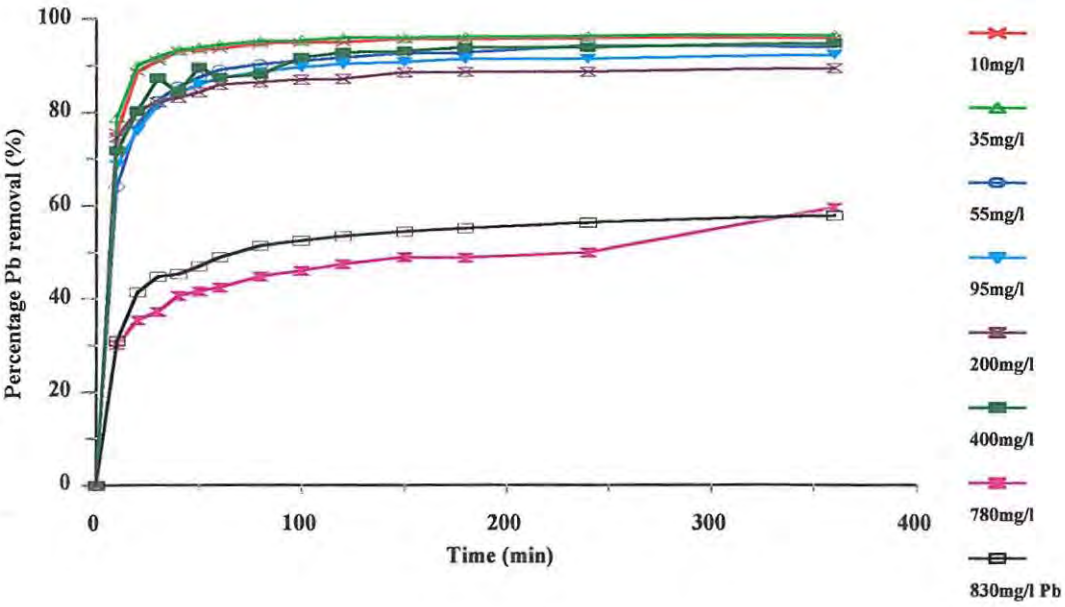

Figure 2.9: Percentage lead removal with time, from aqueous solution with varying initial lead concentrations. pH, 5.7; biomass concentration, 5 g *Azolla* / l solution; temperature, 25 °C; shaking rate, 170 rpm.

2.3.6 Effect of temperature on lead removal

Temperatures ranging from 10 to 50 °C had no notable effect on the rate of lead removal from aqueous solution (figure 2.10) nor on the percentage lead removal from aqueous solution (figure 2.11). Percentage lead removal of between 85 and 90 % was reached within 25 minutes in all cases. The absence of significant effects of temperature on lead removal was probably because, in the temperature range studied, there was very little if any change to the physical integrity of the *Azolla* biomass or the surface structure and chemistry of the groups involved in sequestering the lead ions from solution. Adsorption reactions, particularly with viable biomass, are normally exothermic, with adsorption increasing with decreasing temperature (Weber, 1972). There are some biosorption process which appear to be endothermic, e.g. uranium uptake (Tsezos and Volesky, 1981). In general, optimal biosorption temperatures are between 10-25 °C (Edyvean *et al.*, 1997). However, in this study, temperatures over a range of 10-50 °C had no observed effect on lead removal by the *Azolla* biomass. Temperatures of industrial waste-water environments outside this range are unlikely to exist.

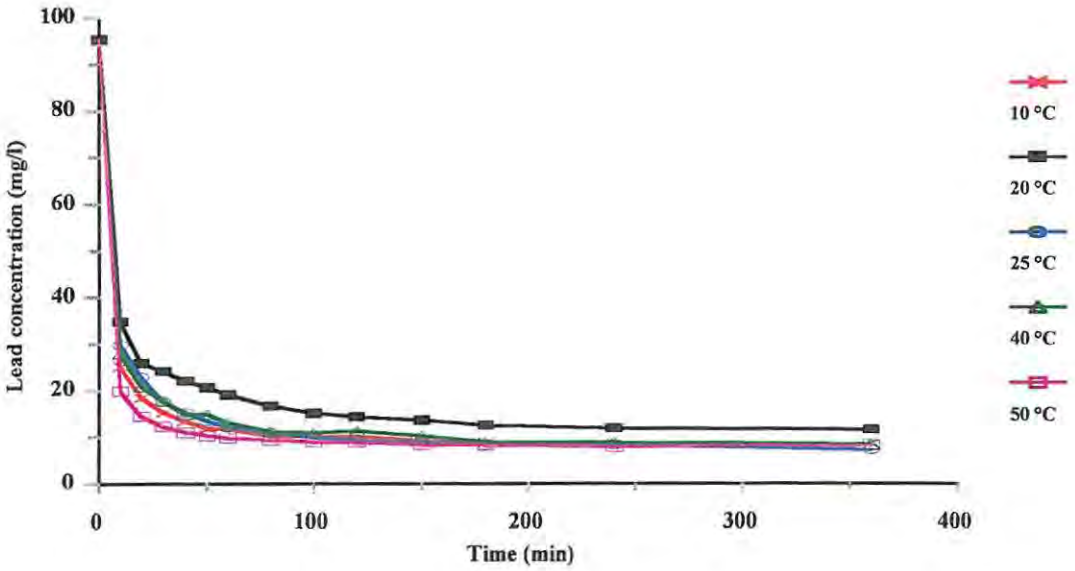

Figure 2.10: Rate of lead removal from aqueous solution with varying temperatures. Initial Pb concentration, 95 mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 4.9; shaking rate, 170 rpm.

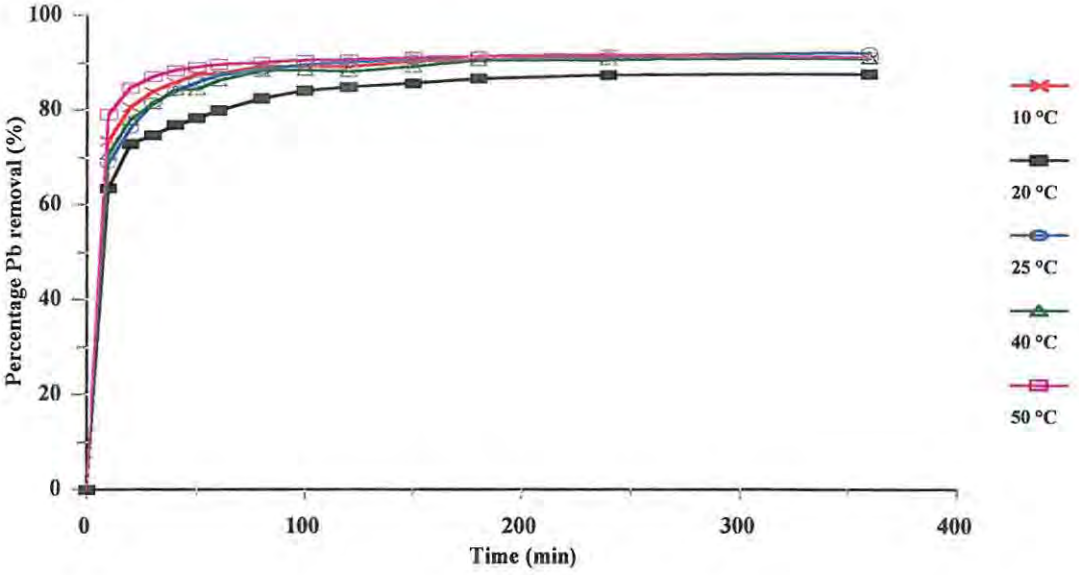

Figure 2.11: Percentage lead removal with time, from aqueous solution with varying temperatures. Initial Pb concentration, 95 mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 4.9; shaking rate, 170 rpm.

2.3.7 Equilibrium sorption isotherm

An equilibrium sorption isotherm for lead by the *Azolla* biomass was generated for a range of initial lead concentrations of 7 to 5000 mg/l at a pH value of 5.2. The equilibrium sorption isotherm is given in figure 2.12. The maximum lead binding capacity (q_{max}) of the *Azolla* biomass for lead was found to be approximately 100 mg/g (mg lead / g *Azolla* biomass).

The value q_{max} is a measure of the binding capacity the biomass has for the metal of interest, in this case lead, and this allows a comparison of potential bioremediation capabilities between different biosorbents. However, to do this, similar conditions need to be employed in each case. Comparison of q_{max} values between biosorbents allows for the selection of the best one, for the removal of a given metal contaminant. Table 2.2 gives a comparison of the lead uptake capacity of several biosorbents and *Azolla* biomass can be seen to be one of the more efficient biosorbents for lead.

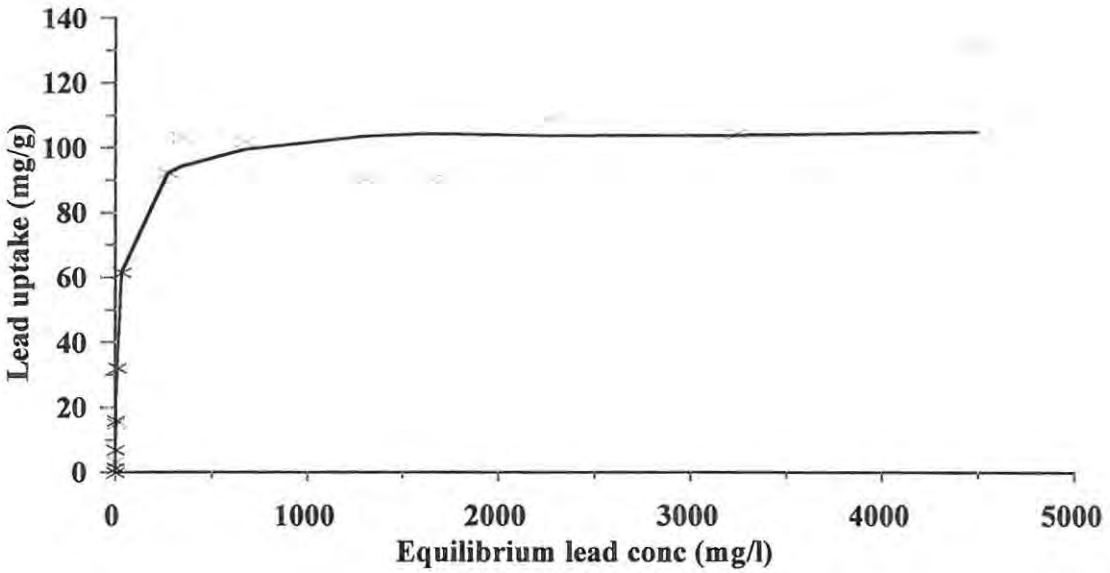

Figure 2.12: Equilibrium sorption isotherm for lead removal from aqueous solution by *Azolla* biomass. pH, 5.2; temperature, 25 °C; shaking rate, 170 rpm.

Table 2.2: Lead uptake capacity of different biosorbents

COMPARISON OF q_{max}			
METAL	**SORBENT**	**q_{max}**	**SOURCE**
Lead	Sphagnum moss peat	30.7	Ho *et al.* (1996a)
	Groundnut husks	39.3	Okieimen *et al.* (1991)
	Sago waste	46.64	Quek *et al.* (1998)
	Tea leaves	78.7	Tan and Khan, (1988)
	Azolla filiculoides	100	This study (1998)
	Penicillium chrysogenum	116	Niu *et al.* (1993)
	Cladophora crispata	251	Özer *et al.* (1994)

q_{max} - maximum metal uptake (mg metal / g biomass)

2.3.8 Effect of different lead salts on lead removal

Figures 2.13 and 2.14 present the effect of four different lead salts in aqueous solution on lead removal by the *Azolla* biomass over three hours. The four lead salts selected for this study were lead monoxide (PbO), lead sulphate ($PbSO_4$), lead chloride ($PbCl_2$) and lead nitrate ($Pb(NO_3)_2$). The rate of lead removal from aqueous solution does not appear to be affected by the lead salt contributing the lead ions as evidenced in figure 2.13. The percentage lead removal from aqueous solution with each of the lead salts was approximately 95 % (figure 2.14).

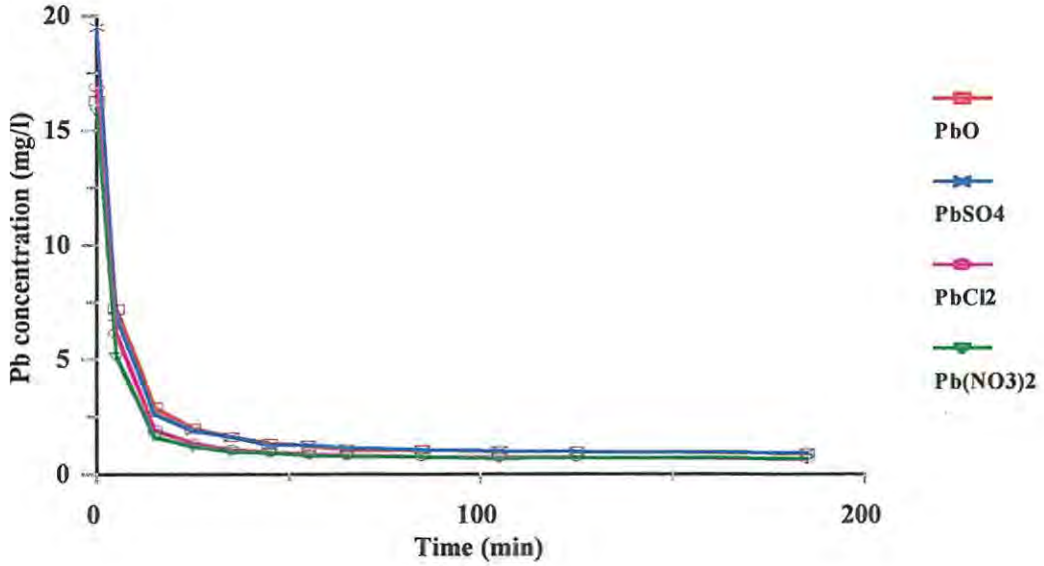

Figure 2.13: Rate of lead removal from aqueous solution with different lead salts. Initial Pb concentration, 17 (±2) mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, 25 °C; shaking rate, 170 rpm.

Although all four lead salts have different solubilities in water, all were made up to the same initial concentration of approximately 17 mg/l, the concentration obtained with least soluble lead salt ($PbCl_2$). The presence of different anions in solution with the lead ion had no significant effect on the percentage lead removal from aqueous solution by the *Azolla* biomass. The different anions in solution do not appear to contribute to or interfere with the lead adsorption system of the biomass.

This suggests that for any lead-containing waste-water, the lead salt contributing the lead ions in solution will not affect the lead removal capacity of the *Azolla* biomass.

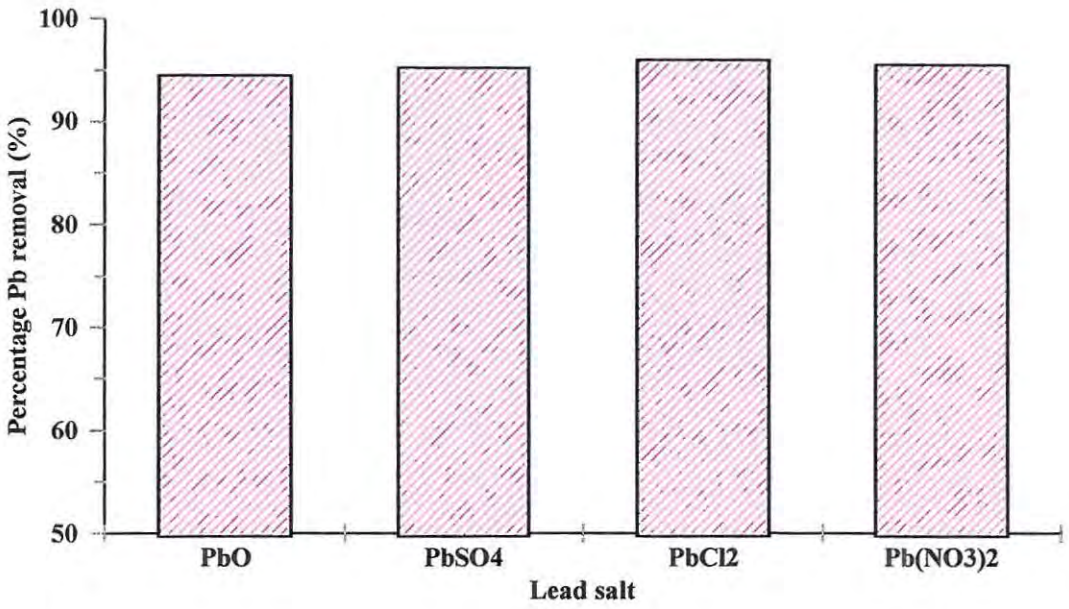

Figure 2.14: Percentage lead removal from aqueous solution after three hours with different lead salts. Initial Pb concentration, 17 (±2) mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, 25 °C; shaking rate, 170 rpm.

2.3.9 Multiple-metal solutions studies

Single metal solutions obviously do not exist in most metal contaminated environments, and so it was important to study the effect of multiple metals in solution. Copper (Cu) and iron (Fe) were chosen for these studies because they were found to be two of the other metals, besides lead, be present in lead-acid battery manufacturing plant effluent, albeit in low concentrations. The source of the copper and iron in lead-acid effluent could not be ascertained, but is thought to result from corrosion of piping by sulphuric acid (H_2SO_4) used in the production of lead-acid batteries, which would explain their variable concentrations observed in different effluent samples. Metal concentration of 40 mg/l was chosen for subsequent competition studies to ensure maximum metal uptake in control samples

with single metal solutions, which would then highlight any competitive effects due to the presence of other metal ions.

2.3.9.1 Precipitation studies

Depending on the lead, copper and iron salt used in the competition studies, some precipitation was observed. Figures 2.15 and 2.16 gives the amount of precipitation of each metal, and most of the metal salt precipitating out of solution was found to be a lead salt. Approximately 45 to 55 % of lead precipitated out of solution when added to a copper sulphate solution (figure 2.15), and approximately 45 to 58 % when added to ferric chloride (figure 2.16). There was some copper and iron that also precipitated out of solution, a maximum of approximately 6 % in the case of copper and 10 % in the case of iron. Final competition studies were done using copper nitrate ($Cu(NO_3)_2$) and iron nitrate ($Fe(NO_3)_2$) as earlier studies with copper sulphate ($CuSO_4$), and ferric chloride ($FeCl_2$) resulted in almost complete precipitation of lead out of solution, probably as lead chloride and sulphate salts, both of which are only sparingly soluble.

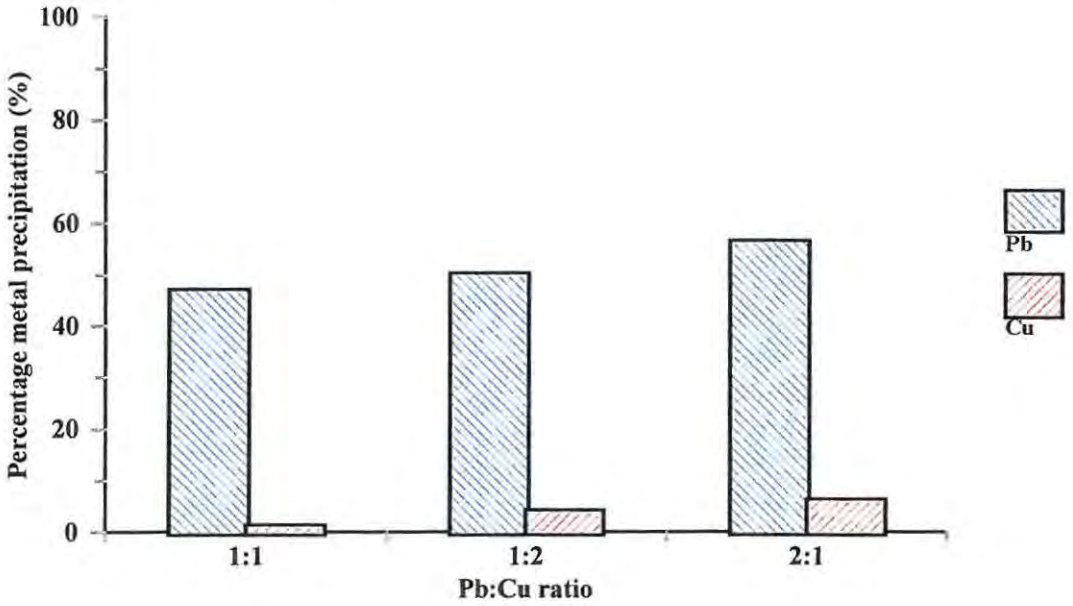

Figure 2.15: Percentage lead and copper precipitation from aqueous solution after 2½ hours. 1 part = 40 mg/l; pH, 5.4; temperature 25 °C, shaking rate, 170 rpm.

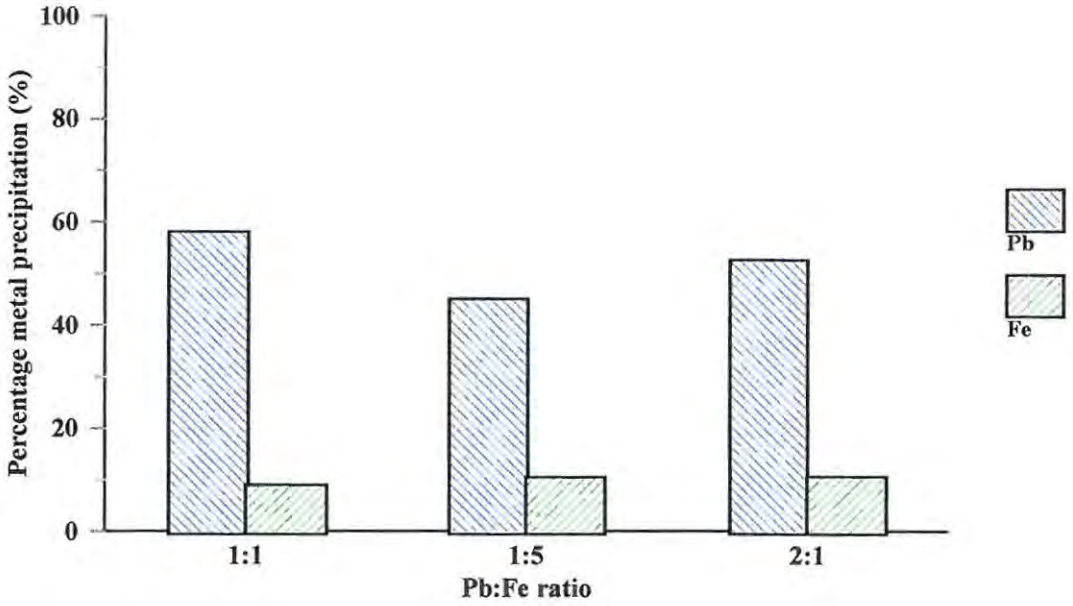

Figure 2.16: Percentage lead and iron precipitation from aqueous solution after 2½ hours. 1 part = 40 mg/l (except for the Pb:Fe=1:5 sample where 1 part = 20 mg/l); pH, 4.2; temperature, 25 °C, shaking rate, 170 rpm.

2.3.9.2 Effect of copper in solution on lead removal

The effect of the presence of another metal ion in solution was investigated, in this case copper (Cu). Figure 2.17 shows the rate of lead removal from aqueous solution in the presence of copper ions. Different ratios of lead to copper ions in solution were found to have little or no effect on the percentage lead removal from aqueous solution at the given metal concentrations. An equilibrium percentage lead removal of approximately 95 % was reached within 25 minutes in each case. This is slightly higher than the equilibrium percentage lead removal value of 92 % observed with only lead ions in solution (figure 2.17).

The results, therefore, suggest no significant competition from copper ions for binding sites on the *Azolla* surface exists. This may be due to the different molecular sizes of the metal ions which favour the uptake of the lead ions.

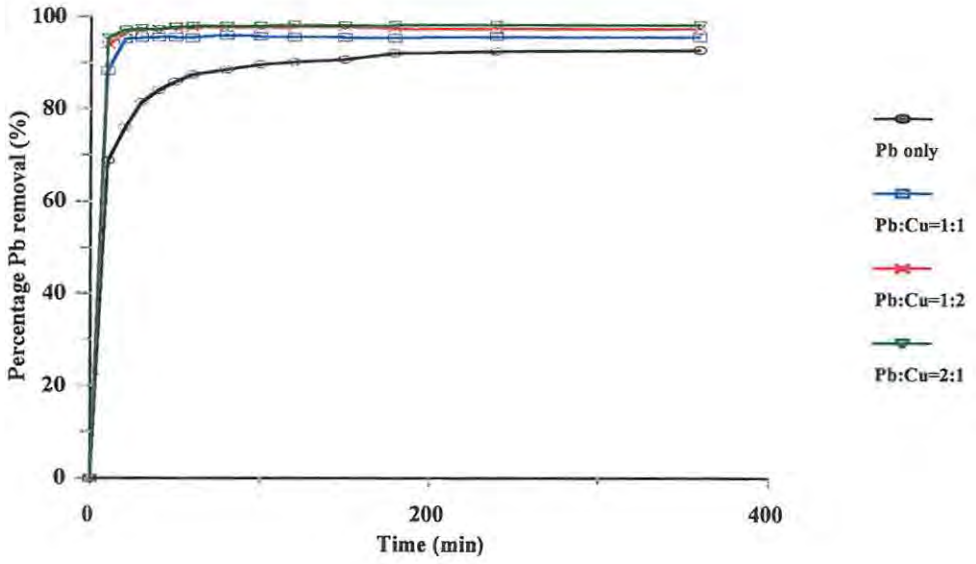

Figure 2.17: Percentage lead removal with time, in the presence of varying initial copper concentrations in aqueous solution. 1 part Pb or Cu = 40 mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 5.1; temperature, 25 °C; shaking rate, 170 rpm.

