DEVELOPMENT OF BIOREACTOR SYSTEMS FOR THE TREATMENT OF HEAVY METAL CONTAINING EFFLUENTS

APM Antunes · D Sanyahumbi · L Nightingale · R Payne · A Maclear · JR Duncan

WRC Report No. 845/1/03

Water Research Commission

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Report to the
WATER RESEARCH COMMISSION

by

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WRC Project No 845/1/03
ISBN No 1-86845-995-0

MARCH 2003

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Printed by Silowa Printers

EXECUTIVE SUMMARY

Heavy metal contamination of waste waters is a major source of environmental pollution and represents a loss of vital resource since water availability and quality will be a significant factor in the future socio-economic growth of South Africa. Numerous studies have shown that biotechnology based processes may provide an effective and inexpensive means of removing heavy metals from water when compared to more traditional methods such as ion-exchange and chemical precipitation.

The objectives of this study were:

1. Evaluate the potential of algae and the water fern, *Azolla*, to accumulate heavy metals from effluents.
2. Exploit the exopolysaccharide production by a number of algae as a means of enhancing metal removal efficiencies.
3. Optimise bioreactor design as well as biomass retention and separation systems for direct on-site application of the technology developed above.
4. In conjunction with (1), to examine systems for cultivation of *Azolla* and preparation of a biosorbent from this biomass.
5. Embark on a capacity building programme as part of the project by involving staff and students.

This study focused primarily on mine effluents as one of the major metal-contaminated waste waters in South Africa. Wastewater from the mining industry is generally extremely complex and contains numerous chemical species which influence the adsorption of the metals to any biomass. A variety of factors needs to be addressed before treatment is considered viable, including establishing the binding characteristics of the metal of interest to maximise its interaction with the biomass to be utilised.

Azolla filiculoides was investigated in the adsorption of gold(III), lead(II), iron(III), copper(II) and platinum(IV).

In batch studies, the optimum biomass and initial gold(III) concentrations were found to be 5 g/L and 8 mg/L respectively with 95-100% removal of gold under these conditions. The adsorption of gold(III) is principally pH-dependent with optimal removal (95-100%) at pH 2. Lead(II), iron(III) and copper(II) did not compete with gold(III) adsorption under equimolar and simulated effluent conditions. Halides, with increasing affinity for gold (chloride < bromide < iodide), can affect gold uptake with the soft base, iodide, exhibiting the most inhibition (25%) and the hard base, chloride, 0%. Mercaptoethanol (soft base) showed no interference in gold(III) adsorption while the presence of sulphate (hard base) and sulphite (borderline base) showed that concentrations in excess of 10 mM may adversely affect gold(III) uptake (25-30% inhibition), most likely due to competition for cationic sites on the biomass.

Column studies, better suited to high volume treatment, indicated that a flow-rate of 5 mL/min and an initial gold(III) concentration of 5 mg/L was optimal with 90-100% removal being achieved. Competitive effects between lead, iron, copper and gold again showed little or no interference. The halides, chloride, bromide and iodide, affect gold(III) uptake similarly to the batch studies (0-25% inhibition), while the bases mercaptoethanol and sulphate minimally affect gold(III) binding with sulphite severely hampering adsorption (70% inhibition).

To optimise gold desorption, preliminary batch studies indicated that a ratio of 1:1 of adsorbent:desorbent was optimal, whilst gas purging of thiourea with oxygen, air and nitrogen decreased gold elution in proportion to decreased amounts of oxygen. A series of desorbents were utilised, in column studies, to optimise and determine the speciation of bound gold. The presence of an oxidant with thiourea enhanced desorption greater than 3 fold when compared with thiourea alone (25-35% recovery). Thiourea desorption studies, aided by the oxidant, suggest that gold is present in the +1 and 0 oxidation states. Ultimately thiourea, perchloric acid and hydrochloric acid was found to be the most optimal elutant for gold (100% recovery).