The effect of lead on percentage copper removal is shown in figure 2.18, and again various concentrations of lead with respect to copper appeared to have little to no effect on the percentage copper removed from aqueous solution. An equilibrium percentage copper removal of approximately 50 % was observed within 25 minutes.

Although these competition studies in batch systems suggested little or no competition between lead and copper ions in solution, this does not agree with some published literature, (de Rome and Gadd, 1991; Bedell and Darnall, 1990; Doyle *et al.*, 1980; Andres *et al.*, 1993) which reports non-specific adsorption to available sites using other biosorbents. There may, therefore, be some selective adsorption of lead from solution by *Azolla* biomass, or separate binding sites available for the two metal ions. Falla and Block, (1993) showed that isolated envelopes of *Pseudomonas fluorescens* had at least two different binding sites with different affinities for cadmium, nickel, copper and zinc ions, which ma be the same for *Azolla* biomass.

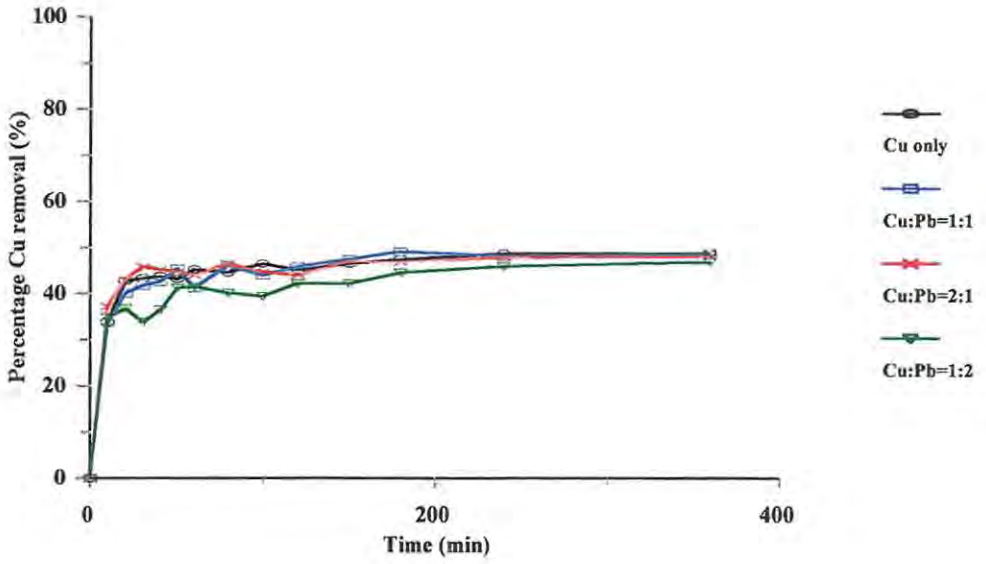

Figure 2.18: Percentage copper removal with time, in the presence of varying initial lead concentrations in aqueous solution. 1 part Cu or Pb = 40 mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 5.1; temperature, 25 °C; shaking rate, 170 rpm.

2.3.9.3 Effect of iron in solution on lead removal

Iron (Fe) in solution at various concentrations with respect to lead concentration had little effect on the percentage lead removal solution which reached an average equilibrium at approximately 95 %. A slight decrease in the percentage lead removal from 98 to 93 % was observed when the ratio of lead ions to copper ions in solution was 1:5 (figure 2.19). Figure 2.20 shows the percentage rate of iron removal from aqueous solution in the presence of various concentrations of lead. Iron percentage removal reached an equilibrium at approximately 70 to 75 % irrespective of the concentration of lead ions in solution.

As with multiple-metal studies involving copper, there does not appear to be any competition between iron and lead ions in solution for binding sites on *Azolla* biomass. This supports the hypothesis that there is selective adsorption of lead or *Azolla* biomass has binding sites with different affinities for different metal ions.

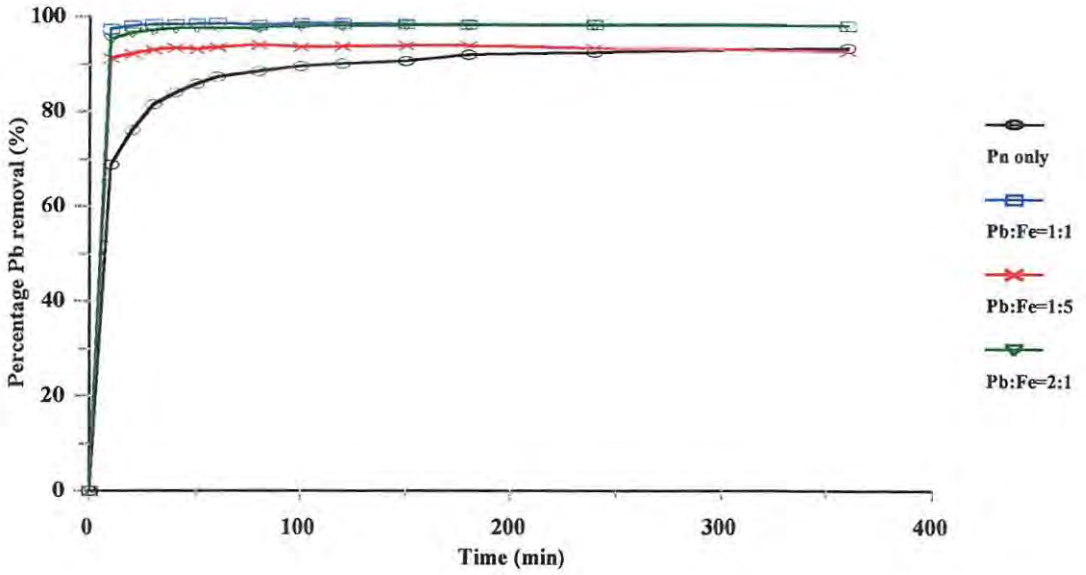

Figure 2.19: Percentage lead removal with time, in the presence of varying initial iron concentrations in aqueous solution. 1 part Pb or Fe = 40 mg/l (except for the Pb:Fe = 1:5 sample where 1 part = 20 mg/l); biomass concentration, 5 g *Azolla* / l solution; pH, 4.7; temperature, 25 °C; shaking rate, 170 rpm.

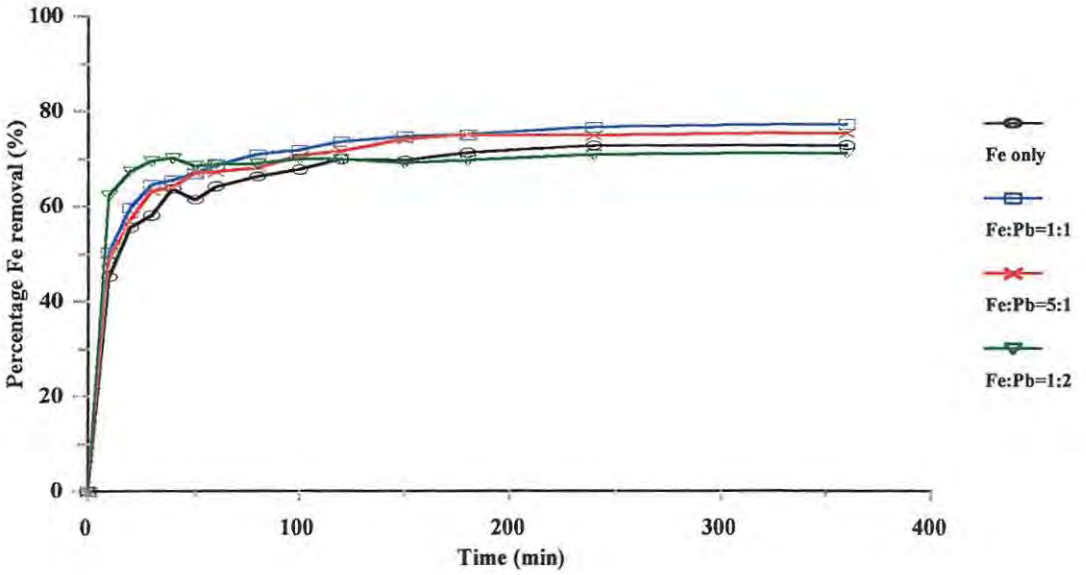

Figure 2.20: Percentage iron removal with time, in the presence of varying initial lead concentrations in aqueous solution. 1 part Fe or Pb = 40 mg/l (except for the Fe:Pb = 5:1 sample, where 1 part = 20 mg/l); biomass concentration, 5 g *Azolla* / l solution; pH, 4.7; temperature, 25 °C, shaking rate, 170 rpm.

2.3.9.4 Effect of both copper and iron in solution on lead removal

The effect of three metal ions in solution was determined in batch systems and the results are given in figure 2.21. The percentage metal removal equilibria for each metal ion was approximately 95, 50 and 75 % for lead, copper and iron respectively. These percentage metal removal equilibria value are very similar to those achieved in single metal ion studies for lead, copper and iron of 92, 48 and 70 % respectively. An Equi-molar metal concentration of 250 μM (approximately 52, 16 and 14 mg/l of lead, copper and iron respectively) was used in this experiment to investigate the competitive effect of equal molar concentrations of the metal ions in solution.

The three metal ions in solution did not appear to compete with the other metal ions for adsorption to the *Azolla* biomass. This further strengthened the hypothesis that the binding sites on the *Azolla* biomass have different affinities for different metal ions.

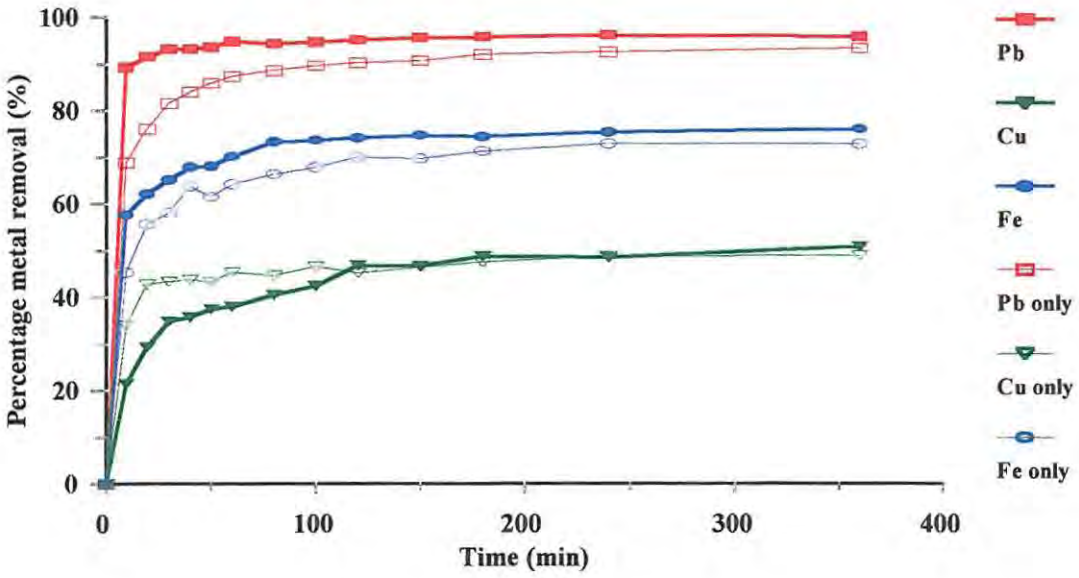

Figure 2.21: Percentage metal removal from a multiple-ions aqueous solution. Pb:Cu:Fe = 1:1:1, one part = 250 μM; single metal concentration, 250 μM; biomass concentration, 5 g *Azolla* / l metal solution; pH, 4.8; temperature, 25 °C; shaking rate, 170 rpm.

2.4 CONCLUSION

The non-viable biomass of the water fern *Azolla filiculoides*, when dried and then ground to a gritty consistency, showed strong physical stability which it maintained through-out the range of conditions that were investigated. No noticeable changes in the *Azolla* biomass' physical integrity were observed at pH values ranging from 1.5 to 9.5, temperatures from 10 to 50 °C, and initial lead concentrations from 10 to 800 mg/l.

Maximum percentage lead removal by the *Azolla* biomass was found to be between pH values of 3.5 and 5.7, therefore if the pH of the metal solutions was found to be within this range of values after being made up, no further pH adjustments were attempted. Aqueous solutions with lead concentrations below 200 mg/l give the most efficient lead uptake by the *Azolla* biomass. There was no effect of temperature observed over the range from 10 to 50 °C. Given these conditions, 5 g *Azolla* / l of metal-containing solution appears to be the optimum biomass concentration.

Competition studies using batch systems showed little or no competitions between lead, iron and copper with either two or three metal ions in solution. However, a relatively greater amount of lead (up to 95 %) was removed from multiple-metal solutions compared to copper or iron (50 and 70 % respectively). This may suggest selective removal of lead ions, or that there is more than one type of binding site on *Azolla* biomass with different affinities for different metal ions. Comparison of these competition studies in batch systems with competition studies using column systems, which are likely to be the preferred systems for industrial application purposes, will later be made.

The high maximum lead capacity of the *Azolla* biomass of 100 mg/g compares favourably with other

values in literature (table 2.2). Remediation studies done at the Hebrew University with dried *Azolla* biomass also gave its uptake capacity to be approximately 100 mg Pb/g *Azolla* (Priel, 1995). This makes *Azolla* a promising candidate for application in bioremediation and strongly supports its potential as an important biosorbent which may be used successfully and efficiently in treating wastewater from some industries.

The subsequent set of experiments investigated the *Azolla* biomass' capacity for lead removal from aqueous solution in column reactors, since that is the likely form of application of this technology in industry.

CHAPTER THREE

LEAD REMOVAL FROM AQUEOUS SOLUTION IN COLUMN SYSTEMS BY *AZOLLA FILICULOIDES*

3.1 INTRODUCTION

Due to their high mobility in natural water ecosystems and the food chain, and their toxicity to microorganisms, the remediation of heavy metal has become a world-wide priority. Industry's adverse impact on water resources is immense, and there is a need to promote effective pollution prevention and waste-water treatment methods to reduce contamination and deterioration of natural water systems (Atkinson *et al.*, 1998).

As discussed earlier, many microorganism and plants have the ability to accumulate heavy metals from their external environments. The efficiency of this process will differ between organisms. The mechanisms involved may also vary from a range of physicochemical interactions such as adsorption and deposition to energy dependent cell processes involving active transport mechanisms (Gadd, 1988). These processes are of industrial importance due to their potential for application in bioremediation of waste-waters. There is evidence that some biological systems are not only cheaper, but more efficient biosorbents of metals from solution, and may provide bioremediation technologies that may provide an alternative or subsidiary method to conventional techniques for metal removal and recovery. Technological application of biosorbent systems may depend on the relative ease of recovery of the bound metal for subsequent re-use or for further containment. Non-destructive recovery may also be necessary to allow regeneration and multiple re-use of the

biosorbent in order to reduce costs (Tsezos, 1984).

Investigations into the capacity of aquatic plants, mainly as viable biomass, to remove metal ions from solution have been made by researchers such as Muramoto and Oki, (1983); Abbasi and Nipaney, (1985); Scott, (1992); Delgado *et al.*, (1993). If viable biomass is to be used in bioremediation processes, the ease of biomass growth and biomass yield have to be taken into account in order to ensure regular availability of the biomass (Jain *et al.*, 1989).

Considerable biosorption studies using viable biomass have been carried out and these were discussed in chapter one, including work done by Sela *et al.* (1988) using viable *Azolla* biomass. However, the use of dead biomass in metal recovery offers several advantages in that the system is not affected by adverse operating conditions or metal toxicity, supply of nutrients is not necessary, adsorption and recovery of the surface-bound metals is relatively simple. For industrial and technical application, freely dispersed biomass has the following disadvantages: it may cause problems in the operation of reactors by blocking flow pipes and clogging filters and separation of biomass and effluent can prove difficult and costly (de Rome and Gadd, 1991). Zhao and Duncan (1997a and b) used non-viable *Azolla* biomass in batch and column reactors to remove hexavalent chromium from solution and electroplating effluent.

When considering industrial application of any biosorption system, uptake onto the microbial biomass constitutes the initial phase of the system, this must be followed by a recovery phase. The simplest and cheapest desorption process is eluting the metal from the biomass surface by means of a desorbing agent such as mineral acids like nitric acid. The efficiency of desorption depends on the H^+ concentration rather than the anionic species present (de Rome and Gadd, 1991).

This study investigated the ability of the *Azolla* biomass to adsorb lead from aqueous solutions with different initial lead concentrations and at different flow rates in column systems. The lead removal potential of the biomass was also investigated in the presence of two competing metal ions. Re-usability of the biomass was determined by repeated adsorption and desorption cycles, after which the percentage lead removal and recovery were determined.

3.2 MATERIALS AND METHODS

3.2.1 Biomass

Azolla filiculoides biomass was obtained locally and prepared as outlined in chapter 2, with the exception that dried whole *Azolla* biomass was used instead of ground biomass in subsequent column studies.

3.2.2 Solutions

All solutions were prepared as detailed in chapter 2.

3.2.3 Metal adsorption and desorption experiments

All experiments were performed in duplicate. A 1000 ml volume of metal solution was pumped through a packed up-flow column containing 5 g of *Azolla* biomass in a bed volume of 49 ml at 2, 5 and 10 ml/min. Samples were collected at regular time intervals using a Gilson fl 204 fraction collector and analysed for the metal of interest using an atomic absorption spectrophotometer.

After 1000 ml of metal solution had been pumped up the column, 50 ml of a 0.1 M mineral acid (HCl or HNO_3) was used to elute the metal off the column under gradient flow, with 5 washes. A volume

of 100 ml of de-ionised water was used to wash the column twice before reconditioning/regenerating the biomass with 4 washes using 50 ml of a 0.05 M basic solution (NaOH or $NaHCO_3$). A final single wash with 200 ml of de-ionised water was carried out and the next adsorption cycle started. The amount of metal in the desorbent, reconditioning basic solution and water washes was analysed using an atomic absorption spectrophotometer.

3.2.4 Metal analysis

Analysis of metal in solution was as described in chapter 2.

3.3 RESULTS AND DISCUSSION

When considering metal removal from solution in column systems, several factors such as initial lead concentration, the biomass's maximum uptake capacity for the metal of interest (results from batch equilibrium sorption isotherm studies) and the flow rate need to be considered. The bed volume in any given system and the flow rate both affect the retention time of the metal solution in the column in contact with the biomass, and therefore the metal removal efficiency of the column system. Break-through points are a measure of the volume at which the percentage metal removal starts to decrease after reaching equilibrium. These points give an idea of how much metal solution can be treated at maximum efficiency at a given flow rate and initial lead concentration. At the break-through point, the binding sites of the biomass become saturated, and are unable to remove any more metal ions from solution. The saturation process can be rapid, in which case the drop in percentage metal removal occurs within a short space of time, or it can be gradual, occurring over a longer period of time. These factors were investigated for the *Azolla* biomass column system for the removal of lead from aqueous solution.

3.3.1 Effect of initial lead concentration at a flow rate of 2 ml/min

No break-through points were observed for initial lead concentrations of 55 and 100 mg/l on passing 1000 ml of metal solution through the column at a flow rate of 2 ml/min (figure 3.1). The percentage lead removal equilibrium at these two lead concentrations was approximately 100 and 98 % respectively. Break-through points were observed for all the subsequent metal solution with a range of initial lead concentrations from 180 to 890 mg/l. Table 3.1 below contains a summary of the data observed in and calculated from figure 3.1.

The break-through values (V_b) generally decreased with increasing initial lead concentrations, with the exception of the 275 and 465 mg/l samples whose break-through points were the same. At initial lead concentrations of 100 mg/l or less there was no break-through point observed, this is probably due to the fact that after 1000 ml of lead solution has been passed through the column with 5 g of

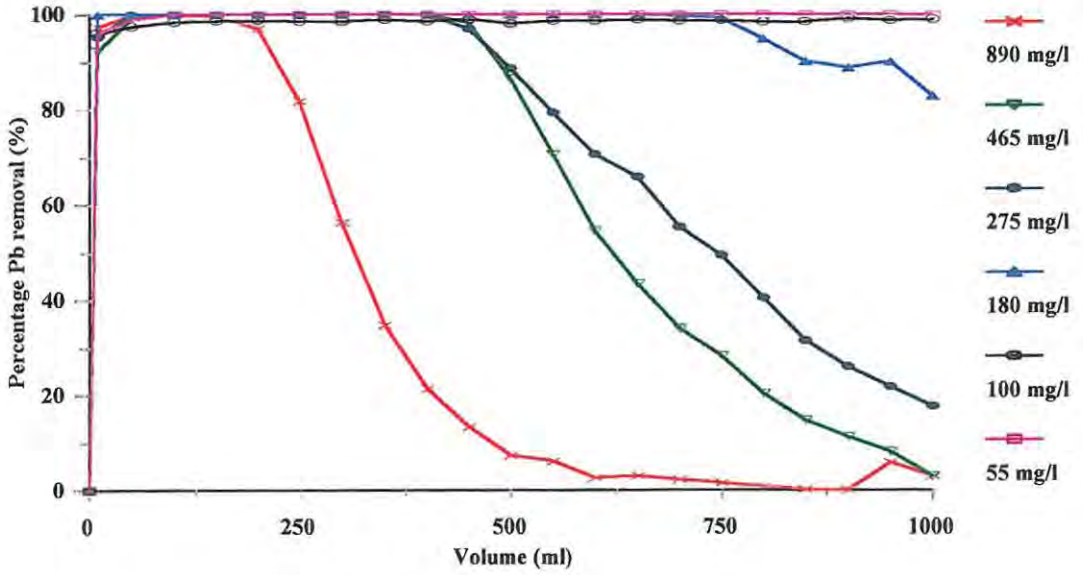

Figure 3.1: Break-through curves for lead removal from aqueous solution, with varying initial lead concentrations. Flow rate, 2 ml/min; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, RT ~ 20 °C, bed volume, 49 ml.

Azolla biomass, assuming 100 % removal, the amount of lead removed by the biomass would be 20 mg/g or less. The maximum lead uptake capacity of the *Azolla* biomass was found to be approximately 100 mg/g, therefore an uptake value of 20 mg/g is well short of this maximum value. This indicates that more than 1000 ml of an aqueous solution with lead at a concentration of 100 mg/l or less can be passed through the *Azolla* biomass column at a flow rates of 2 ml/min, without saturation of the binding sites occurring.

Table 3.1: Lead removal in a column system at 2 ml/min

C_i (mg/l)	C_b (mg/l)	C_f (mg/l)	V_b (L)	q_b (mg/g)	q_f (mg/g)
55	0	0	1	11	11
100	0.8	0.8	1	20	20
180	1.2	31	0.75	27	30
275	7.8	226	0.45	24	10
465	7.5	451	0.45	41	3
890	2.0	860	0.15	27	6

C_i - initial Pb concentration C_b - Pb concentration at break-through point C_f - final Pb concentration

V_b - volume at break-through point q_b - Pb uptake at break-through point q_f - Pb uptake at C_f

$q_b = (C_i - C_b) \times V_b / 5$:(5 = biomass concentration in g (constant))

$q_f = (C_i - C_f) \times V_f / 5$:(5 = biomass concentration in g (constant)) , V_f = final volume(constant) 1 L)

Figure 3.1 also shows that the percentage lead removal equilibrium for all the different initial lead concentrations was found to be between 97 and 100 %. The decrease in the percentage lead removal after the break-through point was gradual, except for the sample with the highest initial lead concentration of 890 mg/l where it was found to be rapid. This was probably because as the

concentration of the lead ions in solution increases, more lead ions are available for interaction with the sequestering groups on the biomass surface and saturation of the sites occurs gradually. The rapid saturation of the biomass observed for the highest initial lead concentration may be due to the very high number of lead ions saturating the system.

3.3.2 Effect of initial lead concentrations at a flow rate of 5 ml/min

Figure 3.2 shows the break through curves for the same initial lead concentrations as those given in figure 3.1, but at a flow rate of 5 ml/min. The trends of the break-through curves were similar to those observed at a flow rate of 2 ml/min, with no break-through point for the solutions at 55 and 100 mg/l of lead.