Preliminary adsorption studies of platinum by *Azolla filiculoides* were conducted. Batch studies indicated that 1 g/L biomass concentration, initial platinum concentration of 20 mg/L and pH 2 are optimal, while the column studies indicated a flow-rate of 10 mL/min and initial platinum

concentration of 20 mg/L as optimal. In the platinum effluent study, platinum showed a removal of 23% and 21% for the batch and column studies respectively. Adsorption was accompanied by H^+ release.

The potential viability of *Azolla filiculoides* as a biosorbent has thus been demonstrated in the removal of gold and platinum from simulated as well as wastewater solutions. A high recovery from synthetic solutions of greater than 99% for gold (2-10 mg/L), and greater than 89% for platinum (20 mg/L) was achieved.

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 suggests that flow rate, and therefore retention time, has 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 effluents. 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 suggests 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, concluded 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.

When the toxic effects of metals were investigated, *Spirulina* was found to have a threshold level of about 30 µM for copper, zinc and lead. These metals appeared to have a direct effect on the photosynthetic pathway, thereby causing a rapid decline in cell growth. Lead on the other hand seemed to affect surface properties and hence took longer to cause deterioration in growth.

Although relatively low concentrations of metal may have a toxic effect on the cyanobacterium, *Spirulina* may have potential as a precipitation agent. The role of *Spirulina* in the precipitation of heavy metals appears to be through its ability to maintain a high pH in the surrounding medium, possibly through the enzyme carbonic anhydrase. The carbonate produced by the enzyme was utilised for metal precipitation studies. Metals precipitated were categorised into 3 groups: hydroxides, carbonates from dissocation of carbonate (Pb and Fe) and from free carbonate in solution (Mg and Ca). The last-named method seems to be the most suitable.

The various technological approaches for treating metal-containing waste water are explored and each offers a cost-effective alternative to the existing treatment strategies. The full exploitation of these potential treatments is dependent on the interest and investment by the various industries.

In terms of achieving the original objectives of the study the water fern, *Azolla*, was found to be an effective accumulator of gold, platinum, lead, iron, and copper from synthetic solutions. Lead, copper and iron could readily be recovered from the biomass while the recovery of gold and

platinum was more complex but achievable. The most optimal bioreactor for continuous and on-line application was an up-flow column reactor, relatively loosely packed with biomass. Cultivation of the *Azolla* biomass can be achieved in simple shallow baths supplied with appropriate nutrients but variable climatic conditions can significantly affect growth rates. The most appropriate and inexpensive method of biosorbent preparation was simple air or sun drying.

While one of the initial objectives was to investigate the accumulation of metals from low volume, high metal concentration effluents this was superceded previously initiated and by on going studies in our laboratory using electroplating and battery effluents. Consequently, this study concentrated on two major metal-containing effluents in the South Africa, *viz.* mine wastewater and battery manufacturing effluents.

A further objective of the project was to exploit the use of algal exopolysaccharides for metal removal but preliminary studies showed that while these compounds were efficient metal accumulators, the difficulties and costs involved in producing and extracting these polymers did not make this a viable option for bioremediation.

Capacity building programmes in collaboration with the Universities of the North and Fort Hare were not successful largely due to the lack of capacity of the staff involved and the low numbers of suitably qualified postgraduate students. A successful collaboration with staff and students at the Vaal Triangle Technikon was established and is ongoing.

While no pilot scale plants are yet in operation either in the laboratory or on-site, data related to this bioremediation process and the related technologies have successfully been transferred to a battery manufacturer in East London as well as Eskom and Anglo Platinum mining and refining operations.

Future research should focus on a detailed examination of the water chemistry related to metal containing effluents as this will largely dictate the most appropriate bioremediation technologies to be utilised and the most suitable operational conditions in terms of pH, anion concentrations, etc. An integration of biological systems for effective metal removal is likely to be the most efficient process to use for metal removal. Biosorption should be investigated as a pre- or

polishing step in such an integrated system. In terms of gold and platinum effluents, biosorption systems offer a great deal of potential for metal removal and recovery but the best conditions for optimum metal removal were to be established and further investigation will need to be undertaken to establish the most appropriate and cost-effective solutions to utilise for metal desorption, recovery and concentration.