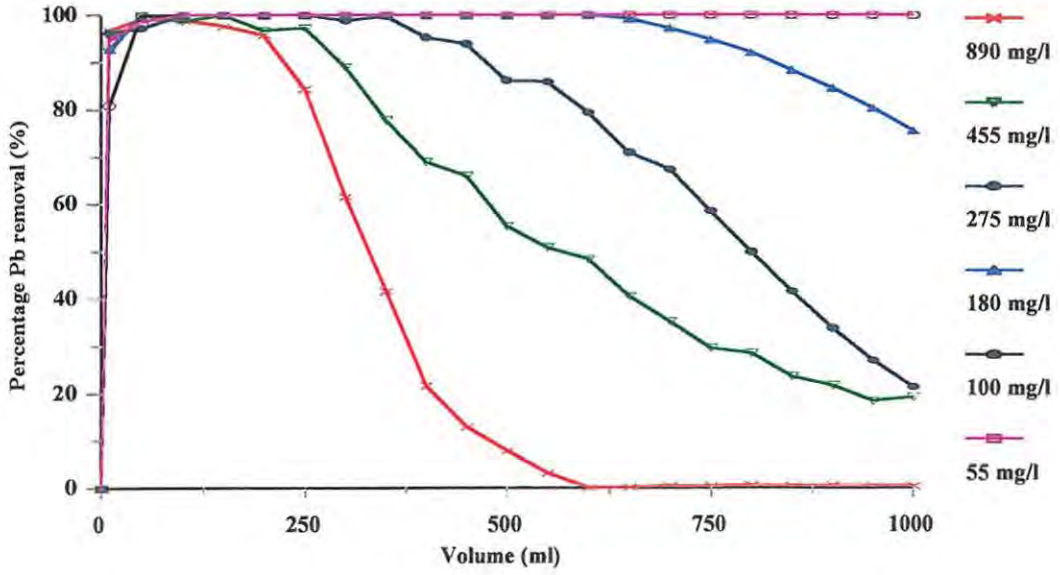

Figure 3.2: Break-through curves for lead removal from aqueous solution, with varying initial lead concentrations. Flow rate, 5 ml/min; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, RT ~ 20 °C; bed volume, 49 ml.

The percentage lead removal equilibrium for both the 55 and 100 mg/l solutions was found to be 100%. The break through points of all but the 890 mg/l solution were seen to occur earlier in this case compared to the break-through points at a flow rate of 2 ml/min (figure 3.1). Table 3.2 below gives the data observed and calculated from figure 3.2.

Table 3.2: Lead removal in a column system at 5 ml/min

C_i (mg/l)	C_b (mg/l)	C_f (mg/l)	V_b (L)	q_b (mg/g)	q_f (mg/g)
55	0	0	1	11	11
100	0	0	1	20	20
180	5.0	43	0.7	25	27
275	0.9	216	0.35	19	12
465	12.6	367	0.25	23	20
890	21.1	884	0.15	26	1

C_i - initial Pb concentration C_b - Pb concentration at break-through point C_f - final Pb concentration

V_b - volume at break-through point q_b - Pb uptake at break-through point q_f - Pb uptake at C_f

$q_b = (C_i - C_b) \times V_b / 5$:(5 = biomass concentration in g (constant))

$q_f = (C_i - C_f) \times V_f / 5$:(5 = biomass concentration in g (constant)) , V_f = final volume(constant) 1 L)

Table 3.3 shows that the break-through values (V_b) within the samples at 5 ml/min decreased as the initial lead concentration increased, a similar trend was observed for figure 3.1 at a flow rate of 2 ml/min. This was probably due to the same reasons as explained for figure 3.1, as was the rapid decrease in the percentage metal removal observed in the case of the 890 mg/l solution, and the gradual decrease at the other lower initial lead concentrations. Percentage lead removal equilibrium for the different samples was between 96 and 100%.

3.3.3 Effect of initial lead concentrations at a flow rate of 10 ml/min

As the flow rate of the column experiments was increased from 2 to 10 ml/min, the general trend in the break-through curves remained very similar. The only difference in each case being in the point at which break-through occurred. Figure 3.3 gives the break-through curves of the range of solutions at different initial lead concentrations from 55 to 890 mg/l. At the lower initial lead concentrations of 55 and 100 mg/l there were again no break-through points observed, and both curves reached a percentage lead removal equilibrium of approximately 100 %. Percentage lead removal equilibria for the other solutions was between 94 and 100%. A percentage lead removal equilibrium value of 94 % for the 275 mg/l lead solution was the lowest in all the samples.

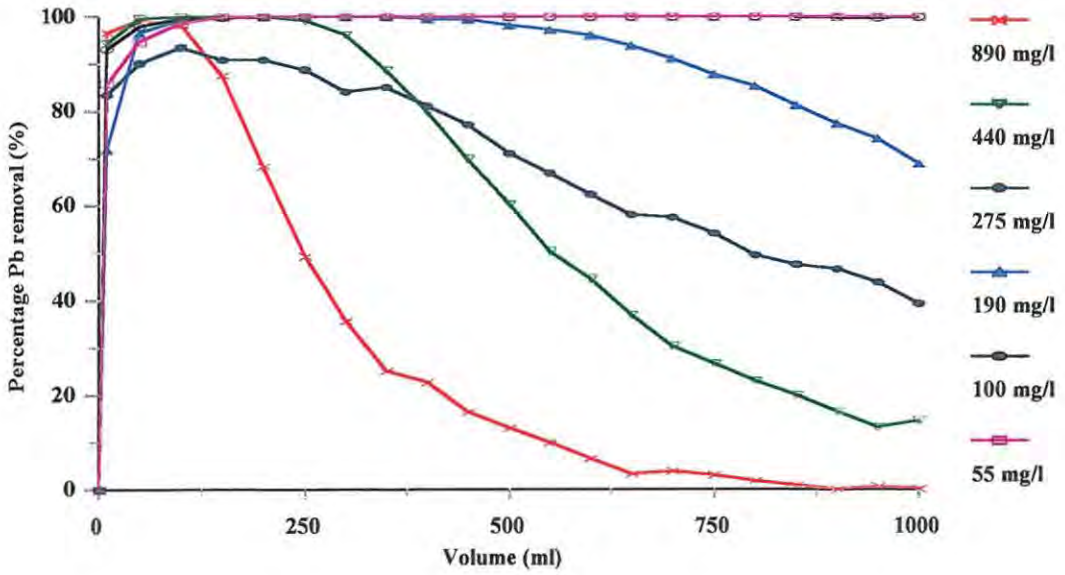

Figure 3.3: Break-through curves for lead removal from aqueous solution with varying initial lead concentrations. Flow rate, 10 ml/min; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, RT ~ 20 °C; bed volume, 49 ml.

The data observed and calculated from figure 3.3 is given in table 3.3 below. The break-through points (V_b) given in table 3.3 show a decrease with an increase in initial lead concentration with the exception of the 465 mg/l sample. The amount of lead uptake (mg/g) at the break-through points were found to increase at the lower initial lead concentrations with corresponding increases in initial lead concentrations. A similar trend was observed with experiments at flow rates of 2 and 5 ml/min.

Table 3.3: Lead removal in a column system at 10 ml/min

C_i (mg/l)	C_b (mg/l)	C_f (mg/l)	V_b (L)	q_b (mg/g)	q_f (mg/g)
55	0	0	1	11	11
100	0	0	1	20	20
180	11.5	58	0.65	22	24
275	17.3	162	0.1	5	23
465	15.3	340	0.3	27	25
890	15.5	888	0.1	17	0.4

C_i - initial Pb concentration C_b - Pb concentration at break-through point C_f - final Pb concentration

V_b - volume at break-through point q_b - Pb uptake at break-through point q_f - Pb uptake at C_f

$q_b = (C_i - C_b) \times V_b / 5$:(5 = biomass concentration in g (constant))

$q_f = (C_i - C_f) \times V_f / 5$:(5 = biomass concentration in g (constant)) , V_f = final volume(constant) 1 L)

It appears, from results from figures 3.1 to 3.3, that the maximum lead uptake capacity of the *Azolla* biomass, and in fact any biomass, in batch systems is greater than that in column systems due to the fact that the retention time and agitation in batch systems allows for optimum interaction between the biosorbent and biosorbate, which is not true in column systems. Therefore the maximum uptake capacity value for a given biomass determined in batch systems can only be used to give an

approximate idea of uptake capacity in column systems. More accurate maximum uptake capacities for a given biomass in column systems can be estimated by comparing values calculated using metal solutions of different initial metal concentrations.

At all three flow rates investigated, solutions with initial lead concentrations above 100 mg/l showed break-through points whose values decreased with an increase in initial lead concentration (tables 3.1-3.3). The relationship was not a perfect inverse one, and discrepancies are probably due to some degree of experimental error, for example the break-through point at initial lead concentration of 275 and 465 mg/l were the same at a flow rate of 2 ml/min. The rapid saturation observed at high initial lead concentrations was more pronounced at the faster flow rates as the stearic hindrance and reduced retention times contribute to limited interaction between the metal ions and the binding sites. Table 3.4, below, summarises the effect of initial lead concentration and flow rate on the amount of lead uptake in the *Azolla* biomass column systems. The maximum amount of lead uptake of 41 mg/g was found to be at a flow rate of 2 ml/min with an initial lead concentration of 465 mg/l. At the lower initial lead concentration values (50 and 100 mg/l, and to a lesser extent 180 mg/l) the flow rate does not appear to have any significant effect on the amount of lead uptake. This is probably because at these low lead concentrations, flow rates of up to 10 ml/min are not limiting, and the number of binding site are probably in excess of the number of metal ions in solution. At these higher flow rates all or most of the metal ions have an opportunity to interact with the binding sites, and the reduction in contact time due to an increase in the flow rate has no effect. At higher initial lead concentrations, the increased flow rate, and therefore reduced contact time, becomes limiting as the same number of binding sites are available to bind a lot more metal ions in solution. The result is a decrease in lead uptake from solution. Therefore, it is only at low initial lead concentrations that flow rate does not affect lead removal by *Azolla*.

Table 3.4: Lead uptake at break-through points at various flow rates and initial lead concentrations

Lead uptake at break-through point (q_b) - mg/g			
	Flow rate		
Initial [Pb] (mg/l)	**2 ml/min**	**5 ml/min**	**10 ml/min**
55	11	10	10
100	19	20	22
180	27	24	23
275	24	19	5
465	41	22	23
890	27	26	18

3.3.4 Multiple-metal solution studies - initial metal concentration of 100 mg/l

Iron and copper were used in multiple-metal studies in column systems because both metals ions were found to be present in lead-acid battery effluent, and in order to compare multiple-metal studies done in column systems to those done previously in batch systems.

The results of the effect of three metal ions, lead (Pb), copper (Cu) and iron (Fe) at an initial concentration of approximately 100 mg/l respectively, on metal removal in column systems by the *Azolla* biomass are given in figures 3.4 and figure 3.5. The control experiments for metal uptake from single-metal solutions (Pb(C), Fe(C) and Cu(C)) showed that there were no apparent break-through points for the lead and iron removal from solution. However, the control curve for copper removal showed a gradual decrease in percentage copper removal (figure 3.5) which was mirrored by an increase in the amount of copper in solution (figure 3.4). The percentage lead removal equilibrium values were approximately 100, 95 and 70 % for lead, copper and iron respectively.

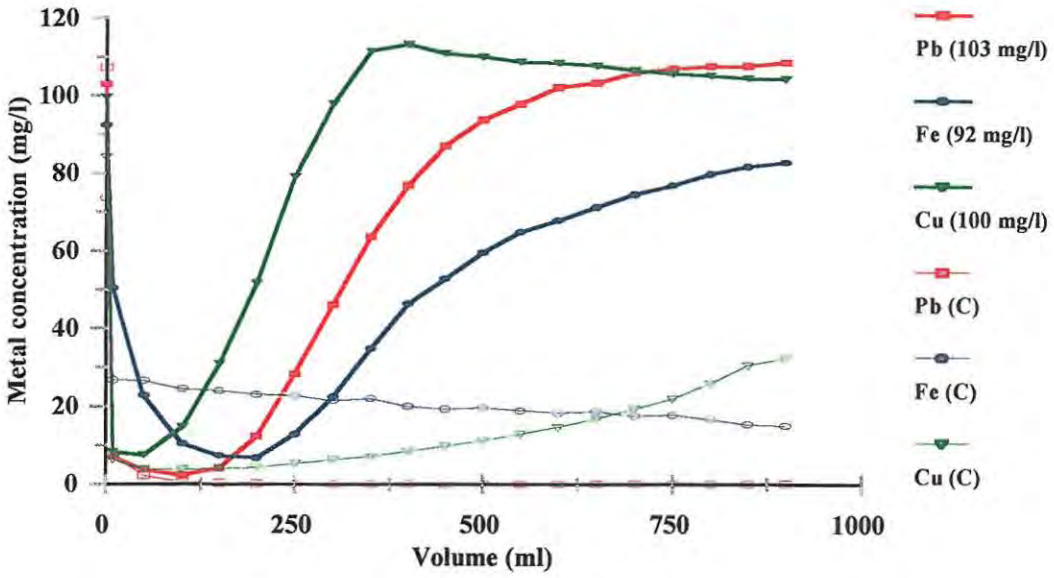

Figure 3.4: Break-through curves for lead, iron and copper removal from multiple-metal aqueous solution at an initial metal concentration of approximately 100 mg/l. Pb(C), Fe(C) and Cu(C) are single-metal control studies. Biomass concentration, 5 g *Azolla* / l solution; pH, 3.0; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

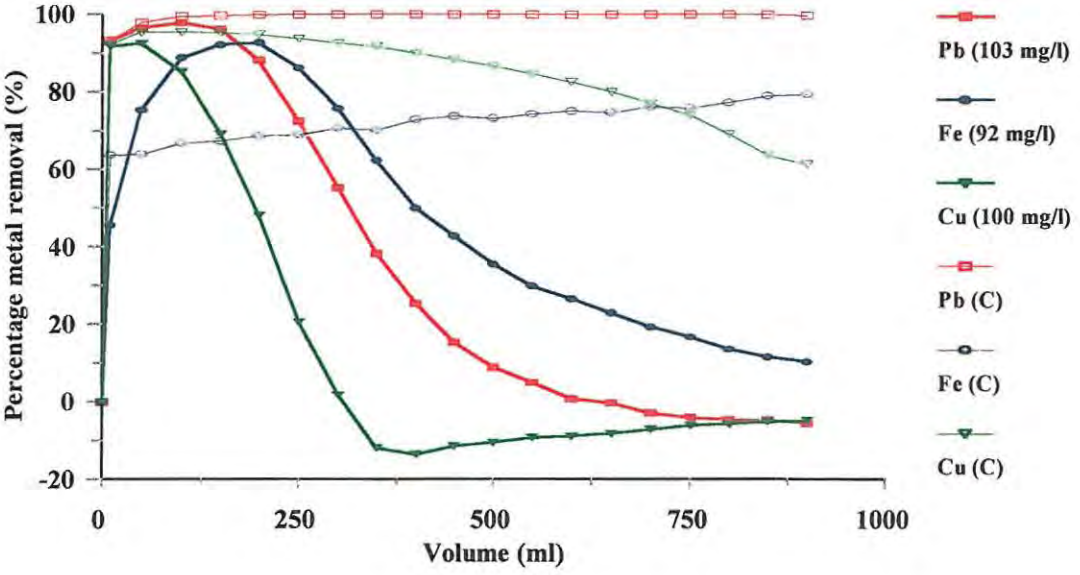

Figure 3.5: Percentage lead, iron and copper removal from multiple-metal aqueous solution at an initial metal concentration of approximately 100 mg/l. Pb(C), Fe(C) and Cu(C) are single-metal control studies. Biomass concentration, 5 g *Azolla* / i solution; pH, 3.0; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

Break-through points for all three metals were observed in metal removal from multiple-metal solutions. This suggested that the presence of other metal ions in solution does to affect the removal from solution of each metal ion compared to uptake from single-metal solutions. The break-through point for copper was the lowest, followed by iron and lead. This may be due to greater competition between lead and copper ions for similar binding sites in column systems. In the case of copper, after approximately 300 ml of multiple-metal solution had been passed through the column, the copper concentration found in solution was higher than that in the eluant. This suggested that previously bound copper ions were displaced from their binding sites on the *Azolla* biomass surface. A similar effect was observed with lead removal after about 550 ml, but not with iron removal (figures 3.4 and 3.5).

3.3.5 Multiple-metal solution studies - initial metal concentration of 50 mg/l

Figures 3.6 and 3.7 show the effect of metal removal from multiple-metal solutions, in this case the initial metal concentrations were reduced to approximately 50 mg/l compared to figures 3.4 and 3.5, where they were 100 mg/l. The trends observed in all three curves were very similar to those observed in figures 3.4 and 3.5. There were break-through points at approximately 350 ml for lead, 200 ml for copper and 450 ml for iron. The decrease in percentage metal removal after these break-through points was more rapid for lead and copper than for iron. This supported the hypothesis that there may be more competition for similar binding sites between copper and lead ions, as was observed with initial metal concentrations of 100 mg/l. In contrast to batch studies with multiple-metal solutions, there appeared to be clear competition between lead, copper and iron for uptake by the *Azolla* biomass. The competition between metal ions observed in column systems may be a reflection of the effect of reduced retention time which is not an issue in batch systems.

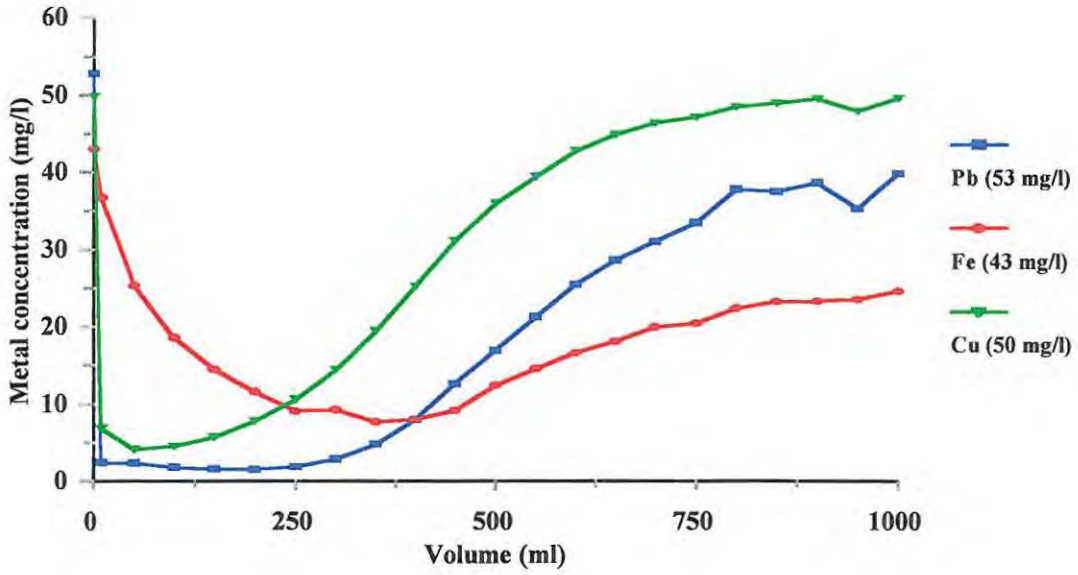

Figure 3.6: Break-through curves for lead, iron and copper removal from multiple-metal aqueous solution, at an initial metal concentration of approximately 50 mg/l. Biomass concentration, 5 g *Azolla* / l solution; pH, 3.0; flow rate, 10 ml/min, temperature, RT ~ 20 °C; bed volume, 49 ml.

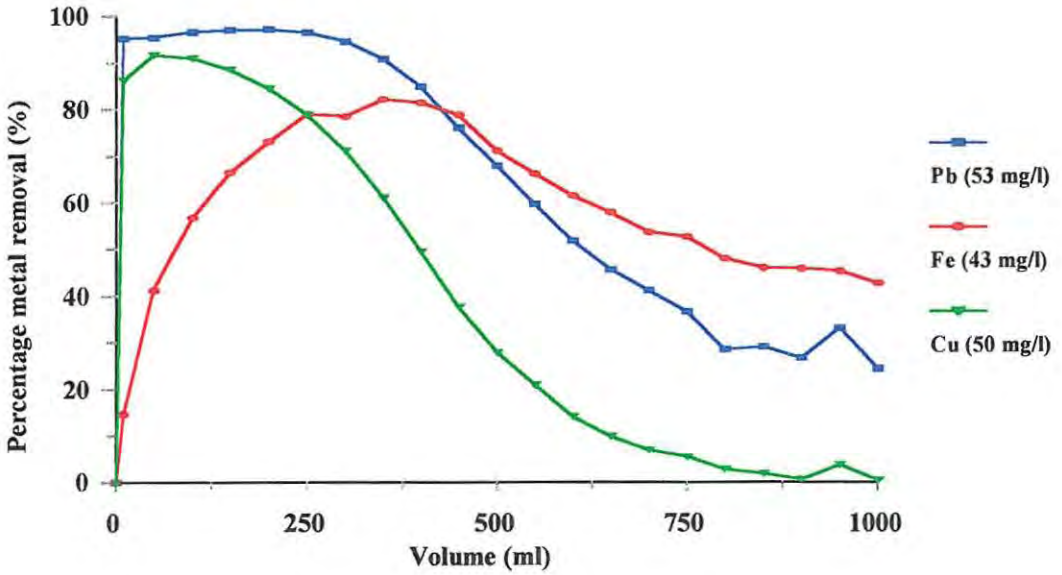

Figure 3.7: Percentage lead, iron and copper removal from multiple-metal aqueous solution at an initial metal concentration of approximately 50 mg/l. Biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; pH, 3.0; temperature, RT ~ 20 °C; bed volume, 49 ml.

Although the presence of competitive effects in these column studies were in contrast to the lack of competition observed between lead, copper and iron in batch studies, the higher percentage lead removal in both systems still suggested some preference of lead ions by the *Azolla* biomass. This may be due to the higher molecular size of lead compared to iron or copper. Table 3.5 below shows the lead uptake capacity (mg/g) of the *Azolla* biomass from single-metal solutions (q_1) compared to lead uptake from multiple-metal solutions (q_2 - initial lead concentration was approximately 50 mg/l and q_3 - initial lead concentration of about 100 mg/l).

Table 3.5: Metal uptake in multiple-metal solutions

Metal sample	Metal uptake (mg/g)		
	q_1	q_2	q_3
Lead	19.3	3.4	3.0
Iron	10.5	3.0	3.4
Copper	9.3	1.7	7.6

q_1 - metal uptake (mg/g) in single-metal solutions (initial metal concentration ~ 90 mg/l)

q_2 - metal uptake (mg/g) in multiple-metal solutions (initial metal concentration ~ 50 mg/l)

q_3 - metal uptake (mg/l) in multiple-metal solutions (initial metal concentration ~ 100 mg/l)

The data shows that there was a higher lead uptake from single-metal solution studies of 19.3 mg lead / g *Azolla* biomass. Iron and copper uptake capacities in single metal studies under the same conditions were 10.5 and 9.3 respectively, almost half of that observed for lead. Studies with multiple-metal solutions saw these values drop to approximately the same value for lead and iron (about 3 mg/g) at initial metal concentrations of both 100 and 50 mg/l. The uptake capacity of the biomass for copper in multiple-metal solutions varied significantly between the system with an initial

metal concentration of 50 mg/l and that of 100 mg/l, and these were approximately 2 mg/g and 8 mg/g respectively. This was probably due to the stronger competition observed between lead and copper ions. There was a decrease in lead uptake from single-metal solutions compared to multiple-metal solutions, from 19.3, 10.5, 9.3 mg/g down to 3.4, 3.0 and 1.7 for lead, iron and copper respectively when the initial metal concentration is approximately 100 mg/l. Very similar values are found with initial metal concentrations of 50 mg/l except for copper. The decrease in the individual amount of metal removal in multiple-metal solutions can be attributed mostly to some form of competition for binding sites. Adsorption by cations onto binding sites on non-viable biomass surfaces has been reported to be of a non-specific nature. The amount of uptake is affected by the concentration and chemistry of each metal ion, the nature of the sequestering groups present and physicochemical factors of the aqueous environment (de Rome and Gadd, 1991). However, in the case of the *Azolla* biomass, the percentage removal of lead from multiple-metal solution as found in figures 3.4 to 3.7 was generally higher than for iron or copper. This suggests some element of selective lead adsorption by certain ligands on the biomass' surface.

3.3.6 Adsorption and desorption cycles - biomass re-usability

The bar graph showing the change in adsorption efficiency of the *Azolla* biomass when reconditioned with 50 ml 0.05 M NaOH following lead desorption with 50 ml 0.1 M HCl, over 10 cycles is given in figure 3.8. Efficiency of the biomass was measured in terms of percentage lead removed from the influent solution and that recovered relative to the amount removed in that cycle. Starting with an initial lead concentration of 100 mg/l at each cycle, the percentage lead removal from aqueous solution was 90 % or more for all 10 cycles. The percentage lead recovered from the biomass by desorption with dilute HCl increased from approximately 50 % at the end of the first cycle to approximately 75 % by the fourth cycle. Percentage recovery in subsequent cycles was over 80 %.