ACKNOWLEDGEMENTS

The research in this report emanated from a project funded by the Water Research Commission and entitled:

"DEVELOPMENT OF BIOREACTOR SYSTEMS FOR THE TREATMENT OF HEAVY METAL CONTAINING EFFLUENTS"

The steering committee responsible for this project, consisted of the following persons:

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Ms. K Wentworth	Water Research Commission (Secretary)
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The financing of the project by the Water Research Commission and the contribution of the members of the steering committee is acknowledged gratefully.

This project was only possible with the co-operation of many individuals and institutions. The authors therefore wish to record their sincere thanks to the following:

Dr. GW Watkins	Department of Chemistry, Rhodes University
Ms. M Progrund	Department of Biochemistry, Microbiology and Biotechnology
Mr. R. Cross & his Staff	Electron Microscopy Unit, Rhodes University
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TABLE OF CONTENTS

CHAPTER 2: REMOVAL OF GOLD(III) BY *Azolla filiculoides*: BATCH STUDIES

Chapter 4: Desorption Studies

INTRODUCTION

Heavy metal contamination of waste waters is a major source of environmental pollution and represents a loss of vital resource since water availability and quality will be a significant factor in the future socio-economic growth of South Africa. The unsuitability of metal contaminated water for reuse in industrial processing is also an important economic consideration for many industries because of the escalating cost of fresh water supplies.

Numerous studies have shown that biotechnology based processes may provide an effective and inexpensive means of removing heavy metals from water when compared to more traditional methods such as ion-exchange and chemical precipitation.

The objectives of this research programme were as follows:

1. Evaluate the potential of algae and the water fern, *Azolla*, to accumulate heave metals from effluents.
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4. In conjunction with (1) to examine systems for cultivation of *Azolla* and preparation of a biosorbent from this biomass.
5. Embark on a capacity building programme as part of the project by involving staff and students from Fort Hare University and possibly the University of the North in these studies.

This report will deal with experimental studies designed to achieve these. An overview of the previous studies and literature related to this field is presented followed by a series of chapters describing the use of the water fern, *Azolla*, to removal and recover gold, platinum and lead from solution and effluents using both batch and column reactors. There are, in addition, two sections dealing with the bioremediation potential of a fungus and algal species. Each chapter has a relevant background (introduction) section followed by detailed materials and methods descriptions, a results and discussion section and a conclusion.

CHAPTER 1

LITERATURE REVIEW

Recent economic growth rates in developing countries, combined with population growth has resulted in an increased consumption of natural resources such as water and metals (Ayres, 1997). These resources may be considered as either renewable or non-renewable.

1.1. Water - A Renewable Resource

A water resource is an ecosystem which includes the physical and structural habitats such as the water and aquatic biota, and the ecological processes which link these habitats. As a semi-arid country, the shortage of water plays an important role in the economic and development sectors in South Africa and is of critical strategic importance. Realising the limiting effect it has on the expansion on the economy this renewable resource needs to be optimally utilised to the benefit of current and future consumers.

Consequently, the limits to the degree of utilisation which can be sustained by a water resource before resilience is lost needs to be recognised. The responsibility for the management of water resources includes the protection of users, which in turn requires protection of the water resources from over-utilisation or a repercussion which causes its deterioration. Sustainability requires that a balance be reached between protection of water resources, water users and the society's requirements for economic growth and development. In South Africa, the National Water Act (Act 36,1998) provides for water to be protected, utilised, developed, conserved and controlled in a sustainable and equitable manner. In other words, the act provides for the regulation of water usage through water licensing, water allocation and water use charges. A change in present water usage patterns is necessary to guarantee all water users that a sustainable water resource is safeguarded for the future.

Some of the reasons contributing to the challenges of ensuring sustainable water resources for the future are:

- While the climate varies from rain forest to desert, the typical climate of SA is semi-arid, with the average rainfall, 475mm per annum, being just over half the global average.
- Rainfall patterns are variable, with droughts followed by floods commonly occurring.
- The distribution of rainfall is uneven, with 60% of the river flow arising from only 20% of the land area.
- Limited groundwater.
- Some metropolitan and industrial growth centres have developed around mineral deposits and are far from major water resources.
- South Africa's average evaporation rate exceeds its precipitation rate.
- A population growth rate of 2-3% per annum.
- A large backlog of housing and service delivery.

In addition, the industrial, mining and power generation sector accounts for more than ten percent of water usage in South Africa. The main industrial, mining and power users are centralised in Gauteng and the surrounding area, although the Western Cape and Kwazulu-Natal are also significant consumers (DWAF, 1998).

1.2 Water resource policy and legislation

Management of natural resources and the environment has progressed rapidly in South Africa in recent years. This is a result of the global trend towards increased knowledge of the environment and the need to protect it, as well as a massive change in the political perspectives in the country (Harris *et al.*, 1999).

The Department of Water Affairs and Forestry has reviewed water laws, both locally and abroad in preparation for new legislation designed to reflect democratic principles and equitable resource access by all. The development of new legislation presented an ideal opportunity to achieve the objectives of providing more equitable access to water resources and consolidate much of the

recent development in water resource thinking (Harris *et al.*, 1999; Singh and Constantinides, 2000).

The Department's slogan of "some for all, forever" succinctly captures the goal for water resource management and service provision in South Africa. It recognises that water is an essential, finite resource and indicates a fundamental commitment to equitable allocation. Finally it acknowledges the goal of sustainable use.

1.2.1 Ecological integrity

Ecosystems have an inherent recovery capacity in response to variable conditions, such as floods and droughts, but this ability is finite. As the maximum recovery capacity is assumed to exist in a natural, unmodified system, the degree to which a specific component of the water resource differs from its natural state is important in estimating its ability to recover. The degree of modification of a particular part of a water resource can be assessed by measuring components of its ecological integrity (Harris *et al.*, 1999).

The ecological integrity of a water source is its ability to maintain a balanced, integrated composition of physico-chemical habitat characteristics and biotic components on a temporal and spacial scale. These should be comparable to the natural characteristics of ecosystems in the region. Ecological integrity implies the ecosystem's structure and functions are not impaired by anthropogenic stresses. A resource will only be able to provide for long term water uses if its ecological integrity is assured (Harris *et al.*, 1999; Singh and Constantinides, 2000).

Many of South Africa's water resources have already been modified by use and development and are no longer in their natural state, but this does not mean they are no longer sustainable. In such cases the effects of these uses need to be determined and those uses that can be sustained by the water resource, so that its integrity remains at an acceptable level, need to be identified (Harris *et al.*, 1999).

The concept of a water "Reserve" has been defined and is intended to protect water resources, so that basic human needs can be met and ecological functions and processes can be sustained. The Reserve consists of two parts, the basic human needs reserve and the ecological reserve. The basic human needs reserve provides for the essential needs of individuals served by the water resource in question and includes water for drinking, for food preparation and for personal hygiene. The ecological reserve relates to the water required to protect the aquatic ecosystem of the water resource. The Reserve for all or part of any significant water resource is determined by the Minister of Water Affairs and Forestry (National Water Act, 1998).

Components of ecological integrity, such as the chemical and physical characteristics of the water, the quantity of water, the habitat and the structure and function of the associated biotic communities are measured to ensure the Reserve does indeed protect the ability to meet human needs and sustain ecological functions. The ongoing monitoring and assessment of the condition of the water resources, their response to impacts and the state of the Reserve are critical to the management and protection of resources. The aim is for management decisions to be made on the basis of sound scientific and technical information and understanding (Harris *et al.*, 1999; Singh and Constantinides, 2000).

1.2.2 Policy for protecting water resources

The enactment of the National Water Act (1998) and the Water Services Act (1997) made various requirements and provisions for the implementation of water conservation and demand management principles. The Water Services Act sets out a framework to ensure the provision of basic water supply and sanitation and a regulatory framework for water services institutions. The National Water Act ensures that the nation's water resources are protected, used, developed, conserved, managed and controlled in ways which take into account, amongst other factors, promoting the efficient, sustainable, and beneficial use of water in the public interests. Furthermore, the Act gives the Minister broad powers to make regulations limiting or restricting the purpose, manner or extent of water use as well as the attachment of conditions relating to water resource protection to every authorisation or licence issued. Finally the Act requires the

establishment of a national water resource strategy and a catchment management strategy, to provide for the development, management and protection of water resources and catchments (DWAF, 1999).