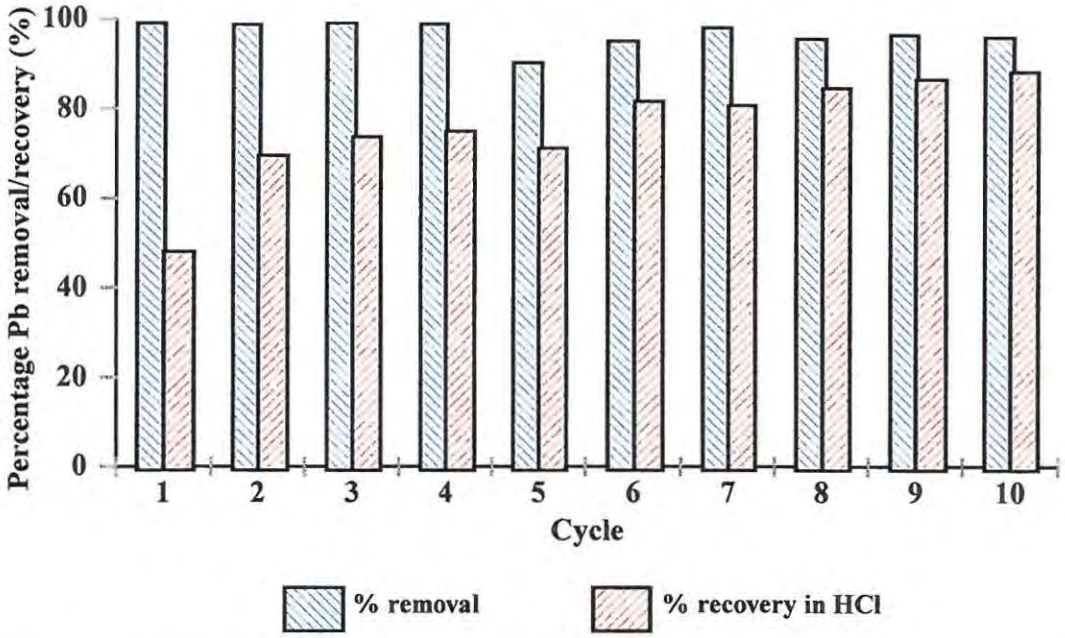

Figure 3.8: Percentage lead removal and recovery for10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50ml 0.1 M HCl as the desorbent. Initial Pb concentration, 100 mg/l; eluant pH, 5.4; biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; reconditioning base, 50 ml 0.05M NaOH, temperature, RT ~ 20 °C, bed volume, 49 ml.

Figure 3.9 shows the adsorption efficiency of the *Azolla* biomass reconditioned with the 50 ml 0.05 M NaOH following lead desorption, this time with 50 ml 0.1 M HNO_3, over 10 cycles. The initial lead concentration was again 100 mg/l at the start of each cycle. The percentage lead removal from aqueous solution was 94 % or more for all but the second cycle, where percentage lead removal was found to be approximately 81 %. There appears to be no explanation for this decrease other than experimental error. However, it was observed that percentage lead recovery after the second cycle was also relatively low compared to the other cycles. The reason for this observed effect was, again, not apparent. Percentage lead recovery from the biomass by desorbing with HNO_3 was found to be consistently over 70 % with the exception of the second cycle were it was approximately 53 %. Compared to desorption with HCl, there appears to be a slighter higher percentage lead removal with HNO_3 desorption, but a slight lower percentage lead recovery.

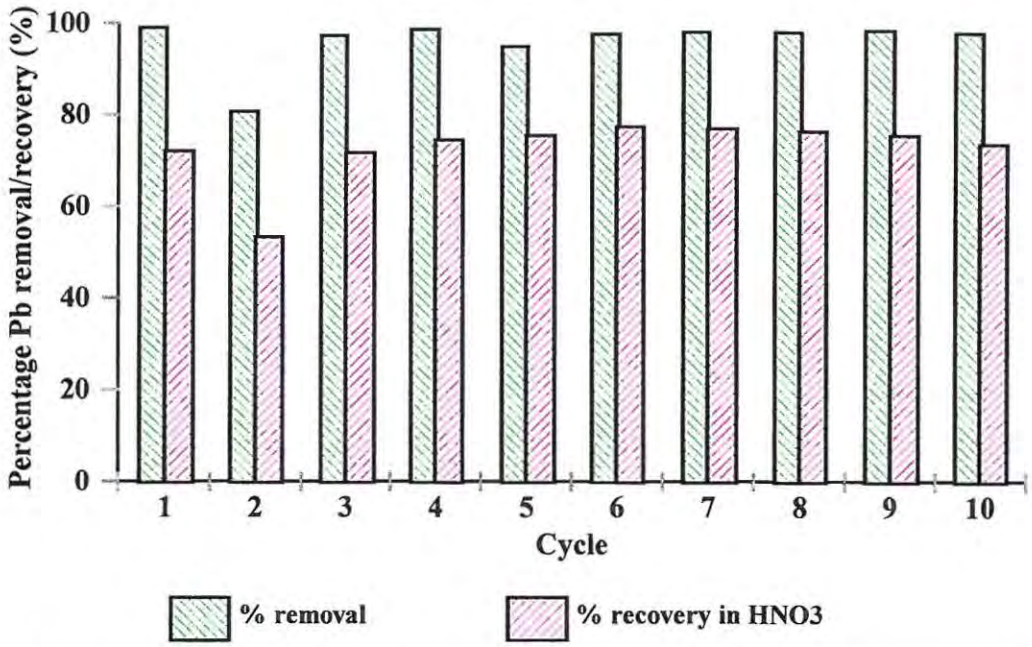

Figure 3.9: Percentage lead removal and recovery for10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml 0.1 M HNO_3 as the desorbent. Initial Pb concentration, 100 mg/l; eluant pH, 5.4; biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; reconditioning base, 50 ml 0.05M NaOH, temperature, RT ~ 20 °C, bed volume, 49 ml.

The effects of changing the reconditioning agent from NaOH to $NaHCO_3$ on the adsorption and desorption of lead over 10 cycles are given in figures 3.10 and 3.11. A 50 ml volume of 0.05 M $NaHCO_3$ was used to recondition the *Azolla* biomass after lead desorption with 50 ml 0.1 M HCl or 0.1 M HNO_3. The percentage lead removal from aqueous solution using HCl (figure 3.10) was found to decrease gradually from the first to the subsequent cycles from about 98 to 69 % lead removal. Percentage lead recovery from the biomass using HCl was more consistent at over 90 % with cycles 1, 5 and 10 being the exceptions, with percentage recovery values of 51, 85 and 53 respectively.

The adsorption and desorption results using HNO_3 are shown in figure 3.11. The percentage lead removal from solution was found to follow a similar trend to that seen using HCl, with a decrease

in percentage lead removal with the number of cycles from 100 % down to 70 %. Percentage lead recovery using HNO_3 was also observed to be over 90 % with the exception of cycles 1, 5 and 10, whose percentage lead recovery were 43, 86 and 50 % respectively.

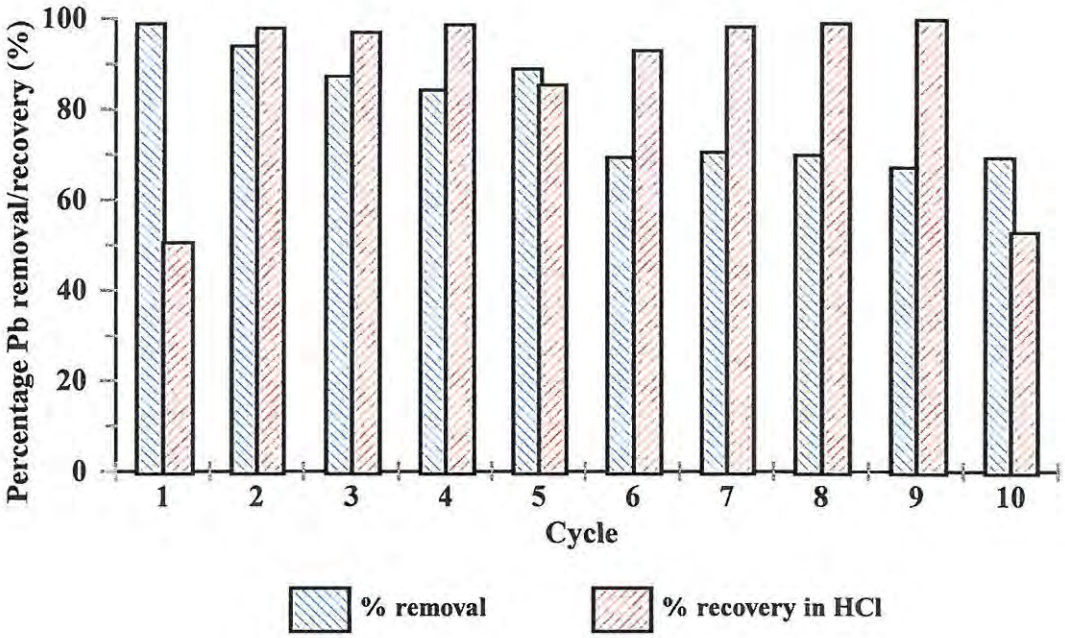

Figure 3.10: Percentage lead removal and recovery for10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml 0.1 M HCl as the desorbent. Initial Pb concentration, 100 mg/l; eluant pH, 5.4; biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; reconditioning base, 50 ml 0.05M $NaHCO_3$, temperature, RT ~ 20 °C, bed volume, 49 ml.

In general, there were no observed adverse effects on lead removal and recovery efficiency following ten repeated adsorption and desorption cycles of lead onto and from *Azolla* biomass columns using NaOH as the reconditioning agent. There was no decrease in percentage lead removal from solution, or percentage lead recovery by desorbing with HCl or HNO_3. Lead recovery using 0.1 M mineral acid solutions resulted in lead recovery percentages for HCl and HNO_3 of approximately 80 % and 94 % respectively. These results suggest that the mineral acids and basic solutions do not adversely affect the ligand structure on the biomass surface.

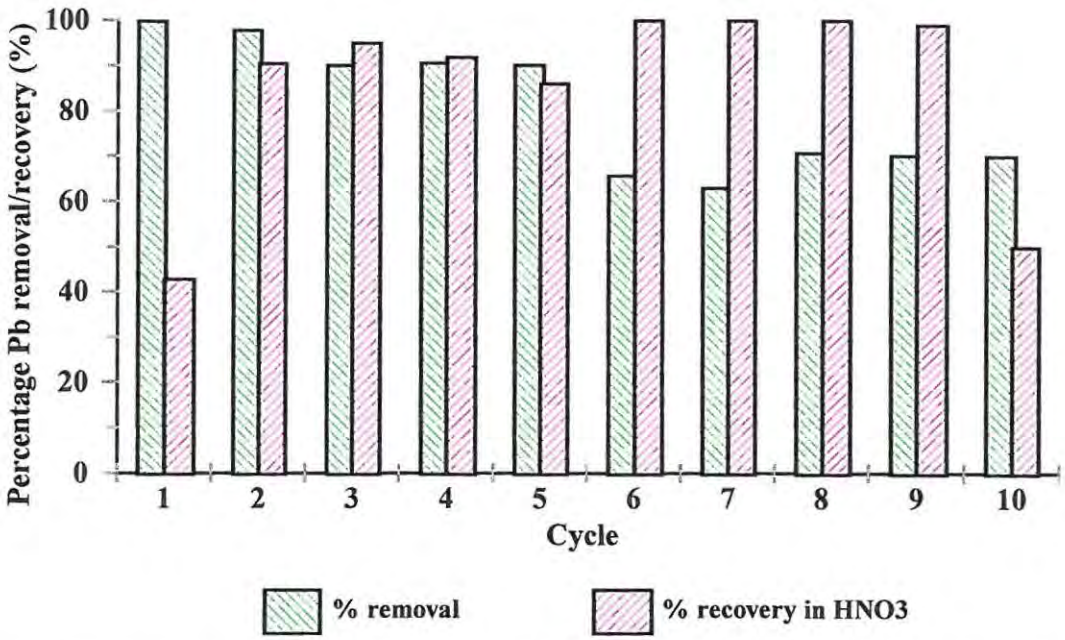

Figure 3.11: Percentage lead removal and recovery for 10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml 0.1 M HNO_3 as the desorbent. Initial Pb concentration, 100 mg/l; eluant pH, 5.4; biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; reconditioning base, 50 ml 0.05 M $NaHCO_3$, temperature, RT ~20 °C, bed volume, 49 ml.

Reconditioning with $NaHCO_3$ did not appear to be as efficient as with NaOH, as the percentage lead removal was found to decrease with each cycle. This is probably because $NaHCO_3$ is a milder base compared to NaOH. The percentage lead recovery with HCl and HNO_3 was generally high at over 90 % recovery. Attempts to evaluate the capacity of sulphuric acid (H_2SO_4) to desorb lead from the *Azolla* biomass were discontinued after precipitation of lead was observed, probably as $PbSO_4$.

In all the adsorption and desorption studies, a 1000 ml volume of lead-containing effluent was passed through the *Azolla* column, and the bound lead desorbed in 50 ml of mineral acid. This translates to a 20 times concentration of the initial lead in solution. The implication of this in industry would be considerable. The ability to desorb bound metal and concentrate it to a large extent would offer opportunities for re-using the desorbed metal in several industries, e.g. electroplating industry, and

for less costly disposal methods in other cases.

Several other researchers have reported similar desorptive effects of mineral acids on metal ions bound onto biomass surfaces. de Rome and Gadd (1991), Garnham *et al.* (1992) and Galun *et al.* (1987) have reported the effects of H^+ in displacing bound metal ions and therefore giving an ion exchange type system to desorb the metal ions. No adverse effect on the physical integrity of the *Azolla* biomass by treatment with the dilute mineral acids or reconditioning bases was observed. Scanning electron microscopy (discussed in a later chapter) was used to check for deterioration and break down of biomass structure and none was observed. Of the reconditioning agents were used to neutralise the pH of the biosorbent following acid desorption steps. NaOH appeared to be a more efficient reconditioning agent compared to $NaHCO_3$ and it also seemed to improve the biomass' metal removal efficiency. Galun *et al* (1987) suggested that biomass treatment with a mineral acids may serve to displace blocking groups like Ca^{2+}, or denature some surface bound molecules to expose more sites for metal binding. Reconditioning with NaOH may serve a similar purpose.

3.4 CONCLUSION

The *Azolla* biomass was able to effectively remove lead from solution, particularly when the initial lead concentration was 100 mg/l or less. Flow rates of up to 10 ml/min did not appear to have any effect on the percentage lead removal of over 95 % at these initial lead concentrations. The high affinity of the *Azolla* biomass for lead, even under competition from other metal ions in solution, makes it a promising candidate for useful application in bioremediation processes. Column systems eliminate the problems associated with developing methods to separate the biomass from the liquid, as in batch systems, following metal removal and recovery.

Recovery of the bound lead from the biomass suggests that lead binding to the *Azolla* biomass is reversible, and dilute mineral acids can be used to desorb and concentrate the bound lead metal effectively. Adsorption and recovery was repeated up to10 times without any significant decrease in percentage lead removal or recovery by the biomass. In considering industrial application of the *Azolla* biosorption system, its efficient metal uptake ability constitutes an important initial phase of the system, and its physical integrity following recovery and regeneration over 10 cycles make it an ideal biosorbent. Therefore, the use of the *Azolla* biomass in bioremediation processes would offer a cheap, efficient and environmentally non-polluting alternative to other systems. Re-usability of the *Azolla* biomass is a promising step toward the possible application of the biosorbent in bioremediation processes in terms of the costs.

The capacity of the *Azolla* biomass to efficiently remove lead from aqueous solution was demonstrated in this and the previous chapter. The following chapter investigated the ability of the *Azolla* biomass to remove lead from industrial effluent.

CHAPTER FOUR

LEAD REMOVAL FROM EFFLUENT BY *AZOLLA FILCULOIDES*

4.1 INTRODUCTION

Metals are among the most commonly used raw materials in industry. Waste-waters from industrial and mining processes are the major sources of pollution by heavy metals. However in developing countries, many industries operate as small or medium scale, sometimes family businesses on residential premises. These businesses can generate considerable pollution loads which, in most cases, are discharged directly into natural water environments without pre-treatment. Waste-water with heavy metal concentrations exceeding acceptable upper limits pose health hazards, but even with concentrations below acceptable upper limits, there is still a potential for long term contamination, as metals are known to accumulate in biological systems (Quek *et al.*, 1998).

The increasing volumes of industrial waste-waters requiring treatment, e.g. mining, smelting, galvanization, combustion, chemical and agricultural industries, has prompted extensive reviews of biomass resources and potential for bioremediation (Fourest *et al.*, 1994).

As indicated in the introductory chapter, conventional methods for removal of heavy metals and radionuclides from waste-water usually employ physicochemical processes which include: precipitation, coagulation, reduction processes, ion ex-change, membrane processes such as ultra filtration, electrodialysis, reverse osmosis, and adsorption. Most conventional methods have been found to have limited application or are expensive and inefficient when considering remediation of waste-water with high metal concentrations in the range of 1-100 mg/l. Adsorption onto activated

carbon is a recognized method for metal removal from waste-water, but its high cost of production limits its application in waste-water remediation (Kapoor and Viraraghavan, 1995).

Royer *et al.* (1992) of the United States have investigated remediation processes of contaminated soils and waste deposits at defunct lead-acid battery recycling sites (LBRS), facilities where battery breaking, secondary smelting, or both operations are performed for the primary purpose of reclaiming the lead from spent lead-acid batteries. Metallic lead and lead compounds are generally the main contaminants of concern in soils and waste deposits during LBRS remediation, however, other metal contaminants include cadmium, iron, copper and others. The remedial options usually selected for lead contaminated sites include: no action, containment, immobilization and separation with lead recovery option. In spite of the toxicity of lead even at low concentrations, the relative immobility of lead and risks involved in remediation at contaminated sites usually mean the option of no action or containment rather than remediation may be chosen.

Many low-cost biosorbents are being investigated for their possible potential in metal-remediation processes, these include microbial biomass, peat, compost, leaf mould, palm press fibre, coal, straw, wool fibre, rice-milling by-products, sago waste and saw-dust. Recently, Lee *et al.* (1998) reported on the use of modified apple residues, consisting of processed skins, seed and stems, to remove copper, lead and cadmium from solution. The apple residue had more affinity for lead ions compared to copper and cadmium ions. Saturated column systems gave almost complete metal desorption using 3 or 4 washes of a 0.5 N HCl solution (Lee *et al.*, 1998).

The present study investigated the use of non-viable *Azolla* biomass in the removal of lead from effluent from two lead-acid battery manufacturers. The two effluents are referred to henceforth as

effluent A and B. Due to the different metal constituents in each of the samples collected over 2 years, there was a need to distinguish between samples. A sample number was therefore designated, e.g. effluent A1 and effluent A2 refers to effluent from the same manufacturer collected at different times, and therefore the metal ion constituents of the 2 samples are not necessarily the same, and in most cases, the pH also varies between samples.

4.2 MATERIALS AND METHODS

4.2.1 Biomass

Azolla filiculoides biomass was obtained locally and prepared as described in chapters 2 and 3 for batch and column studies.

4.2.2 Solutions

Solutions were prepared as detailed in chapter 2, and lead-acid battery effluent was obtained from two local lead-acid battery manufacturing companies.

4.2.3 pH profiles

The pH of the effluent was determined prior to use in each experiment, and the pH of collected samples was measured using a CyberScan 2500 pH meter.

4.2.4 Metal removal experiments in batch systems

Experiments were performed in duplicate. *Azolla* biomass (5 g biomass / l effluent) was added to 100 ml volumes of the effluent in 300ml Erlenmeyer flasks. A 2 ml sample was immediately taken and the flasks were placed in a shaking incubator at 25 °C (or room temperature (RT)~ 20 °C) at 170

revolutions per minute (rpm). Samples of 2 ml volumes were taken every 10 minutes for the first hour, every 20 minutes for the second hour, and every 30 minutes for the third hour. Each sample was filtered using a millipore filter system with a 25 mm diameter, 0.45 μm pore size cellulose acetate filter and analysed for the metal of interest using an atomic adsorption spectrophotomer (AAS).

4.2.5 Metal removal experiments in column systems

All experiments were performed in duplicate. A 1000 ml volume of effluent was pumped through a packed up-flow column containing 5 g of *Azolla* biomass in a bed volume of 49 ml at 2, 5 and 10 ml/min. Samples were collected at regular time intervals using a Gilson fl 204 fraction collector and analysed for the metal of interest using an atomic absorption spectrophotometer.

For lead adsorption and desorption cycles, after 1000 ml of metal solution had been pumped up the column, 5 washes using 50 ml of a 0.1 M mineral acid (HCl or HNO_3) were carried. This was followed by 2 washes of the biomass with 100 ml of de-ionised water. Reconditioning/regeneration of the biomass was done using 4 washes with 50 ml of 0.05 M NaOH. A final single wash with 200 ml of de-ionised water was carried out before the next adsorption cycle was started. The amount of lead in the desorbent (mineral acid), reconditioning solution (NaOH) and water washes was analysed using an atomic absorption spectrophotometer.

4.2.6 Sulphate (SO_4^{-2}) analysis

To a 5 ml filtered effluent sample, 1 ml of buffer A was added, followed by 1 ml (or more until sulphates precipitation was complete) of a 20 g/l barium chloride ($BaCl_2$) solution. After mixing for one minute, the absorbance of the mixture was read on a UV spectrophotometer and the sulphate concentration determined with reference to a standard curve (see appendix A).

Buffer A : 3 g magnesium chloride hydrate($MgCl_2.6H_2O$); 0.5 g sodium acetate, anhydrous ($NaO_2C_2H_3$); 0.1 g potassium nitrate (KNO_3); 2 ml glacial acetic acid; made up to 100 ml with de-ionised water (Hattingh, 1980).

4.2.7 Chloride (Cl^-) analysis

The effluent was analysed for chloride content using the method as described in the LIRI Technologies Introductory Course in Wastewater Management manual (LIRI technologies, 1990). An 5 ml aliquot of filtered sample was pipetted into a 250ml Erlenmeyer flask, and 50ml of distilled water added. The pH of the solution was adjusted with sodium carbonate, where necessary, to 6.5 or above, and titrated with 0.1 N $AgNO_3$ using potassium chromate indicator (5 drops), until the point of colour change from yellow to orange-red.

Calculation: mg/l Cl^- = (titration volume x 0.003546 x 10^6)/aliquot

4.2.8 Metal analysis

Analysis of metal in solution was done as described in chapter 2.

4.3 RESULTS AND DISCUSSION

4.3.1 Metal composition of the lead-acid battery effluent

Table 4.1 shows the range of concentrations encountered for lead, copper, iron, chlorides and sulphates in the battery effluents. The concentration of sulphates and chlorides in the effluent was determined to get a better understanding of the status of the effluent. Knowledge of the physicochemical properties of the effluent, and the dissolved elements present therein, helps in determining the speciation of metal ions and any precipitation that may result in the course of an

experiment. However, the concentration of anions in solution rarely affects the adsorption of metals by biosorbents (Garnham, 1997). There are probably other compounds present in the battery effluent, but of primary importance to the present study was the lead, due to its toxicity even at low concentrations. The source of the other two metal compounds, copper and iron, was not confirmed, but may be a result of corrosion of metal piping at the battery manufacturing plants by the mineral acids used in battery production, thus explaining the variability observed between samples. The stated concentrations of copper and iron are within acceptable levels for drinking water, however, it was important to establish their effect, if any, on the removal of lead from solution by the *Azolla* biomass.

Table 4.1: Concentrations of metal ions in battery waste-water

Metal Analysed	Effluent A (mg/l)	Effluent B (mg/l)	Maximum limit in drinking water (mg/l)
lead	10 - 95	3 - 20	0.1
copper	0 - 3	0 - 1	10
iron	70 - 700	10 - 20	1000
sulphates	1200 - 2300	1930 - 2708	300
chlorides	51	32	1000
pH	2.5 - 2.8	1.4 - 7.4	-

(Dept. of Environmental Affairs, South Africa)

4.3.2 Batch Experiments

Batch experiments were carried out using effluents A and B, from different lead-acid battery manufacturers, to generate curves of adsorption rates, pH profiles and percentage lead removal. Since several effluent samples with different lead concentrations were collected from the lead-acid

battery manufacturers at different times, the concentration of lead in each sample is given in the legend of the relevant graph, as is the pH and other experimental parameters.

4.3.2.1 Lead removal from effluent samples A1 and B1

Sorption curves for the rate of lead removal from effluent A1 were similar to those for the rate of lead removal from aqueous solution by the *Azolla* biomass.

In figure 4.1, the lead concentration in the effluent rapidly decreases from 7 mg/l to approximately 1.5 mg/l in 25 minutes. There was very little lead removal from the effluent for the remainder of the experiment (150 minutes). This is because the adsorption onto the biomass surface, and saturation of the binding sites is a rapid process. There is obviously no active (energy-dependant) uptake of the lead by the non-viable *Azolla* biomass, therefore a single metal adsorption phase was expected and that is what was observed.