The policy integrates resource-directed measures for protection (such as resource quality objectives) with source-directed measures (such as effluent standards). It includes:

- Setting water resource-based objectives which clearly define acceptable values for water resources for each of the components (chemical, physical, biological) of ecological integrity.
- Use of source-directed standards which clearly define acceptable values for waste discharge or impact generation and encourage movement towards minimisation of waste disposal and impacts.
- Where source-directed standards cannot be met in the short term, a temporary exemption from the standards could be considered if an impact assessment indicates that the water resource-based objectives could still be met.

Before any potentially hazardous waste can be disposed of, a waste disposal site permit needs to be issued by the Department of Water Affairs and Forestry. The applicant is required to adhere to a series of "minimum requirements" before a permit is issued. The objectives of the minimum requirements are to ensure the protection of SA's water resources, to ensure a nationally uniform approach to waste disposal and to make South African waste management practices internationally acceptable (DWAF, 1998).

The minimum requirements are enforceable under the National Water Act (1998) and the Environment Conservation Act (1989). They allow for the suspension and revocation of disposal permits and the recovery of remediation costs from any person responsible for the pollution of water resources.

A cornerstone of the minimum requirements is the determination of the estimated environmental

concentration (EEC) for particular hazardous substances. The EEC is calculated based on acceptable risk criteria. The aquatic environment has been chosen to quantify the risk to man and the environment. Since the aquatic environment is extremely sensitive to contamination and pollution it is possible to prove that if the aquatic environment is not at risk (being within acceptable risk), that man and more robust ecosystems should also not be exposed to unacceptable risk. The acceptable risk levels have been set at one tenth of the LC_{50} (the concentration determined to kill 50% of specific warm and cold water fish or aquatic invertebrate species) and have been statistically shown to result in a mortality incidence of 1 in 300 000 in the aquatic environment (DWAF, 1998). The acceptable risk values for the elements of interest to this study are listed in appendix A.

1.2.3. Water Pollution

It is also necessary to manage and regulate water resources to achieve long term protection of the water quality. The presence of metal ions in natural, drinking and waste waters can have two different effects. The metal(s) may have positive effects especially when the metals present in the water are essential to life such as calcium (Ca) and zinc (Zn), alternatively the metal(s) may have deleterious effects for both consumption and the environment, such as lead (Pb), mercury (Hg) and iron (Fe) (Galvin, 1996). The concentration of metals in water are a function of their particular chemical and electrochemical behaviour, the hydrological environment conditions as well as disposal levels in effluents. The need to monitor, control and clean up heavy metal pollution has become increasingly important over recent years, with the development of various chemical and physical treatment processes. Awareness of the sensitivity of the environment to the toxicity of these elements has allowed for stricter regulations and control of pollution (Krishnan *et al.*, 1987). Prevention of pollution is a particular long term goal since waste emission in the environment can not be entirely eliminated. The requirements of water quality are determined according to the procedures set out by the South African Water Quality Guidelines and are of significance to this study, the guidelines, in terms of permissible metal concentrations, are indicated in Table 1.1 (DWAF, 1998).

Table 1.1. Maximum permissible limits for the disposal of hazardous metal wastes in water systems for South Africa (DWAF, 1998).