An increase in pH is observed from pH 2.5 until equilibrium is reached at a pH value of approximate 3. Some change always appears to take place in the pH of the system on coming into contact with the *Azolla* biomass, probably due to ligands on the biomass surface contributing to the overall chemistry of the system. The *Azolla* systems tends to effect a slight increase in pH if the initial pH value is lower than 5 and a decrease in pH if the initial pH value is above 5.

Figure 4.2 shows that lead removal from effluent A1 reaches an equilibrium lead removal of approximately 90 % in the first 25 minutes. This implies that saturation of the binding sites on the biomass occurred after taking up 90 % of the lead from solution.

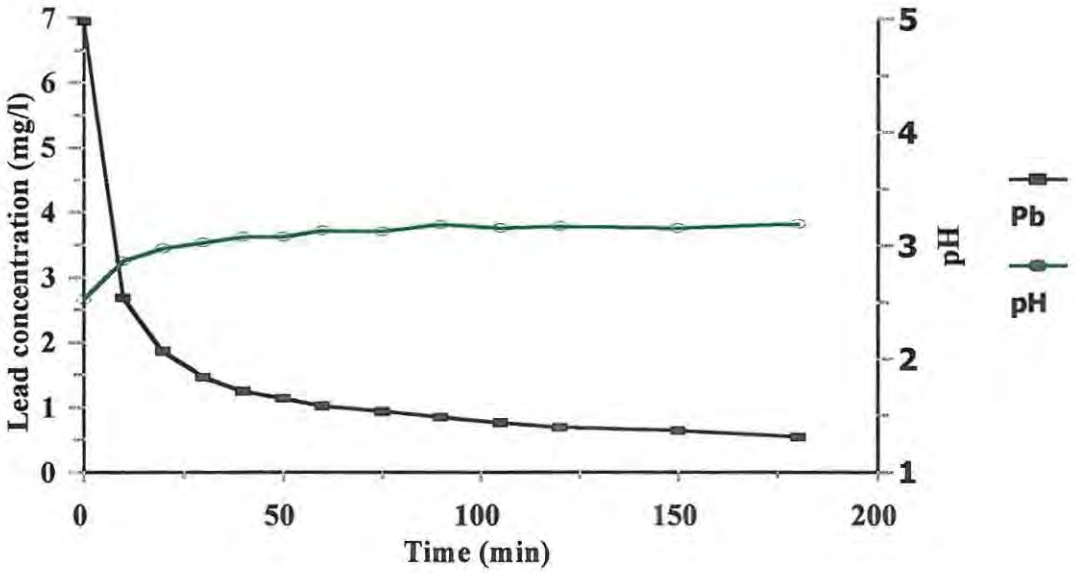

Figure 4.1: Rate of lead removal from effluent A1, and pH profile. Initial Pb concentration, 7.0 mg/l; initial pH, 2.5; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

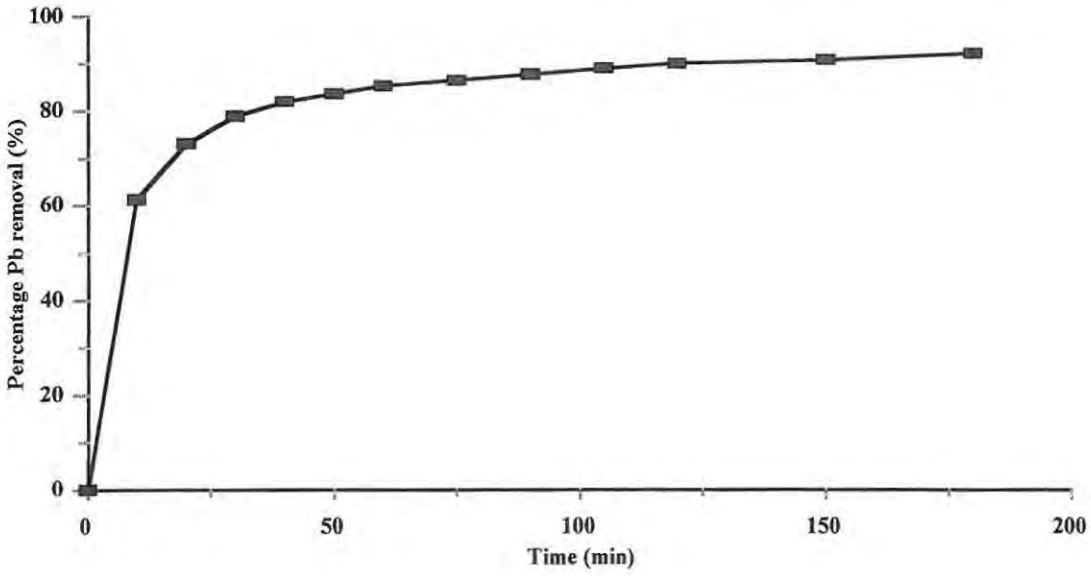

Figure 4.2: Percentage lead removal from effluent A1. Initial Pb concentration, 7.0 mg/l; initial pH, 2.5; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

Effluent B1 had a lower lead content (4 mg/l) and a higher initial pH value of 6.3 compared to effluent A1. Figure 4.3 shows the decrease in lead concentration in the effluent over time, and again the initial rapid lead removal resulted in the lead concentration decreasing from approximately 4 to 0.5 mg/l in about 20 to 25 minutes. Precipitation of lead from solution was found to occur between pH 5 and 7, and literature (Forster and Wase, 1997) gives a precipitation value of 6.3 for $Pb(OH)_2$ (discussed in chapter 2), however there was some lead in solution in effluent B1 at a pH of 6.3. This may be due to incomplete precipitation of the lead in the effluent, resulting in a small amount of lead remaining in solution. Percentage lead removal reached an equilibrium at about 85 %, which was not that much lower than the value obtained for effluent A1 (figure 4.4). This suggests that a range of pH values of 2.5 - 6.3 has little effect on lead removal from the battery effluent. The relatively lower percentage lead removal from effluent B1 maybe due to the difference in solution chemistry as a result of different pH values.

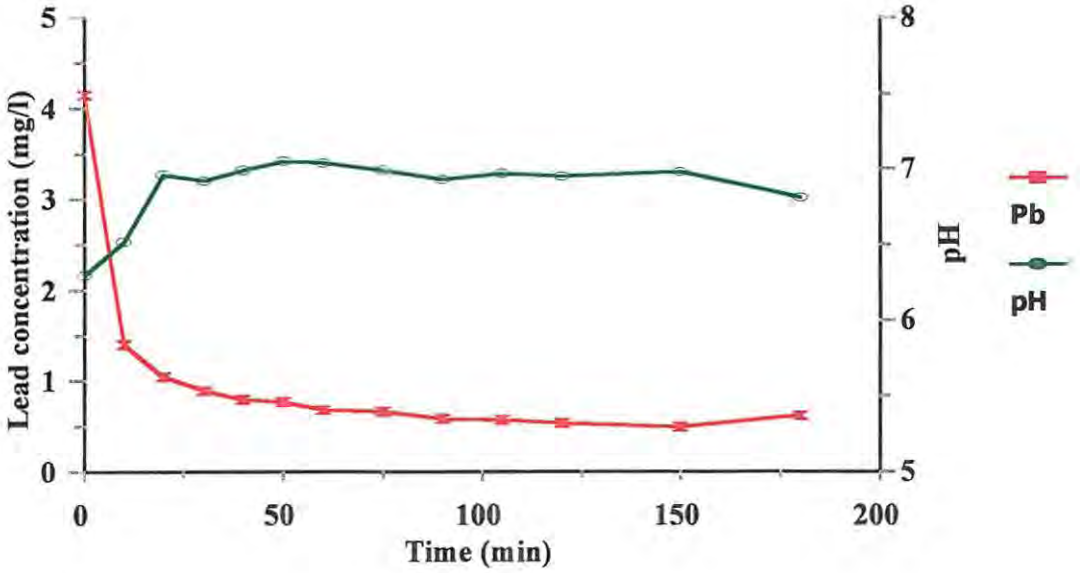

Figure 4.3: Rate of lead removal from effluent B1, and pH profile. Initial Pb concentration, 4.1 mg/l; initial pH,6.3; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

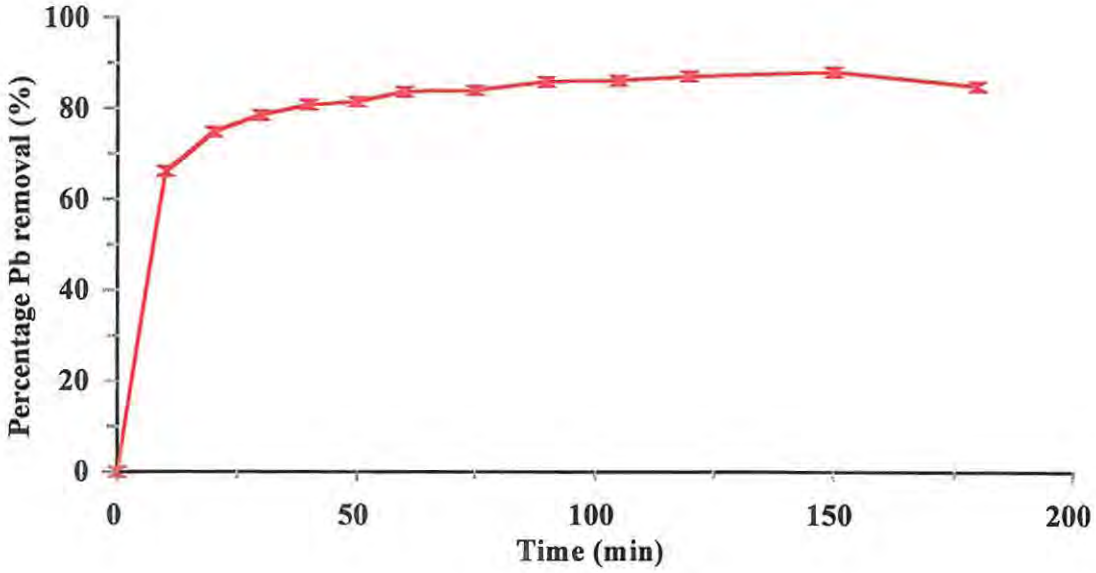

Figure 4.4: Percentage lead removal from effluent B1. Initial Pb concentration, 4.1 mg/l; initial pH, 6.3; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

4.3.2.2 Lead removal from effluent samples A2 and B2

Figures 4.5 and 4.6 give the rate of lead removal from effluent A2 and the percentage lead removal from the same effluent over time. The rate of lead removal from effluent A2 was higher than that of A1 decreasing from approximately 7 to 1 mg/l in 15-20 minutes, observed as a steeper initial slope on the curve. Effluent A2 was very similar to A1 in the initial lead concentration and pH, so there was no significant difference in the percentage lead removal from effluent A2 compared to A1 by the *Azolla* biomass. Percentage lead removal equilibrium for effluent A2 was approximately 85 % compared to approximately 90 % for A1. Slight differences observed in the initial rate of lead removal and the percentage lead removal between effluent A1 and A2 may have simply been due to random experimental error.

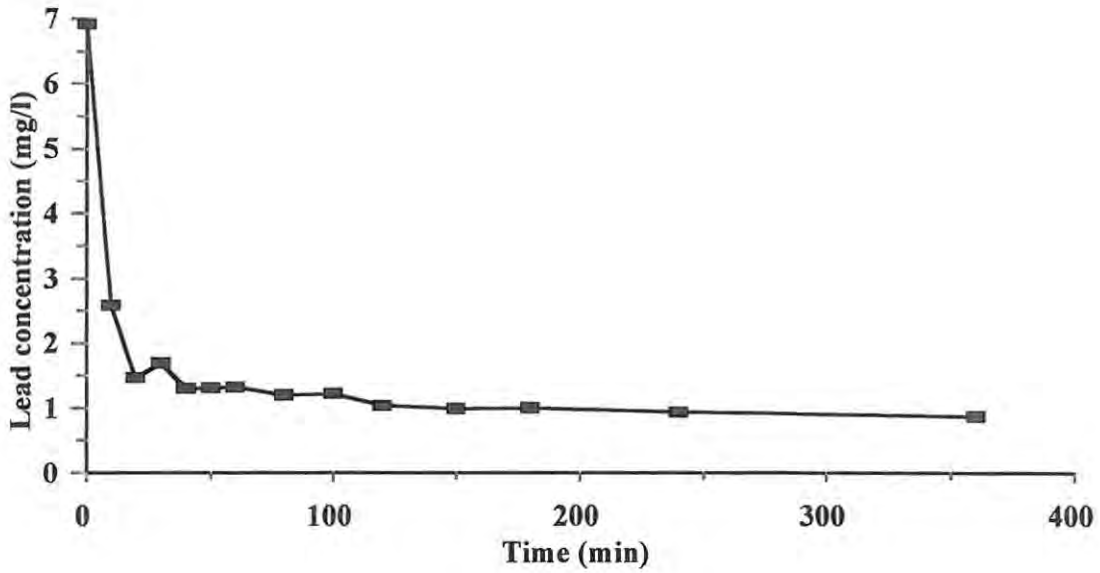

Figure 4.5: Rate of lead removal from effluent A2. Initial Pb concentration, 6.9 mg/l; initial pH, 2.6; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

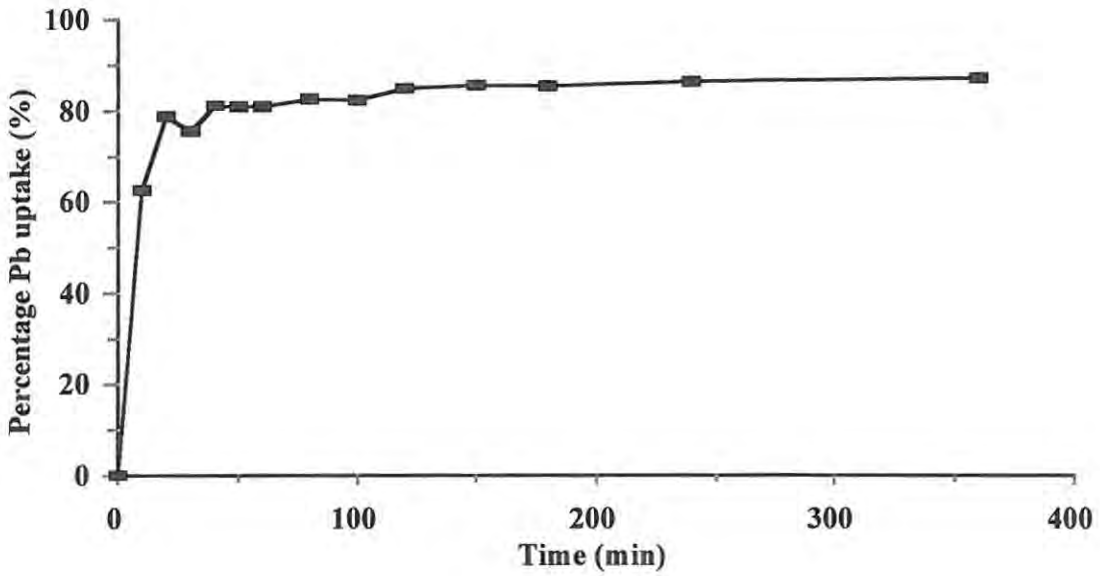

Figure 4.6: Percentage lead removal from effluent A2. Initial Pb concentration, 6.9 mg/l; initial pH, 2.6; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

In figures 4.7 and 4.8 there was a slight change in the trend of the curves for lead removal from effluent B2 which had a very low pH compared to effluent B1. Rapid initial lead removal from the effluent was still observed, with a decrease in lead concentration from 5 to approximately 2.5 mg/l. However, after approximately 80 minutes the amount of lead in solution was found to increase from 2.5 to about 3.5 mg/l (figure 4.7). The percentage lead removal equilibrium of approximately 50 % is achieved for effluent B2 (figure 4.8). The increase in lead concentration in solution after an initial uptake is probably due to the effect of the low pH of the effluent B1 of 1.4. Afer 50 % lead removal, the effluent's acidic nature starts to affect lead removal by the *Azolla* biomass and H^+ ions in the system probably start to displace lead ions bound to the biomass surface and release them back into solution. A similar effect was observed with aqueous solution with very low initial pH values.

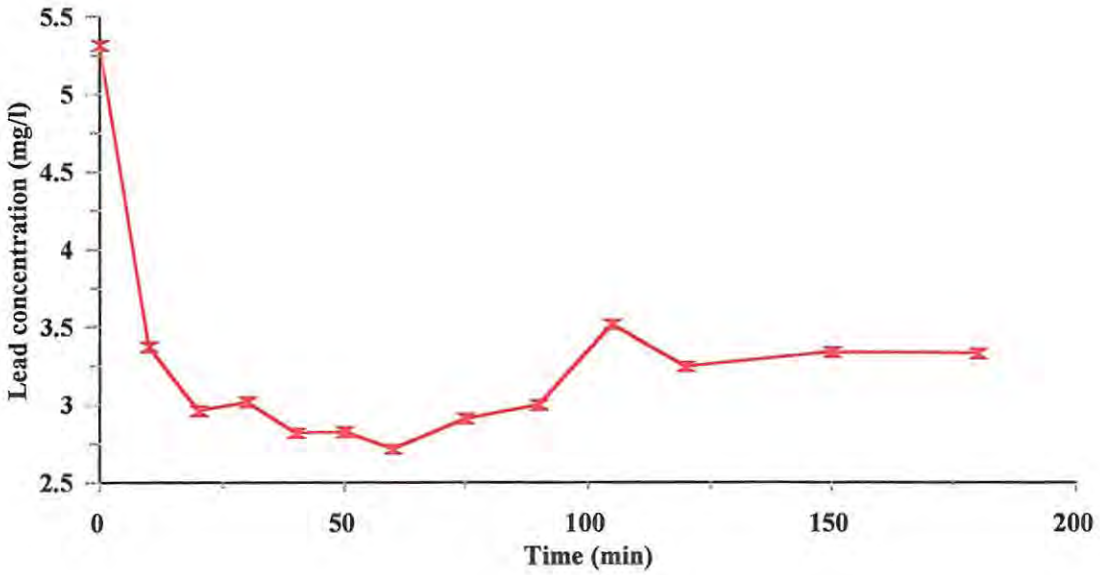

Figure 4.7: Rate of lead removal from effluent B2 by *Azolla* biomass. Initial Pb concentration, 5.3 mg/l; initial pH, 1.4; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C; shaking rate, 170 rpm.

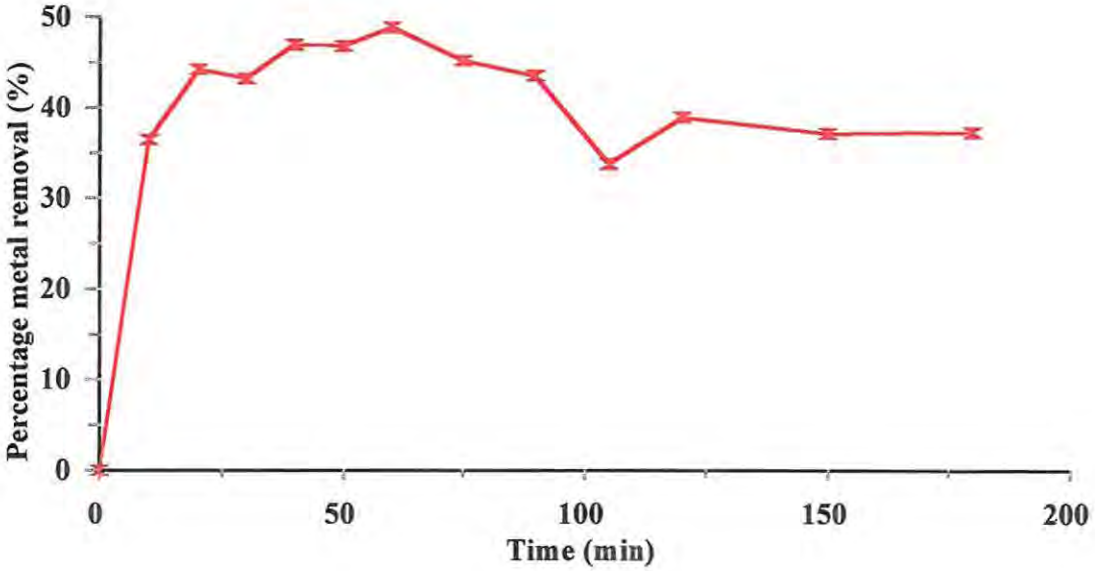

Figure 4.8: Percentage lead removal with time, from effluent B2 by *Azolla* biomass. Initial Pb concentration, 5.3 mg/l; initial pH, 1.4; biomass concentration; 5 g *Azolla* / l effluent; temperature, 25 °C; shaking rate, 170 rpm.

4.3.3 Column experiments

Column experiments were carried out to determine the effects of flow rate and initial pH on lead removal from the battery effluent. Break-through curves for lead, copper and iron removal from the effluent were generated, as were pH profiles of some of the effluent column systems. Finally, adsorption and desorption cycles were repeated 10 times to evaluate the re-usability and change in efficiency in lead removal from effluent and recovery from biomass on regenerating the biomass.

Mineral acids, hydrochloric acid (HCl) and nitirc acid (HNO_3) were investigated for their efficiency in desorption of lead off the *Azolla* biomass. Initial experiments with sulphuric acid (H_2SO_4) had shown poor lead desorption, probably due to lead sulphate ($PbSO_4$) precipitating out of solution and being trapped in the biomass matrix. Therefore, no further experiments were carried out using H_2SO_4.

4.3.3.1 Lead removal from effluent in a column system

Figure 4.9 gives the percentage lead removal from effluents A3 and B3. A flow rate of 2 ml/min was chosen in the preliminary column experiments because previous column experiments (chapter 3) with aqueous solution had also used the slowest flow rate of 2 ml/min. A break-through point at approximately 700 ml was found to occur for effluent A3. Although the system with effluent A3 was not completely saturated over the 1000 ml volume treated, there was a steady decrease in the percentage lead removal. The maximum lead removal equilibrium value of approximately 100 % decreased down to about 90 % at the end of 1000 ml.

There was no break-through point observed for effluent B3 which maintained a maximum lead removal value of approximately 98 % over the 1000 ml volume of effluent pumped up the column.

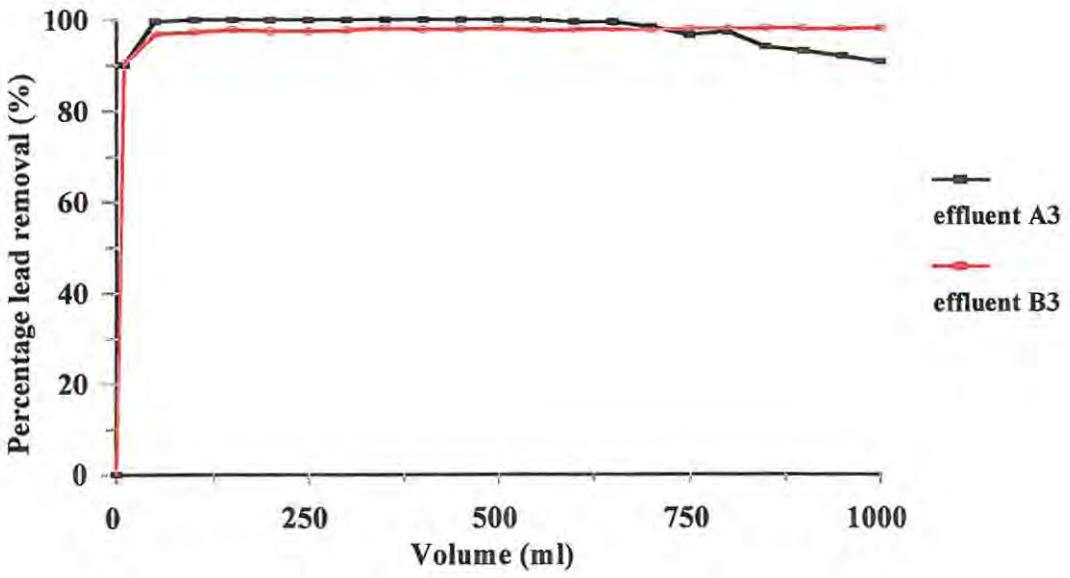

Figure 4.9: Percentage lead removal from effluents A3 and B3, by *Azolla* in column systems. Biomass concentration, 5 g *Azolla* / l effluent; flow rate, 2 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml; initial Pb concentration, 7.5 mg/l (A), 16.6 mg/l (B); initial pH, 2.6 (A), 4.8 (B).

The difference in pH between the two effluents, A3 and B3, appears to be the most probable explanation for reaching a break-through point in lead removal from effluent A. Initial lead removal is affected to a lesser extent by the pH, as the metal-binding groups on the surface of biomass probably contribute to buffering the effect of any H^+ ions present in the effluent. However, as the binding sites are saturated by competing metal and H^+ ions, there is reduced removal of lead from the effluent. The presence of other metal ions in solution probably also introduces other competing ions for the binding sites. This effect is probably not as great as that of H^+ ions, although effluent A samples generally have about 3 times the concentration of copper and up to 35 times the concentration of iron compared to effluent B samples (figure 4.1).

4.3.3.2 Effect of different flow rates on lead removal

The effects of flow rates of 2, 5 and 10 ml/min were investigated for both effluents A3 and B3. Figure 4.10 gives the break-through points and percentage lead removal from effluent A by the *Azolla* biomass. Break-through points are observed at all three flow rates at approximately 400, 450 and 650 ml for the 10, 5 and 2 ml/min systems respectively. However, the percentage lead removal equilibrium value of approximately 100 % was reached at all three flow rates before the break-through point.

At low initial lead concentrations, the break-through points are probably due to saturation of the binding sites rather the increase in flow rate or a decrease in the retention time. Break-through points occurred slightly earlier in the column run with an increase in flow rate, probably due to the binding sites on the biomass becoming saturated quicker at higher flow rates. Saturation may have largely been due to the low pH of effluent A resulting in donation of H^+ ions which competed for and

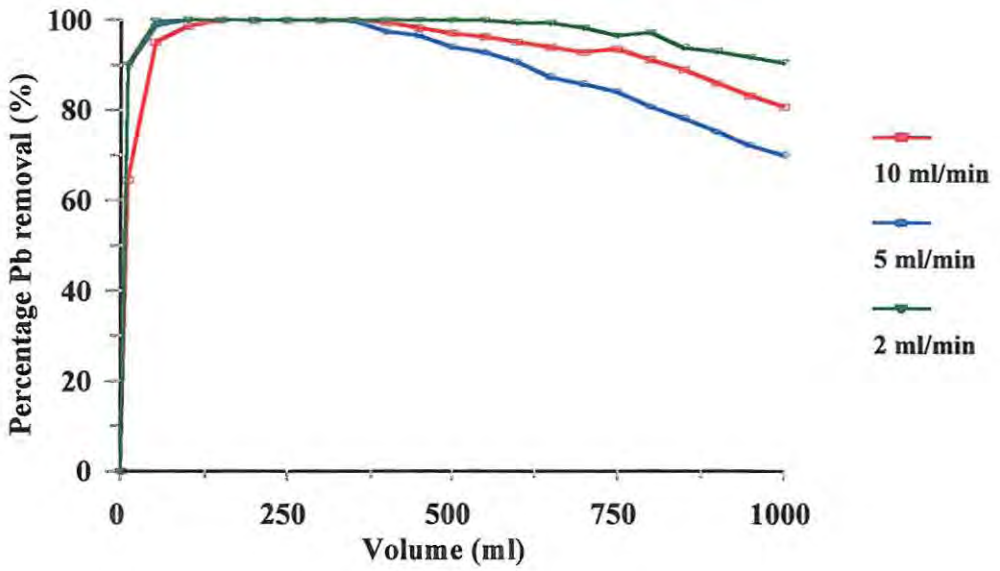

Figure 4.10: Percentage lead removal from effluent A3, at varying flow rates. Initial Pb concentration, 7.5 mg/l, initial pH, 2.6; biomass concentration, 5 g *Azolla* / l effluent; temperature, RT ~ 20 °C; bed volume, 49 ml.

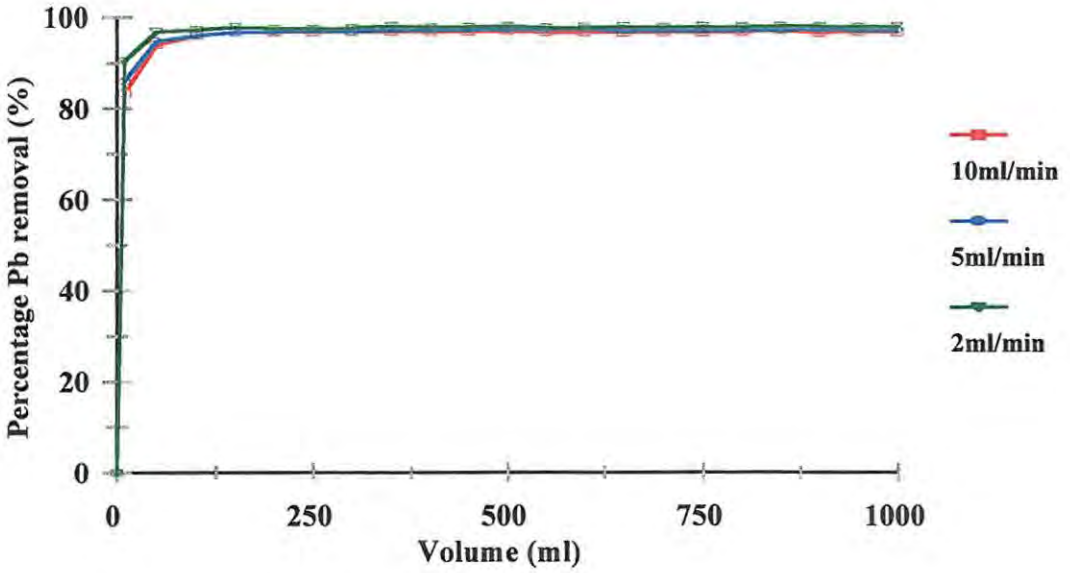

Figure 4.11: Percentage lead removal from effluent B3, at varying flow rates. Initial Pb concentration, 16.6 mg/l, initial pH, 4.8; biomass concentration, 5 g *Azolla* / l effluent; temperature, RT ~ 20 °C; bed volume, 49 ml.

displaced lead ions on the *Azolla* binding sites. The fact that there were no break-through points observed with effluent B at all three flow rates (figure 4.11), suggests that the flow rate, initial lead concentration and other metal ions in the effluent played very little or no role in biomass saturation in this case.

4.3.3.3 Effect of flow rate on lead uptake capacity

Figures 4.12 and 4.13 show the effect of flow rate on the lead uptake capacity of the *Azolla* biomass, from effluent. The graphs compare the maximum possible lead uptake (qmax), calculated for a system assuming 100% lead uptake by the *Azolla* biomass, to the uptake capacity, qb, calculated at the break-through point or, in the case of effluent B where no break-through point was observed, at the equilibrium point.

In figure 4.12 for effluent A3, the slowest flow rate of 2 ml/min was found to give the most efficient lead uptake of 1.3 mg lead / g *Azolla* at the break-through point, which was 91 % of the maximum possible lead uptake capacity for the system. The lead uptake capacity at the other two flow rates was approximately 50-60 % of the maximum possible value. This may be due to the fact that at the faster flow rates the effects of the reduced retention time and low pH meant less time for the lead ions to interact with ligands at the binding sites and that there was some competition from H^+ ions.

There was very little difference between qmax and qb values for the three flow rates for effluent B3 (figure 4.13). The *Azolla* biomass was able to take up over 95 % of the maximum possible lead uptake at each flow rate. These results showed that a flow rate of up to 10 ml/min had no effect on lead removal from effluent B3, and pH probably has the most significant effect on lead removal by the *Azolla* biomass.

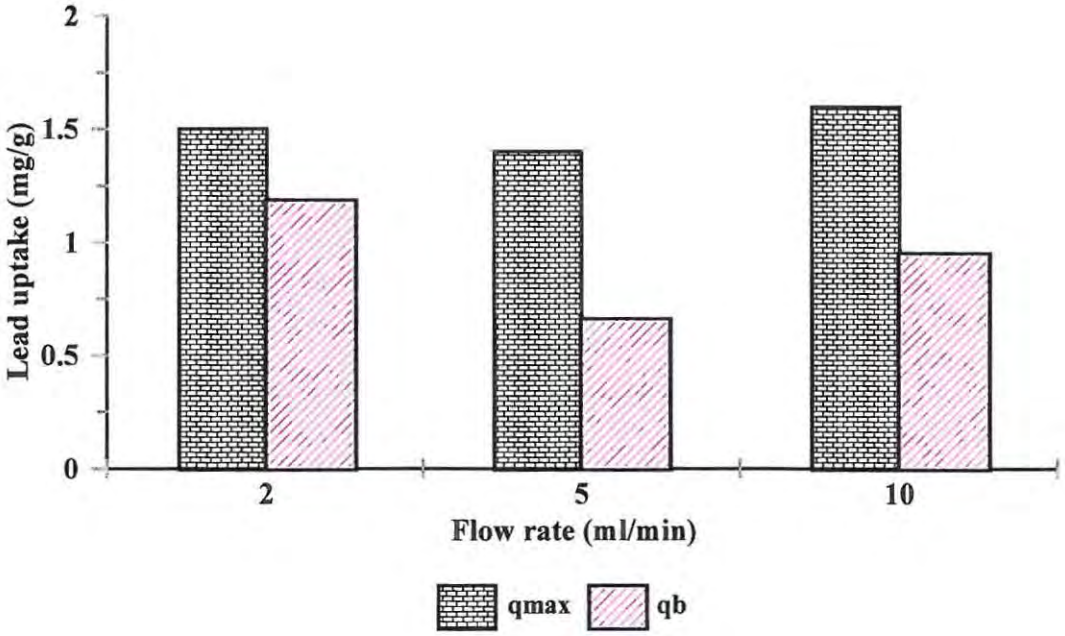

Figure 4.12: Lead uptake from effluent A3, at varying flow rates. Initial Pb concentration, 7.5 mg/l, initial pH, 2.6; biomass concentration, 5 g *Azolla* / l effluent; temperature, RT ~ 20 °C; bed volume, 49 ml; qmax = maximum lead uptake possible; qb = uptake at break-through point or equilibrium.

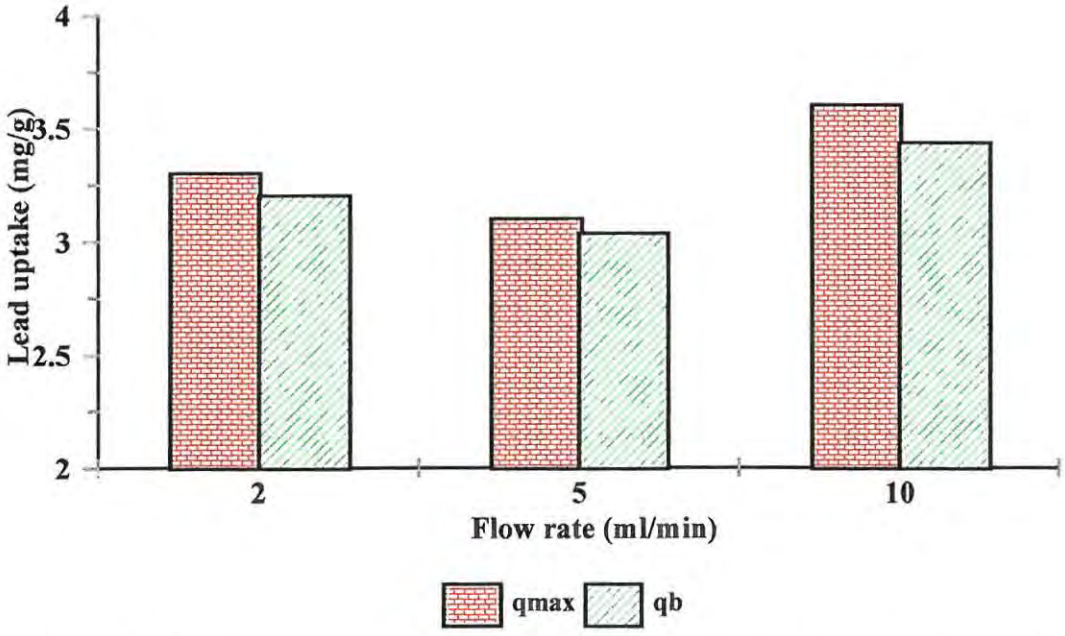

Figure 4.13: Lead uptake from effluent B3, at varying flow rates. Initial Pb concentration, 16.6 mg/l, initial pH, 4.8; biomass concentration, 5 g *Azolla* / l effluent; temperature, RT ~ 20 °C; bed volume, 49 ml; qmax = maximum lead uptake possible; qb = uptake at break-through point or equilibrium.

4.3.3.4 Removal of lead, copper and iron from effluent

Figures 4.14 and 4.15 give the percentage removal of copper and iron compared to lead, from battery effluents A4 ans B4, by the *Azolla* biomass, and the pH profile throughout the experiment. The maximum percentage metal removal values from effluent A4 were 99, 52 and 70 % for lead, copper and iron respectively. The pH profile for effluent A4 showed that the initial pH value of 2.8, increased to about 6 and then decreased and reached an equilibrium at an approximate pH value of 3.4.

The percentage metal removal curves for effluent B4, and pH profile, are given in figure 4.15. Percentage lead removal value is still highest at 82 %, with copper and iron at 70 and 73 % respectively. The initial pH value of 7.5 of effluent B4 was found to decrease and even out at a pH value of approximately 6.5.

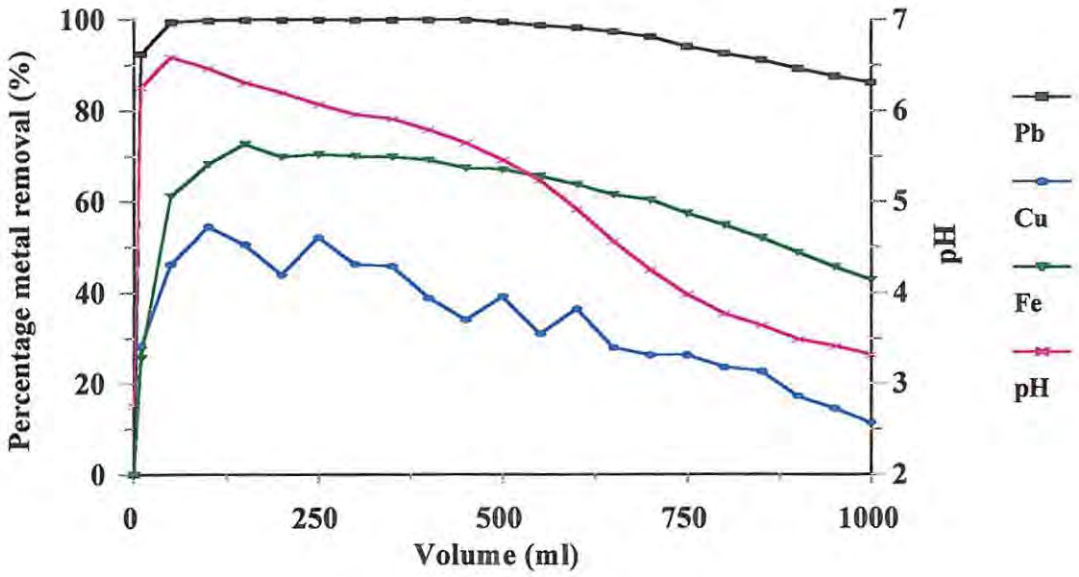

Figure 4.14: Percentage lead, copper and iron removal from effluent A4, Initial metal concentration, 17.2 mg/l - Pb, 0.3 mg/l - Cu, 6.1 mg/l Fe; initial pH, 2.8; biomass concentration, 5 g *Azolla* / l effluent; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

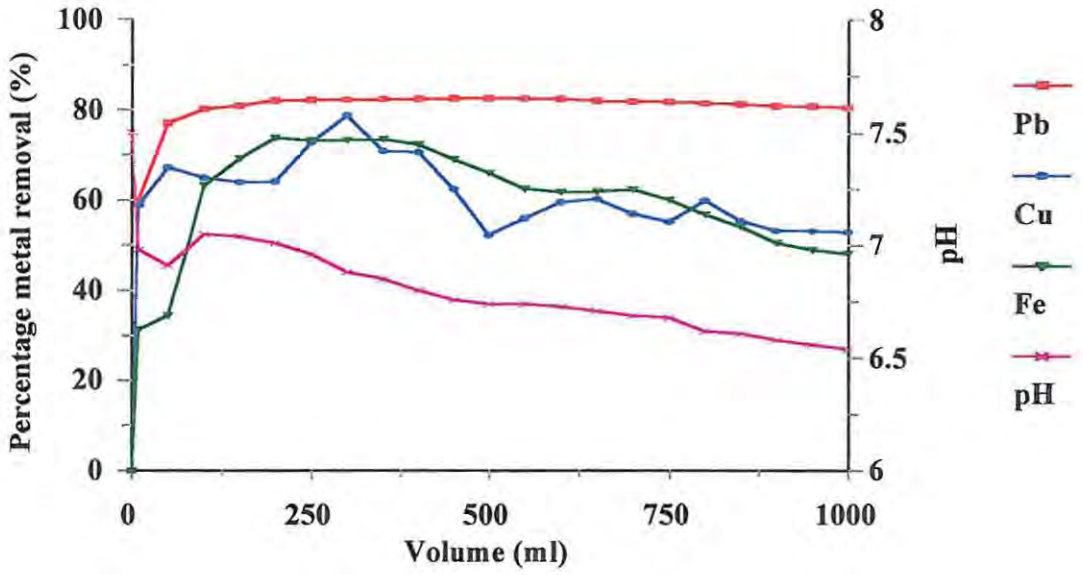

Figure 4.15: Percentage lead, copper and iron removal from effluent B4. Initial metal concentration, 79 mg/l - Pb, 0.5 mg/l - Cu, 0.9 mg/l Fe; initial pH, 7.5; biomass concentration, 5 g *Azolla* / l effluent; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

Lead, copper and iron appear to be taken up at differing amounts from the effluent by the *Azolla* biomass, a trend also found to occur with aqueous solutions. As in previous chapters percentage lead removal, from both effluent A and B, generally appears to be greater than the percentage copper or iron removal from effluent. This suggests a possible selective uptake of the lead ions to some degree, particularly since the lead removal in both cases was considerably higher than that of copper and iron. The percentage copper and iron removal from effluents A and B was found to differ. In effluent A, percentage iron removal was greater compared to that of copper, whereas in effluent B, the percentage copper and iron removal were similar. This may be due to differing metal concentrations and the effect of the different pH values favouring the adsorption of one metal ion over the other. The effect of pH on the chemistry of the effluent and the binding sites on the biomass surface would result in reduced percentage metal removal as H^+ ions compete and displace metal ions from metal binding sites.

pH has been reported to affect to a large extent the formation of metal-biosorbent complexes by modifying the speciation and availability of metallic elements in solution, and the chemical state of the sequestering groups on the biomass (Fourest *et al.*, 1994).

4.3.3.5 Lead recovery from the biomass

Table 4.2: Percentage lead adsorption and desorption with different mineral acids

Mineral acid	Effluent A3		Effluent B3	
	% adsorbed	% recovered	% adsorbed	% recovered
0.1 M HCl	81	29	97	20
0.1 M HNO_3	70	42	95	52
0.5 M HNO_3	76	65	95	71

Table 4.2 gives the results of percentage lead adsorption and desorption in column systems using 50 ml (5 % of the treated effluent volume) of 0.1 M HCl, 0.1 M HNO_3 and 0.5 M HNO_3. Although the values of the percentage lead adsorbed from both effluent A3 and B3 were high, the *Azolla* biomass, however, appeared to favour lead adsorption from effluent B, where the percentage lead adsorbed from the effluent was 95 % or more, compared to 70-80 % from effluent A. This was probably due to the lower pH (2.6) of effluent A3 introducing H^+ ions which would have competed with metal ions for binding sites.

Lead recovery with HCl gave the lowest percentage recovery values, with 29 % being the highest percentage lead recovery. The 0.1 M HNO_3 gave better percentage lead recovery of 52 % from effluent B, however the 0.5 M HNO_3 gave the best percentage recovery values of 65 and 71 % for effluent A and B respectively. The increase in acid concentration resulted in an increase in H^+ ions, hence better metal desorption as the H^+ ions displaced the metal ions off the biosorbent. The effect

of mineral acids on metal adsorption appears to be two-fold; the decrease in pH alters the chemistry of the sequestering groups by protonation, which reduce the number of anionic sites available for metal ion binding. The higher the concentration of H^+ ions available the great the protonation of these sites. The mineral acids also appear to contribute H^+ ions which displace bound metal ions and release them back into solution. The solubility of most metal ions is increased at lower pH values. The decreased lead recovery from effluent compared to that from aqueous solution (chapter 3) may be due interference from other ions present in the effluent system. HNO_3 at 0.5 M concentration was used in subsequent experiments.

4.3.3.6 Adsorption and desorption cycles

The results on the percentage adsorption and desorption of lead over 10 cycles, using 0.5 M HNO_3 to desorb the lead from the *Azolla* biomass, and reconditioning of the biomass with 0.05 M NaOH are given if figures 4.16 and 4.17. De-ionised water was used to wash the biomass 4 or 5 times in-between desorption and reconditioning and after reconditioning of the biomass in order to remove excess acid or base in each case.

The initial lead concentration of effluent A5 was approximately 15 mg/l, and percentage lead removal over 10 cycles was 90 % or more. A percentage lead recovery value of that removed of approximately 25 % was maintained over the 10 cycles.

The initial lead concentration in effluent B5 was approximately 44 mg/l, and the percentage lead adsorption was 97 % or more throughout the 10 cycles of desorption, reconditioning and adsorption. Percentage lead recovery with 0.5 M HNO_3 was maintained at approximately 40 %, with the exception of the third cycle where the percentage lead recovery decreased to approximately 20 %.

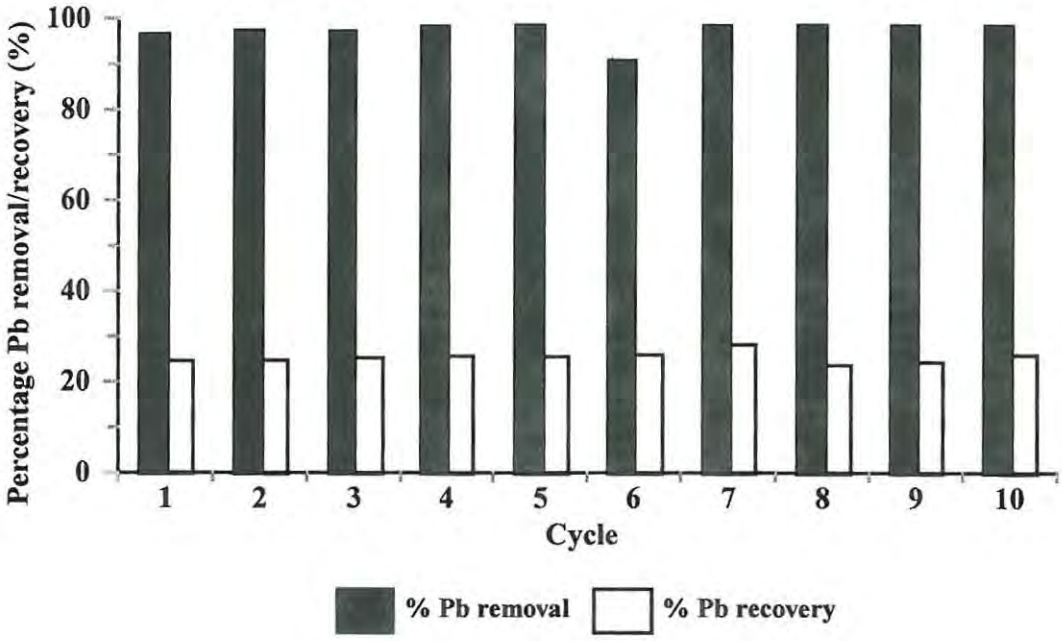

Figure 4.16: Percentage lead removal and recovery from effluent A5, for 10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml, 0.5 M HNO_3 as the desorbent and 50 ml, 0.05 M NaOH to regenerate the biomass. Initial Pb concentration, 14.7 mg/l; initial pH, 2.7; biomass concentration, 5 g *Azolla* / l effluent; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

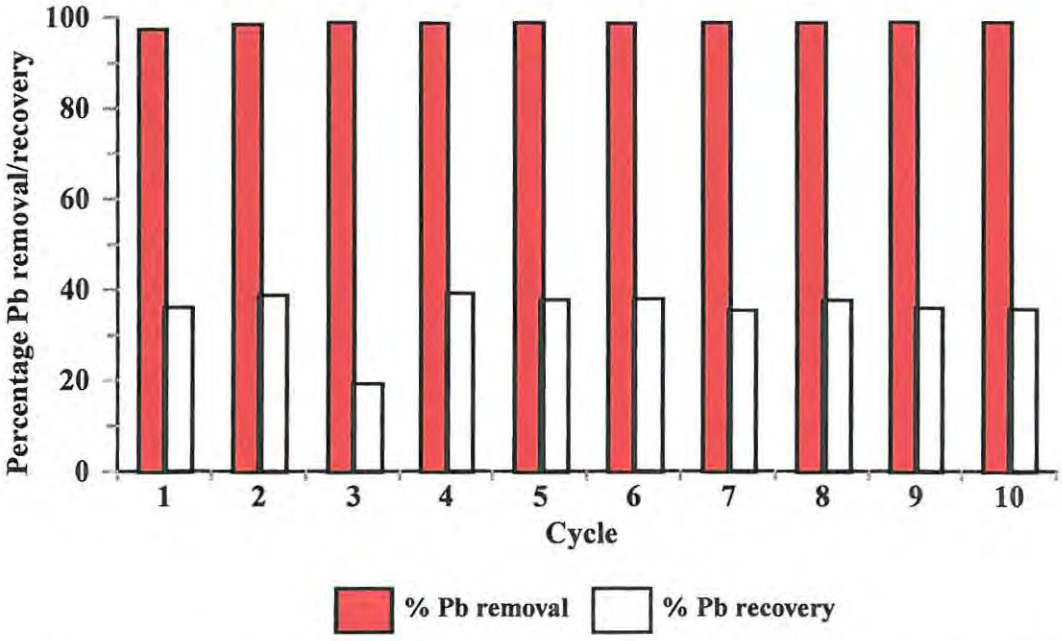

Figure 4.17: Percentage lead removal and recovery from effluent B5, for 10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml, 0.5 M HNO_3 as the desorbent and 50 ml, 0.05 M NaOH to regenerate the biomass. Initial Pb concentration, 43.7 mg/l; initial pH, 7.5; biomass concentration, 5 g *Azolla* / l effluent; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

Although 0.5 M HNO_3 was found to be the more efficient of the mineral acids investigated for the desorption of lead from the *Azolla* biomass (table 4.2), it gave much lower desorption results in the adsorption and desorption cycles study. The reasons for this were not clear, as the percentage lead removal did not appear to be affected. Reduced lead adsorption would be expected if lead desorption decreased. Regenerating the *Azolla* biomass by desorbing bound lead with HNO_3 and reconditioning it with NaOH, with some water washes in between, showed that the biomass could be re-used up to ten times with little or no significant loss in lead removal or recovery efficiency (figures 4.16 and 4.17).