Metal	*Maximum Limit (mg/L)*
Aluminium	0.39
Antimony	0.07
Barium	7.80
Cadmium	0.03
Chromium	4.70
Cobalt	6.90
Copper	0.10
Cyanide	0.0053
Iron	9.00
Lead	0.10
Manganese	0.30
Mercury	0.022
Nickel	1.14
Selenium	0.26
Silver	2.00
Zinc	0.70

1.3. Minerals - A Non-Renewable Resource

Metals are amongst the most commonly used raw materials in today's industrial world (Anon., 1987). Enormous quantities of metal ores are extracted from the earth's crust and every metal extracted is a potential waste with the exception of scarce or valuable metals such as platinum or gold that are currently being recycled (Ayres, 1997). Mining, metal-refining, the use of metals in manufacturing, and final disposition of manufactured products constitute industrial activities which have resulted in metal losses (Anon., 1987). Metal resources are non-renewable and natural reserves are becoming depleted. It is therefore imperative that metals of technological importance and strategic significance, in terms of economic value or potential hazard, be recovered using an appropriate treatment (Atkinson *et al.*, 1998).

With regards to the tonnage of material being handled and processed in the mining industry, the technology used for concentrating the ore is of importance environmentally. For example, in the mining of gold (Au), concern is focused on the areas of heap leaching and the use of potassium or sodium cyanide as a reagent to concentrate the gold ore. The cyanides remain in the impoundments and sometimes leak into the groundwater. In the gold mining industry there is also an older and much more dangerous process of gold recovery, namely, the use of mercury (Hg) to amalgamate gold particles in the low grade ores (4-20 ppm). The gold-mercury amalgam is then heated, vaporising the mercury and leaving the gold. The mercury is partially oxidised in the atmosphere, eventually condensing on the soil or vegetation, washing into the rivers and ultimately entering into the food chain as toxic methylmercury. Large areas have been rendered uninhabitable in locations where this process was used in the past. This process is still being used today by small illicit gold miners in Africa and in other third world countries (Hamer, 1993).

1.4. Metal Wastes

Metal wastes not only represent a critical loss of non-renewable resources but pose increasing environmental problems such as a serious health hazard (Volesky, 1999). They accumulate in the food chain with humans at the top of the chain. Thus a significant opportunity exists for recovering these metal contaminants to moderate the threat of toxic, heavy metal contamination of the environment (Anon., 1987).

The principal driver of change in the mining and metallurgical processing sectors in the forthcoming decades will be the awareness of environmental problems. Much of historical pollution can be traced to waste management practices that promoted disposal rather than treatment. Previously legislation was designed to protect the environment as a response to the out-of-control pollution rather than as a pre-emptive measure. Presently, legislature requires a better understanding of the pollutants in our environment, to minimise the effects of past pollution and to act immediately should an industrial accident occur (Hamer, 1993). Stricter environmental regulations with regard to metal discharges are now being enforced for industrialised areas (Volesky, 1999).

Technological aspects of metal recovery from industrial waste waters must be re-evaluated. Effluent treatment processes are designed so that waste waters discharged into natural waters have no adverse effects. Possible effects depends on the volume and composition of the effluent discharged. The impact of industry on water resources is enormous and only through strict regulations and prevention measures will the deterioration/contamination of the water reservoirs diminish. Proposals for resource recovery and waste minimisation should be constantly analysed to reduce the generation of chemicals and hazardous wastes (Atkinson *et al.*, 1998).

1.4.1. Treatment strategies

1.4.1.1. Chemical precipitation

Chemical treatment strategies for AMD remediation have two primary goals, the neutralisation of pH and the precipitation of heavy metals. The most commonly employed chemical treatment method is the addition of lime. The lime may be added in the unslaked, hydrated ($Ca(OH)_2$) or dolomitic ($CaMg(CO_3)_2$) form. Each form has associated advantages and disadvantages, for example hydrated lime reacts faster than the dolomitic form, but is almost three times as expensive and produces greater sludge volumes (Maree *et al.*, 1992).

All heavy metals encountered in AMD waters form complexes with hydroxide species. These complexes affect the solubility of the particular mineral. Considering the generic divalent metal ion (M^{2+}) it is clear that up to three hydroxide complexes can be formed (MOH^+, $M(OH)_2^0$ and $M(OH)_3^-$) depending on the pH. While the positive charged complex has meaning considering the aqueous phase alone, the remaining two only have significance in determining metal ion solubility (Loewenthal *et al.*, 2001). The two phase equilibrium diagram (Figure 1.1) for ferrous iron illustrates this phenomenon. This has implications for lime dosing, with a relatively specific amount needing to be added to achieve optimal metal removal. From Figure 1.1 it is evident that optimal $Fe(OH)_2$ precipitation is only achieved in the region of pH 11 and that below pH 10 the Fe^{2+} and $Fe(OH)^+$ species dominate.