4.4 CONCLUSION

The variability of the pH and metal composition of lead-acid battery effluent makes its bioremediation problematic. The pH of the effluent samples (both A and B) ranged from 1.4 up to 7.5. Since pH affects the speciation of the sequestering ligands and solubility of the different ions in solution, such a wide range of pH values makes consistently high percentage metal removal difficult, as each biosorbent has an optimum pH where maximum adsorption takes place. The competition for binding sites that was observed in column systems (chapter 3), and the extent of competition probably depends on metal ion concentrations. Therefore the variable composition of the lead-acid effluent also makes the prediction of such competitive effects difficult. However, despite the variable nature of the lead-acid effluent, the *Azolla* biomass was able to remove up to 95 % of the lead in solution in different samples, from both effluents A and B, with no observed adverse effects on the physical integrity of the biomass. Lead uptake capacity from both effluents was between 80 and 99 % of the possible maximum uptake (mg/g), complete lead removal being the possible maximum uptake.

Flow rates from 2 to 10 ml/min had little or no effect on the maximum percentage lead removal from the battery effluents. Break-through points that were observed would serve to identify the point at which regeneration of the biomass should occur when applying the system to effluent bioremediation. They would also give information on the possible optimum operating parameters for bioremediation of that effluent and on what volume sizes can be treated with a given amount of biomass.

Although the presence of other ions in solution does result in some degree of competition for binding sites and/or interference with lead removal, the *Azolla* biomass' capacity to remove lead ions from the battery effluent was still relatively high and appears to be selective to some extent. No other biosorption studies using lead-acid battery effluent are available, hence a direct comparison with the *Azolla* system can not be made. Priel (1995) has reported on waste-water treatment studies done at the Hebrew University where dry *Azolla* biomass was found to remove 99.9 % of lead from an industrial waste-water with 1000 mg/l of lead, and further studies showed percentage metal removal values of approximately 99 % for cadmium, chrome and uranium from aqueous solution.

The re-usability of the *Azolla* biomass due to its ability to maintain its physical integrity even after repeated washes with HNO_3 and NaOH make it a very promising candidate for possible application in waste-water bioremediation. Column experiments yielded useful results, as column-type reactors are the form in which the *Azolla* biomass is likely to be applied in bioremediation processes. The highest lead uptake capacity from lead-acid effluent was approximately 9 mg/g from effluent B5, cycle 10, which represented 99% of the maximum possible uptake capacity. The low percentage lead desorption is probably due to the complexity of the effluent introducing other factors in the system which interfere with the desorption process. More efficient desorption methods need to be investigated, e.g. using more concentrated mineral acid solution.

The desorption and regenerations steps are important processes in considering industrial application of any biosorbent system. This means the biomass needs to be able to maintain its physical integrity without breaking up, even after several acidic and basic washes. In the next chapter, scanning electron microscopy was used to try and elucidate if there was any apparent adverse effect due to desorption and regeneration processes on the physical structure and integrity of the *Azolla* biomass.

CHAPTER FIVE

SCANNING ELECTRON MICROSCOPY OF *AZOLLA FILICULOIDES*

5.1 INTRODUCTION

The toxic effects of metal ion bioaccumulation by viable biomass means that reuse of the biomass is not possible and continual replenishing of the biomass is necessary. For application purposes in bioremediation processes, constant replacement of the biomass becomes costly. Regeneration of viable biomass is usually not possible as inactivation of metabolic processes involved in heavy metal bioaccumulation is not reversible. The use of non-viable biomass offers the potential for recycling of the biomass. This is because the non-viable biomass is generally able to withstand otherwise adverse regeneration processes such as desorption of metal ions by mineral acids followed by neutralisation with a basic solution. The maintenance, therefore, of structural integrity is an important factor in determining the potential to regenerate and re-use a particular biomass. An attempt was made in this study to use scanning electron microscopy to elucidate the effect of lead adsorption and desorption using a dilute mineral acid and regeneration of the biomass with NaOH on the physical integrity of the *Azolla* biomass.

5.2 MATERIALS AND METHODS

5.2.1 Biomass

Azolla filiculoides biomass was obtained locally and prepared as described in chapter 2. The biomass was divided into three groups and used for subsequent experiments: whole *Azolla*, ground *Azolla* (< 2 mm, determined by screening), and finely ground *Azolla* (< 1 mm determined by screening).

5.2.2 Solutions

All solutions were prepared as detailed in chapter 2 and 3 and lead-acid battery manufacturing effluent was obtained from two lead-acid battery manufacturers in the Eastern Cape, South Africa.

5.2.3 Experimental procedure

An *Azolla* biomass preparation before adsorption studies was set aside as sample 'a'. A 1000 ml volume of effluent A6 was pumped through a packed up-flow column containing 5 g of *Azolla* biomass in a bed volume of 49 ml at 10 ml/min. Biomass sample 'b' was collected after the 1000 ml volume of effluent had been pumped through the column. The next biomass sample 'c' was collected after column elution with five washes of 50 ml 0.1 M HNO_3 under gradient flow. *Azolla* sample 'd' was collected following two washes of the column with 100 ml of de-ionised water. NaOH (four washes) were used to neutralise and regenerate the column, after which sample 'e' was collected. After a final single wash with 200 ml of de-ionised water was carried out, the last biomass sample 'f' was collected. All samples were dried for 72 hours in a 50 °C oven, before preparation for SEM.

5.2.4. Sample preparation for scanning electron microscopy (SEM)

The initial fixation and dehydration steps required with wet and fragile biomass were not necessary with the *Azolla* biomass after it was dried for 72 hours in a 50 °C oven. No further pre-treatment of the *Azolla* samples was necessary as they were small enough and adequately dehydrated. The *Azolla* samples were mounted onto copper stubs, and then gold coated in a sputter chamber and subsequently observed with a JEOL JSM-840 scanning electron microscope at 100 times magnification.

5.3 RESULTS AND DISCUSSION

5.3.1 Whole Azolla biomass micrographs

Figure 5.1 shows six micrographs of whole *Azolla* biomass at the six different stages of adsorption/desorption and biomass regeneration. An attempt was made to try and observe similar structures of the whole *Azolla* biomass with each samples, in order to see if there was any apparent break- down in the physical integrity of the biomass.

Although the electron micrographs were difficult to interpret, and observations were subjective, it was clear that there was very little, if any, structural or physical difference in the biomass with the series of micrographs from samples a-f. There was, therefore, no observed deterioration in the physical integrity of whole *Azolla* biomass after treatment with HNO_3 and NaOH. This is probably because the strong cellulosic structure of the *Azolla* biomass is resistant to any disruptive effects of weak acids and bases.

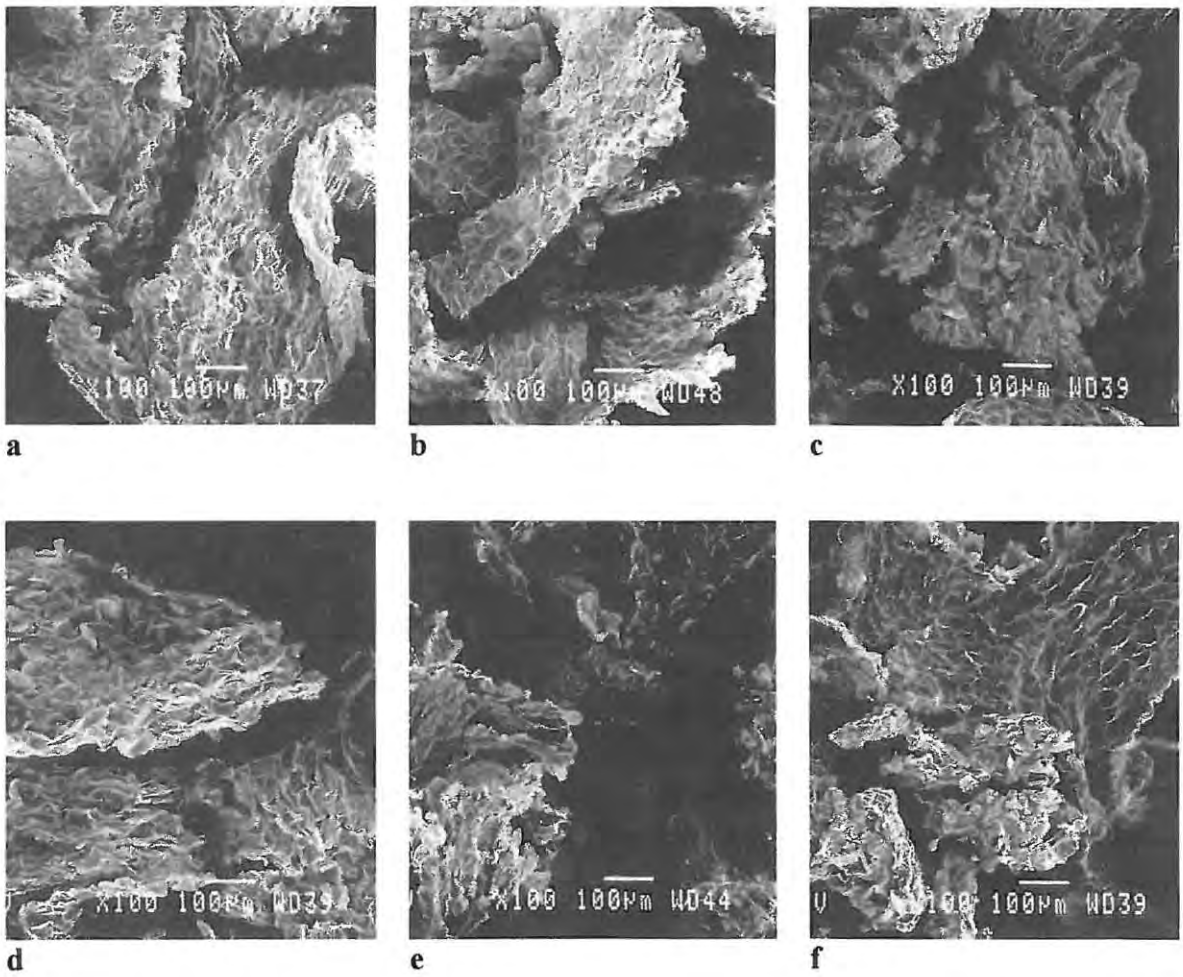

Figure 5.1: Scanning electron graphs showing the physical state of whole *Azolla* biomass over the various stages of biomass regeneration. *Azolla* biomass after: **a** - drying and before lead adsorption experiment; **b** - passing through a 1000 ml volume of lead-battery effluent (pH, 2.7; Pb concentration, 14.7 mg/l); **c** - 5 washes with 50 ml, 0.5 M HNO_3; **d** - 2 washes with 100 ml de-ionised water; **e** - 4 washes with 50 ml, 0.05 M NaOH; **f** - 1 wash with 200 ml de-ionised water.

5.3.2 Ground *Azolla* biomass micrographs

The micrographs taken for ground *Azolla* biomass are given in figure 5.2. Attempts to observe the same biomass structures, in this case, proved even more difficult. However, it was still clear that there was not disruption or break-down of the structure or physical integrity of the ground *Azolla* biomass. This may be due to the fact that the *Azolla* biomass structure is sturdy and can withstand treatment with mild mineral acids and bases. The cellulosic structure of the *Azolla* biomass, again, gives it a robust and highly resistant structure which is not easily disrupted.

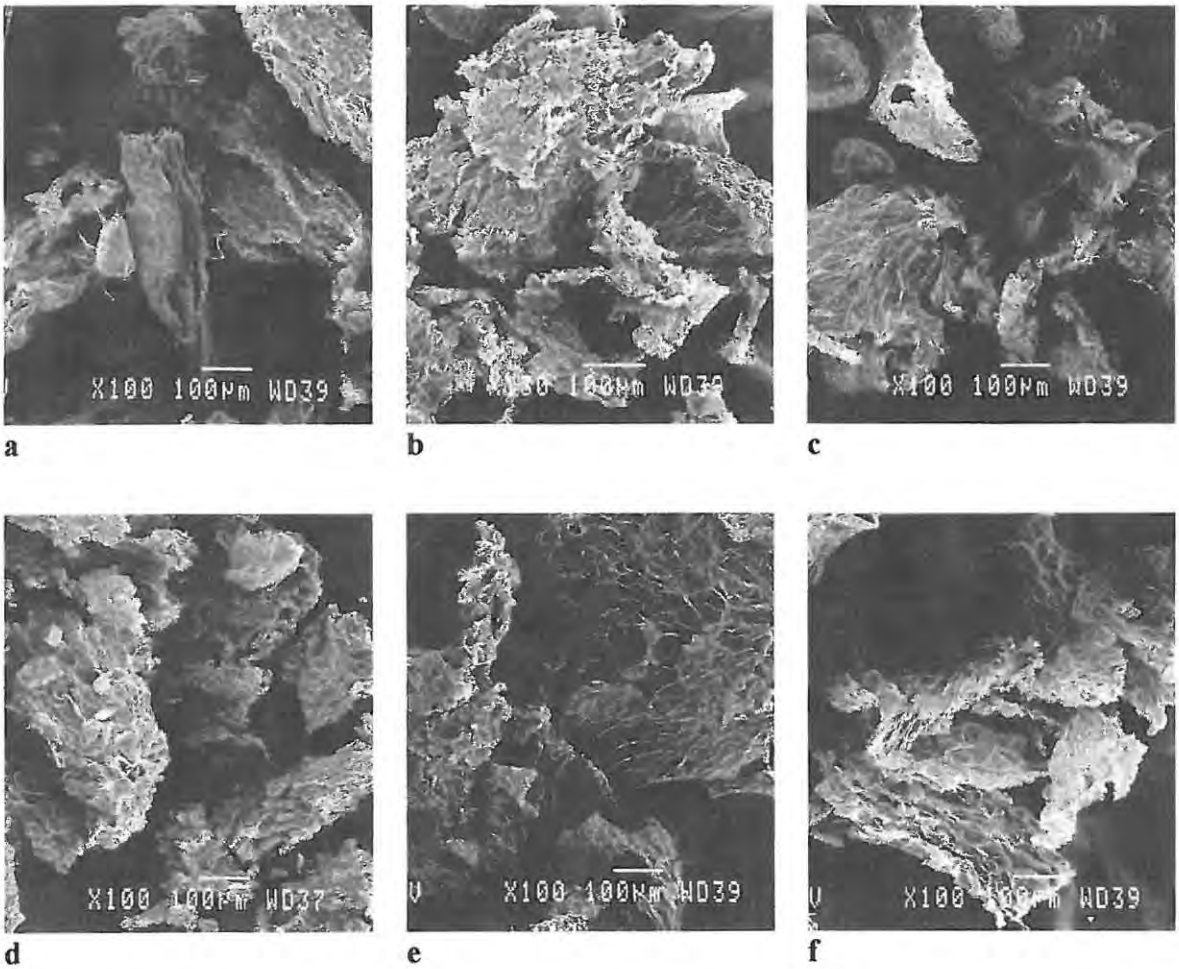

Figure 5.2: Scanning electron graphs showing the physical state of ground (< 2 mm) *Azolla* biomass over the various stages of biomass regeneration. *Azolla* biomass after: **a** - drying and before lead adsorption experiment; **b** - passing through a 1000 ml volume of lead-battery effluent (pH, 2.7; Pb concentration, 14.7 mg/l); **c** - 5 washes with 50 ml, 0.5 M HNO_3; **d** - 2 washes with 100 ml de-ionised water; **e** - 4 washes with 50 ml, 0.05 M NaOH; **f** - 1 wash with 200 ml de-ionised water.

5.3.3 Finely ground *Azolla* biomass micrographs

Figure 5.3 shows the micrographs of finely ground *Azolla* biomass and the effect of dilute acid and base treatment on them, as carried out in adsorption/desorption and regeneration processes. The results are very similar to those observed with whole and coarsely ground *Azolla* biomass. There was no obvious break down in the physical integrity of the biomass. The *Azolla* at the end of the

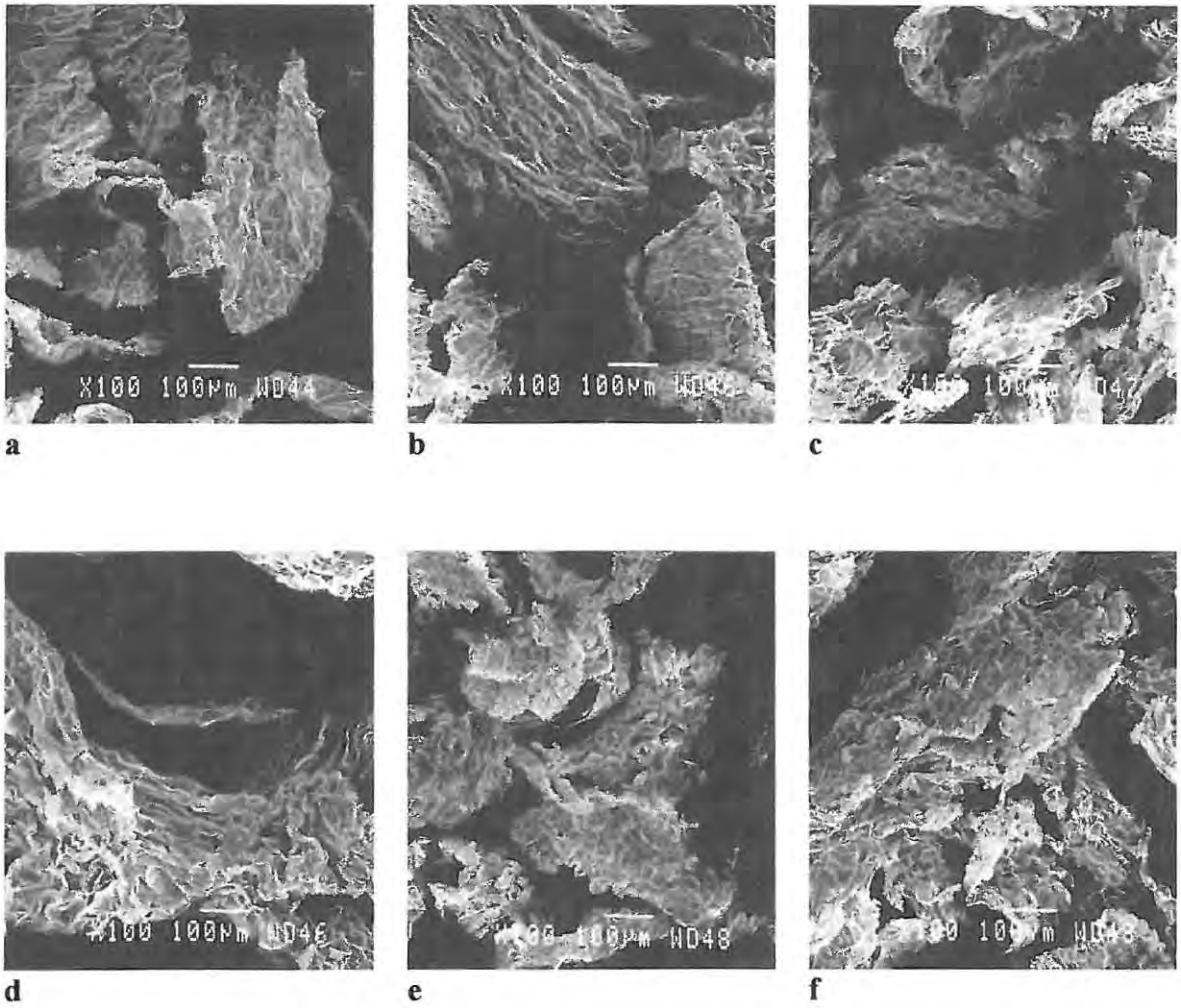

Figure 5.3: Scanning electron graphs showing the physical state of finely groung (< 1 mm) *Azolla* biomass over the various stages of biomass regeneration. *Azolla* biomass after: **a** - drying and before lead adsorption experiment; **b** - passing through a 1000 ml volume of lead-battery effluent (pH, 2.7; Pb concentration, 14.7 mg/l); **c** - 5 washes with 50 ml, 0.5 M HNO_3; **d** - 2 washes with 100 ml de-ionised water; **e** - 4 washes with 50 ml, 0.05 M NaOH; **f** - 1 wash with 200 ml de-ionised water.

regeneration process (micrograph 'f') looked very similar to the *Azolla* before any treatment or lead adsorption (micrograph 'a'). As before, this is attributed to the strong physical structure of the dried *Azolla* biomass.

5.4 CONCLUSION

In terms of industrial application, non-viable biomass appears to be favoured over viable biomass due to the ease of handling, increased uptake and relatively cheaper costs (Volesky, 1990). However, the ability to re-use a given biomass places it in an even more favourable light for application in bioremediation processes. The regeneration of biomass with little, or no, loss in metal removal or recovery efficiency depends on its ability to withstand treatment with generally mild acids and bases, without the sequestering groups being blocked by other ions or stripped off the biomass surface.

Dried *Azolla* biomass' strong physical structure allows it to meet all the above desirable criteria for a good biosorbent. It was able to maintain its physical integrity over a wide range of adverse physical conditions with no observed structural deterioration.

Larsen and Schierup (1981) reported that another cellulosic material, straw, could be re-used up to five times or more with the development of an optimum regeneration technique. However, they did report that there was some leaching of organic constituents of the biomass in basic conditions. Cellulosic material such as bleached bamboo pulp, jute fibres and dyed sawdust were found to undergo up to ten regenerations with no observed loss in adsorption capacity (Shukla and Sakhardande, 1992). Zhao and Duncan (1997a), have also demonstrated the viability of *Azolla* biomass re-usability in the removal and recovery of nickel from electroplating rinse effluent, thus confirming the potential for application of this form of biomass for bioremediation purposes.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION

Recent years have seen an increase in concern over the pollution of natural watercourses by heavy metals from industrial effluents, most of which are toxic to aquatic life and, eventually, higher life forms through the food chain. Therefore, restrictions on the discharge of effluents containing heavy metals have been intensified in the past few years (Wilson and Edyvean, 1994). This project evaluated the capacity and efficiency of the non-viable biomass of the water fern, *Azolla filiculoides*, as a biosorbent for the removal and recovery of lead from aqueous solution and from lead-acid battery manufacturing effluent.

Conventional methods for metal removal from waste-waters prior to discharge include chemical precipitation, ion-exchange and electrochemical methods. The conventional method of treatment of lead-acid battery manufacturing effluents is chemical precipitation using lime, while more recently a form of activated clay has been used with limited success. However, industry as a whole has been interested in biosorbents which are as efficient, if not more so, as conventional metal removal methods, since they are cleaner and more 'environmentally friendly' technologies, whose commercial application is likely to be cheaper than conventional methods.

The harmful effects of lead are well known, and there are a wide variety of sources which contribute to lead poisoning of natural ecosystems. However, lead poisoning is preventable to a large extent. A 1994 Summit of the Presidents of the Americas, saw an agreement being signed for the phasing out of lead from fuels. This was seen as an important step toward reducing lead contamination into

the atmosphere. Toxic effects of lead in adults and children have been reported for concentrations of lead less than the recommended limit of 0.1 mg/l (Romieu *et al.*, 1997).

Dead biomass as biosorbents for metals, in general, seems to have greater advantages over living biomass. For improved industrial use, it is generally agreed that immobilized or pelleted biomass should be coupled with recovery involving a cheap stripping agent. Some described biological processes are competitive in cost and operational efficiency with existing conventional processes (Gadd, 1990a). Ongoing research with non-viable *Azolla* biomass as a biosorbent has served to better characterize its capacity and efficiency in metal binding, and its potential for application in bioremediation.

Azolla biomass which is readily available and therefore cheap, was found to effectively adsorb lead from aqueous solutions and lead-acid battery manufacturing effluents in batch experiments. The effects of factors such as pH, temperature, initial lead concentration and biomass concentrations were investigated. An understanding of the effects of these parameters is important before the *Azolla* technology can be applied to remediation at an industrial level, where all these factors vary between different effluents. The effects of flow rate and lead concentrations in column reactors was also investigated because column reactors appear to be the most appropriate form in which biosorbent technology for remediation is best applied in industry, as opposed to batch reactors.

Lead removal from solution by the *Azolla* biomass was effective and rapid, reaching saturation within 25 minutes in batch systems. pH had the most pronounced effect on lead removal by *Azolla* biomass, with an optimum uptake range between pH values of 3.5-5.7. Initial lead concentrations of less than 400 mg/l, in batch studies, had little effect on the percentage lead removal capacity from aqueous

solution of the *Azolla* biomass, as did a range of temperatures from 10-50 °C. Biomass concentration of approximately 5 g *Azolla* / l solution was determined to be optimum within the range of parameters investigated for lead remediation from both aqueous solutions and lead-acid battery effluents. The maximum lead uptake capacity for lead was found to be approximately 100 mg lead/g *Azolla*.

The variability in the composition of lead-acid battery effluent makes application of any biosorbent technology for its remediation difficult. However, despite the variation in pH and composition of the lead-acid effluent, it appears that the *Azolla* biomass still showed good lead removal. The effective use of the *Azolla* biomass in removing lead from solution in packed column studies makes it a promising and potent candidate for application in semi-continuous adsorption processes. The regeneration studies with *Azolla* biomass were encouraging, increasing the possible economic benefits of its application in bioremediation processes. Scanning electron microscopy, although not conclusive, showed no apparent break-down in the physical structure of the *Azolla* biomass with repeated adsorption and desorption cycles.

The future development of biosorbent processes for metal removal and recovery depends on factors like uptake capacities, biosorbent selectivity, ease of recovery, comparative effectiveness and cost with existing technology, and insensitivity to operating conditions. For it to be competitive, it has been stated that a biosorbent's removal efficiency should be greater than 99 %, with loading capacities greater than 150 mg metal / g biomass dry weight. However, bioremediation processes need not necessarily replace existing treatment technology, but may be used in addition, as a supplementary or polishing steps to inefficient processes. Many kinds of biomass have been shown have very high metal uptake capacities, but selectivity may be a problem. Recovery may also be

selectively controlled by using an appropriate elution protocol (Gadd, 1990a).

With continued pollution of the biosphere with toxic heavy metals and radionuclides, it is clear that biosorbent-based technologies may have an important role in environmental protection (Gadd, 1990a). Much work still needs to be done to assess the scale-up factors and economics of *Azolla* biomass application in the bioremediation of metal-contaminated effluents. Progress in reactor design and scaling-up will facilitate commercial development of the process.

The complex nature of effluents makes selective bioremediation of target compounds problematic. The competitive effects of other metal ions in effluents, their varying concentrations and pH values between samples of the same effluent, dependent on the time of sampling or stage in the industrial process, all contribute to the complexity of an effluent system. All these factors need to be taken into account when deciding on the effluent which is best suited to a given biosorbent. Promising applications of the *Azolla* biomass in bioremediation appear to be in low volume-high concentration industrial effluents such as electroplating and lead-acid battery manufacturing waste-waters. A further possible application of the *Azolla* biomass would be in down stream processing of high volume-low concentration waste-waters as a polishing step. As with any other biosorbent process for metal remediation, further studies on economical feasibility are required before the '*Azolla* technology' can be commercialized. The diversity of microbe/metal interaction and the potential for technological innovations should allow for increased use of biosorbent-based bioremediation processes in mining, mineral-processing and waste-water industries (Hutchins *et al.*, 1986).

It may be concluded that *Azolla* biomass is an efficient biosorbent for lead and has promising potential for industrial application in remediation of lead-containing waste-waters.

APPENDICES

APPENDIX A

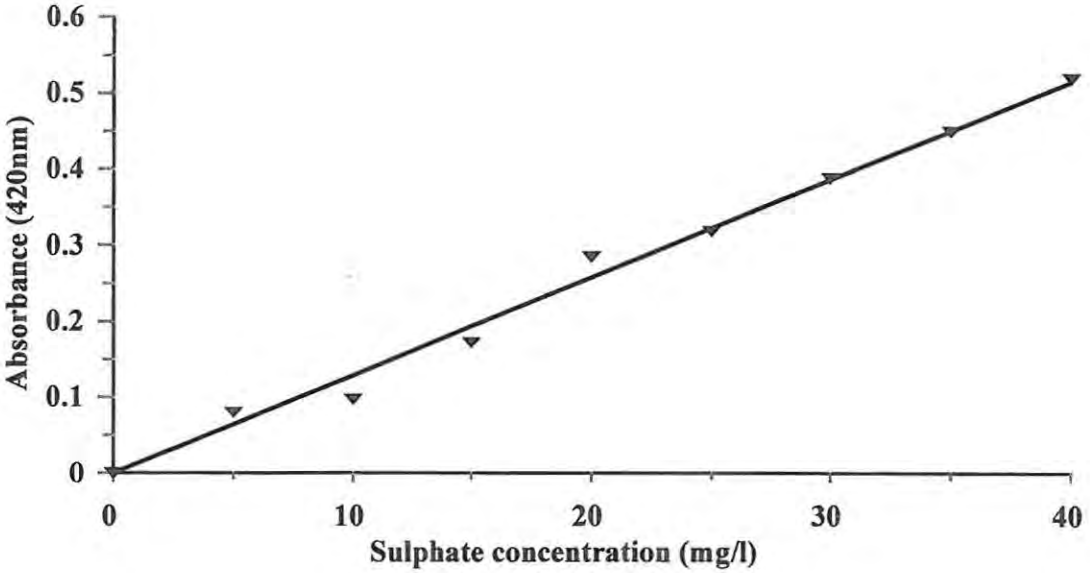

Figure A: Sulphate standard curve. $R^2 = 0.990$

Table A: Data for sulphate standard curve

Sulphate concentration (mg/l)	Absorbance (420nm)	Regression calculated absorbance (420nm)
0	0	0
5	0.063	0.065
10	0.087	0.129
15	0.173	0.194
20	0.282	0.259
25	0.344	0.323
30	0.398	0.388
35	0.477	0.452
40	0.545	0.517

REFERENCES

Abbasi, S.A. and Nipaney, P.C. (1985) Wastewater treatment using aquatic plants. Survivability and growth of *Salvinia molesta* (Mitchell) over waters treated with zinc(II) and the subsequent utilization of the harvested weeds for energy (biogas) production. *Resources and Conservation*, **12**, 47-55.

Andres,Y., MacCordick, H.J. and Hubert, J.C. (1993) Adsorption of several actinide(Th, U) and lanthanide (La, Eu, Yb) ions by *Mycobacterium smegmatis, Appl. Microbiol. Biotechnol.*, **39**, 413-417.

Artola, A. and Rigola, M. (1992) Selection of optimum biological sludge for zinc removal from wastewater by a biosorption process. *Biotechnol. lett.*, **14**, 1199-1204.

Ashton, P.J. and Walmsley, R.D. (1976) The Aquatic fern *Azolla* and it's *Anabaena* symbiot. *Endeavour*, **35**, 39-45.

Atkinson, B.W., Bux, F. and Kasan, H.C. (1998) Considerations for application of biosorption technology to remediate metal-contaminated industrial effluents. *Water SA.*, **24**, No. **2**, 129-135.

Babich, H. and Stotzky, G. (1978) Influence of chemical speciation on the toxicity of heavy metals to the microbiota. *In:* Aquatic toxicology. Nriagu, J. (ed.), John Wiley & Sons Inc., New York, p 2-37.

Bedell, G.W. and Darnall, D.W. (1990) Immobilization of nonviable, biosorbent, algal biomass for the recovery of metal ions. *In:* Biosorption of heavy metals. Volesky, B. (ed.), CRC Press, Inc., Florida, USA., p 321.

Brady, D. (1992) Bioaccumulation of Metal Cations by Yeast Cell Components. Un-published Ph.D thesis. Rhodes University South Africa. p 64, 47-50.

Brady, D., Letebele, B., Duncan, J.R. and Rose, P.D. (1994a) Bioaccumulation of metals by *Scenedesmus, Selenastrum* and *Chlorella algae. Water SA.*, **20**, No. **3**, 213-218.

Brady, D., Glaum, D. and Duncan, J.R. (1994b) Copper tolerance in *Saccharomyces cerevisiae. Lett. Appl. Microbiol.*, **18**, 245-250.

Brady, D. and Duncan, J.R. (1994a) Bioaccumulation of metal cations by *Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol.*, **41**, 149-154.

Brady, D. and Duncan, J.R. (1994b) Cation loss during accumulation of heavy metal cations by *Saccharomyces cerevisiae. Biotechnol lett.*, **16**, No. **5**, 543-548.

Chang, J., Law, R. and Chang, C. (1997) Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. *Wat. Res.*, **31**, No. **7**, 1651-1658.

Davies, A.G. (1983) The Effect of metals upon natural marine phytoplankton populations. *Prog. Phycol. Res.*, **2**, 113-145.

D'Avila, J.S., Matos, C.M. and Cavalcanti, M.R. (1992) Heavy metal removal from wastewater by using activated peat. *Wat. Sci. Technol.*, **26**, No. **9-11**, 2309-2312.

De Rome, L and Gadd, G.M. (1991) Use of pelleted and immobilized yeast and fungal biomass for heavy metal and radionuclide recovery. *J. Ind. Microbiol.*, **7**, 97-104.

De Wet, L.P.D., Schoonbee, H.J., Pretorius, J. and Bezuidenhout, L.M. (1990) Bioaccumulation of selected heavy metals by the water fern, *Azolla filiculoides* Lam. in a wetland ecosystem affected by sewage, mine and industrial pollution. *Water SA.*, **16**, No.**4**, 281-286.

Dean, J.G., Bosqui, F.L. and Lanouette, K.H. (1972) Removing heavy metals from waste water. *Environ. Sci. Technol.*, **6**, No. **6**, 518-522.

Dean, R.B. and Lund, E. (1981) Water Reuse: Problems and solutions. Dean, R and Lund, E (eds.).Academic Press Inc., London. p 1-3.

Delgado, M., Bigeriego, M. and Guardiola, E. (1993) Uptake of Zn, Cr, and Cd by water Hyacinths. *Wat. Res.*, **27**, No. **2**, 269-272.

Department of Water Affairs. (1986) Management of the water resources of the Republic of South Africa. CTP Book Printers, Cape Town, p 1.3-1.24.

Doyle, R., Matthews, T.H. and Streips, U.N. (1980) Chemical basis for selectivity of metal ions by the *Bacillus subtilis* cell wall. *J. Bacteriol.*, **143**, 471-480.

Edyvean, R.G.J., Williams, C.J., Wilson, M.W. and Aderhold, D. (1997) Biosorption using unusual biomasses. *In:* Biosorbents for metal ions. Forster, C. and Wase, J. (eds.), T.J. International Ltd, Padstow, Great Britain, p 174.

Falla, J. and Block, J.C. (1993) Binding of Cd^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} by isolated envelopes of *Pseudomonas fluorescens, FEMS Microbiol. Lett.,* **108**, 347-352.

Forster, C.F. and Wase, D.A.J. (1997) Biosorption of heavy metals: An introduction. *In*: Biosorbents for metal ions. Forster, C. and Wase, J (eds.), T.J. International Ltd, Padstow, Great Britain, p 7.

Fourest, E., Canal, C. and Roux, J. (1994) Improvement of heavy metal biosorption by mycelial dead biomasses (*Rhizopus arrhizus, Mucor miehei* and *Penicillium chrysogenum*): pH control and cationic activation. *FEMS.,* **14**, 325-332.

Gadd, G.M. and Griffiths, A.J. (1978) Microorganisms and heavy metal toxicity. *Microbial Ecology*, **4**, 303-317.

Gadd, G.M. (1986) Fungal responses towards heavy metals. *In*: Microbes in extreme environments. Herbet, R.A. and Codd, G.A. (eds.). Academic Press Inc., London, p 84-110.

Gadd, G.M. (1988) Accumulation of metals by microorganisms and algae. *In*: Biotechnology, Volume 6b. Rehm, H.J. and Reeds (eds.) Weinheim, V.C.H. p 401-430.

Gadd, G.M. (1990a) Heavy metal accumulation by bacteria and other microorganisms. *Experentia,* **46**, 834-840.

Gadd, G.M. (1990b) Metal tolerance. *In*: Microbiology of extreme environments. Edwards, C. (ed.). Alden Press., UK, p 179-205.

Gale, N.L. (1986) The role of algae and other microorganisms in metal detoxification and environmental clean-up. *Biotechnol. Bioeng. Symposium*, **6**, 171-180.

Galun, M., Galun, E., Siegel, B.Z., Keller, P., Lehr, H. and Siegel, S.M. (1987) Removal of metal ions from aqueous solutions by *Penicillium* biomass: kinetic and uptake parameters. *Water, Air and Soil Pollution,* **33**, 359-371.

Galvin, R.M. (1996) Occurrence of metals in waters: An overview. *Water SA.*, **22**, 7-18.

Garnham, G.W. (1997) The use of algae as metal biosorbents. *In:* Biosorbents for metal ions. Wase, J. and Forster, C. (eds.). T.J. International Ltd, Padstow, Great Britain, p 22.

Garnham, G., Codd, G.A. and Gadd G.M. (1992) Accumulation of cobalt, zinc and manganese by the estuarine green microalga *Chlorella salina* immobilised in alginate microbeads. *Environ. Sci. Technol.*, **26**, 1764-1770.

Gonçalves, E.P. and Boaventura, R.A.R. (1998) Uptake and release kinetics of copper by the aquatic moss *Fontinalis antipyretica. Wat. Res.*, **4**, 1305-1313.

Hattingh, W. (1980) Standard Methods for the Examination of Water and Wastewater. 15th edn., APHA, AWA, WPCF, American Public Health Ass., Washington DC, USA, p 439.

Ho, Y.S., Wase, D.A.J. and Forster, C.F. (1996) Removal of lead ions from aqueous solution using sphagnum moss peat as adsorbent. *Water SA.*, **22**, 219-224.

Holan, Z.R. and Volesky, B. (1994) Biosorption of lead and nickel by biomass of marine algae. *Biotechnol. Bioeng.*, **43**, 1001-1009.

Horikoshi, T., Naka jima, A. and Sakaguchi, T. (1979) Studies on the accumulation of heavy metals elements in biological systems IV. Uptake of uranium by *Chlorella regularis*. *Agric. Biol. Chem.*, **43**, 617-623.

Hutchins, S.R., Davidson, M.S., Brierley, J.A. and Brierley, C.L. (1986) Microorganisms in reclamation of metals. *Ann. Rev. Microbiol.*, **40**, 311-336.

Jain, S.K., Vasudevan, P. and Jha, N.K. (1989) Removal of some heavy metals from polluted water by aquatic plants: studies on Duckweed and Water velvet. *Biological Wastes*, **28**, 115-126.

Jain, S.K., Vasudevan, P. and Jha, N.K. (1990) *Azolla pinnata* R.Br. and *Lemna minor* L. for removal of lead and zinc from polluted water. *Wat. Res.*, **24**, No. **2**, 177-183.

Kapoor, A. and Viraraghavan. (1995) Fungal biosorption - an alternative treatment option for heavy metal bearing wastewaters: A review. *Bioresource Technol.*, **53**, 195-206.

Kasan, H.C. and Baecker, A.A.W. (1989) An assessment of toxic metal biosorption by activated sludge from the treatment of coal-gasification effluent of a petrochemical plant. *Water Res.*, **23**, 795-800.

Krishnan, S.S., Cancilla, A. and Jervis, R.E. (1987) Industrial wastewater treatment for toxic heavy metals using natural materials as adsorbents. *J. Radioanal. Nuc. Chem.*, **2**, 373-378.

Kroon, N. (1996) Aliens on the water. *Environ. Plan. Man.*, **7**, No. **1**, 330-335.

Kumar, P. and Dara, S.S. (1980) Removal of toxic heavy metal ions from wastewaters using modified agricultural waste materials. *Prog. Wat. Tech.*, **13**, 353-361.

Kuyucak, N. and Volesky, B. (1988) Biosorbents for recovery of metals from industrial solutions. *Biotechnol. Lett.*, **10**, No. **2**, 137-142.

Larsen, V.J. and Schierup, H. (1981) The use of straw for removal of heavy metals from waste water. *J. Environ. Qual.*, **10**, No. **2**, 188-193.

Laxen, D.P.H. and Harrison, R.M. (1983) Physico-chemical speciation of selected metals in the treated effluent of a lead-acid battery manufacturer and in the receiving river. *Water Res.*, **17**, 71-80.

Lee, S.H., Jung, C.H., Chung, H., Lee, M.Y. and Yang, J. (1998) Removal of heavy metals from aqueous solution by apple residues. *Proc. Biochem.*, **33**, No. **2**, 205-211.

Lester, J.N. and Sterritt, R.M. (1985) Microbial accumulation of heavy metals in wastewater treatment processes. *J. App. Bact., Symposium Supplement.* p 141s-147s.

LIRI technologies. (1990) Introductory course in wastewater management: Lecture 3.3. LIRI technologies, Grahamstown. South Africa. p 1-4.

MacKay, H.M. (1998) Towards a classification system for water resources in South Africa. WISA Conference paper. Institute for water studies, Department of Water Affairs and Forestry. Cape Town, April 1998.

Mishra, B.B., Nanda, D.R. and Misra, B.N. (1987) Accumulation of mercury by *Azolla* and it's effect on growth. *Bull. Environ. Contam. Toxicol.*, **39**, 701-707.

Mowll, J.L. and Gadd, G.M. (1983) Zinc uptake and toxicity in the yeasts *Sporobolomyces roseus* and *Saccharomyces cerevisiae*. *J. Gen. Microbiol.*, **129**, 3421-3425.

Muramoto, S. and Oki, Y. (1983) Removal of some heavy metals from polluted water by water hyacinth (*Eichhornia crassipes*). *Bull. Environ. Contam. Toxicol.*, **30**, 170-177.

Nakajima, A. and Sakaguchi, T. (1986) Selective accumulation of heavy metals by microorganisms. *Appl. Microbiol. Biotechnol.*, **24**, 59-64.

Niu, H., Xu, X.S. and Wang, J.H. (1993) Removal of lead ions from aqueous solutions by *penicillium* biomass. *Biotechnol. Bioeng.*, **42**, 785-787.

Norris, P.R. and Kelly, D.P. (1979) Accumulation of metals by bacteria and yeasts. *Develop. Ind. Microbiol.*, **20**, 299-308.

Okieimen, F.E., Okundia, E.U. and Ogbeifun, D.E. (1991) Sorption of cadmium and lead ions on modified groundnut (*Arachis hypogea*) husks. *J. Chem. Tech. Biotechnol.*, **51**, 97-103.

Özer, D., Aksu, Z., Kutsal, T. and Caglar, A. (1994) Adsorption isotherms of lead (II) and chromium (VI) on *Clapdophora crispata*. *Environ. Technol.*, **15**, 439-448.

Perkins, J.C. (1998) National Water Bill 1998; Water use authorisation. WISA Conference paper. Water Resources, Department of Water Affairs and Forestry. Cape Town, April 1998.

Pretorius, P., van der Merwe, P. and Branford, J. (1998) Paper on the practical implementation of the Department of Water Affairs and Forestry's proposed raw water pricing policy. WISA Conference paper. Department of Water Affairs and Forestry. Cape Town, April 1998.

Priel, A. (1995) Purification of industrial wastewater with the *Azolla* fern. *World Water and Environmental Engineering*, Special Report, **October**, 1.

Quek, S.Y., Wase, D.A.J. and Forster C.F. (1998) The use of sago waste for the sorption of lead and copper. *Water SA.*, **24**, No. **3**, 251-256.

Romieu, I., Lacasana, M., McConnell, R. and the Lead Research Group of the Pan-American Health Organization. (1997) Lead exposure in Latin America and the Caribbean. *Environ. Health Perspective*, **105**, No. 4, 398-405.

Rothery, E. (1980) AA-1275 and AA1475 Series spectrophotometers : Operation Manual. Varian Techtron Pvt. Ltd. Mulgrave, Australia., p 1-15.

Royer, M.D., Selvakumar, A. and Gaire, R. (1992) Control technologies for remediation of contaminated soil and waste deposits at Superfund lead battery recycling sites. *J. Air Waste Manage. Assoc.*, **42**, No. 7, 970-980.

Sağ, Y., Özer, D. and Kutsal, T. (1995) A comparative study of the biosorption of lead(II) ions to *Z. ramigera* and *R. arrhizus. Proc. Biochem.*, **30**, No. 2, 169-174.

Sarkar, A. and Jana, S. (1987) Effects of combinations of heavy metals on Hill activity of *Azolla pinnata. Water, Air and Soil Pollution*, **35**, 141-145.

Schenck, R.C., Tessier, A. and Campbell, P.G.C. (1988) The effect of pH on iron and manganese uptake by green algae. *Limnol. Oceanog.*, **33**, 538-550.

Scott, C.D. (1992) Removal of dissolved metals by plant tissue. *Biotech. Bioeng.*, **39**, 1064-1068.

Sela, M., Tel-Or, E., Fritz, E. and Huttermann, A. (1988) Localization and Toxic effects of cadmium, copper and uranium in *Azolla. Plant Physiol.*, **88**, 30-36.

Shukla, S.R. and Sakhardande, V.D. (1992) Column studies on metal ion removal by dyed cellulosic materials. *J. Appl. Polymer Sci.*, **44**, 903-910.

Shumate, S.E., Stranberg, G.W. and Parrott Jr, J.R. (1978) Biological removal of metal ions from aqueous process streams. *Biotechnol. Bioeng. Symposium*, **8**, 13-20.

Stokes, P.M. (1983) Responses of freshwater algae to metals. *Prog. Phycol. Res.*, **2**, 87-112.

Tan, W.T. and Khan, R.M. (1988) Removal of lead, cadmium and zinc by waste tea leaves. *Environ. Technol. Lett.*, **9**, 1223-123.

Thompson, G.A. and Watling, R.J. (1987) Bioaccumulation potential of heterotrophic bacteria for lead, selenium and arsenic. *Bull. Environ. Contam. Toxicol.*, **38**, 1049-1054.

Trevors, J.T. Stratton, G.W. and Gadd, G.M. (1986) Cadmium transport, resistance and toxicity in bacteria, algae and fungi. *Canadian J. Microbiol.*, **32**, 447, 451-464.

Tsezos, M. (1985) The selective extraction of metals from solution by micro-organisms. A brief overview. *Can. Metal. Quart.*, **24**, No. **2**, 141-144.

Tsezos, M. and Volesky, B. (1981) Biosorption of uranium and thorium. *Biotechnol. Bioeng.*, **23**, 583-604.

Tyagi, R.D. (1985) Effects of heavy metals on biological waste treatment in the activated sludge process. *Proc. Biochem.*, **20**, 194-198.

Volesky, B. (1987) Biosorbents for metal recovery. *TIBTECH*, **5**, 96-101.

Volesky, B. (1990) Removal and recovery of heavy metals by biosorption. *In:* Biosorption of heavy metals. Volesky, B. (ed.),CRC Press, Inc., Florida, USA, p 12-13, 23,.

Volesky, B. (1992) Removal of heavy metals by biosorption. *Amer. Chem. Soc.*, 462-466.

Volesky, B. and Holan, Z.R. (1995) Biosorption of Heavy Metals : Review. *Biotechnol. Prog.*, **11**, 235-250.

Weber, W.J., Jr., (1972) Adsorption. *In:* Physicochemical processes for water quality control. Webber, W.J., Jr., (ed.), John Wiley and Sons, New York, p 199-260.

Wilson, M.W. and Edyvean, R.G. (1994) Biosorption for the removal of heavy metals from industrial wastewaters. *Environ. Biotechnol.*, **4-6 July**, 89-91.

Yakubu, N.A. and Dudeney, A.W.L. (1986) Biosorption of uranium with *Aspergillus niger*. *In*: Immobilization of ions by biosorption. Eccles, H and Hunt, S (eds). Ellis Horwood, Chichester, p 183-200.

Zhao, M. and Duncan, J.R. (1997a) Column sorption of hexavalent chromium from aqueous solution and electroplating effluent using *Azolla filiculoides*. *Res. Environ. Biotechnol.*, **2**, 51-64.

Zhao, M. and Duncan, J.R. (1997b) Batch removal of hexavalent chromium by *Azolla filiculoides*. *Biotechnol. App. Biochem.*, **26**, 179-182.