As ferrous iron is the dominant heavy metal in most AMD discharges and the efficient precipitation of $Fe(OH)_2$ requires a high pH, a modified process is most commonly employed. The high density sludge (HDS) process involves the initial oxidation of the ferrous iron to the ferric form at a high oxygen concentration and a relatively low pH. The ferric iron subsequently precipitates and settles (at pH 6) as amorphous ferric hydroxide which upon dehydration changes to FeOOH and Fe_2O_3 as the final products. The sludge is recycled and contacted with lime in order to induce crystallisation. This process has proven effective for iron removal, but has little capacity to remove heavy metals and salinity (Loewenthal *et al.*, 2001).

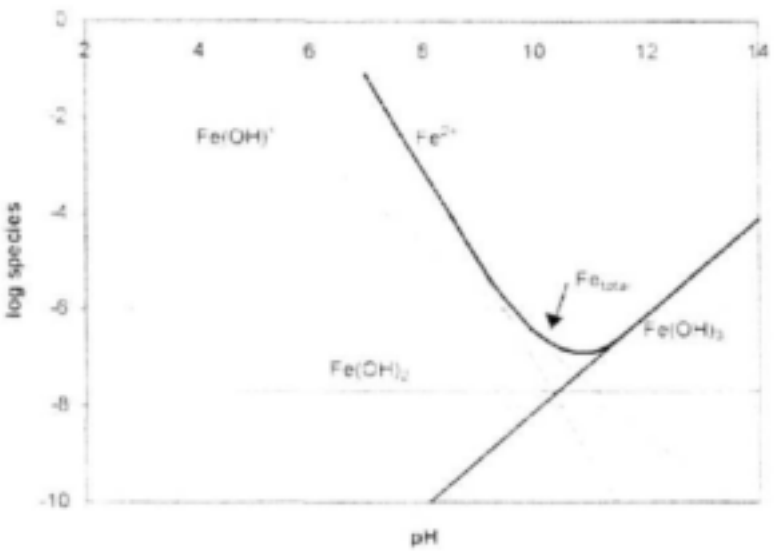


Figure 1.1: Aqueous and solid phase equilibrium for ferrous hydroxide at infinite dilution (Loewenthal *et al.*, 2001).

An alternative to the HDS process is a sequential process where neutralisation and the precipitation of some heavy metals as hydroxides is achieved by lime addition and ferrous iron is removed by sulphide addition. A range of sulphide salts has been utilised, but barium sulphide (BaS) is preferential as the barium will not persist in solution in the presence of sulphate ions. Therefore, the use of BaS can reduce the salinity of the AMD (Larson *et al.*, 1973). The precipitation of ferrous iron by sulphide addition is not without disadvantages, the main one being the formation of a colloidal precipitate that has poor settling characteristics. The potential of biologically generated hydrogen sulphide has also been investigated and will be discussed in more

detail later.

Based on the shortcomings of the above processes a number of authors have proposed an alternative system, the Ferrite Process, which is capable of removing iron and heavy metals in a single process, without the necessity of further sludge treatment (Wang *et al.*, 1996; Barrado *et al.*, 1998; Loewenthal *et al.*, 2001). The term "ferrite" refers to magnetic oxides containing other metals in addition to iron. The general chemical formula of ferrites is MFe_2O_4, where M is any divalent ion with an unhydrated ion radius of between 0.6 an 1.0Å.

Ferrite is formed via partial oxidation of ferrous species at pH values of greater than 7. The reaction has been shown to proceed through two pathways, depending on the pH and rates of oxidation. At pH values of 7-10 and relatively slow oxidation rates, ferrites form through the Green Rust (GR) path. At pH values of 10.5 and greater they form from the FeOOH phase. Green rusts are unstable green-blue compounds consisting of a mixture of ferrous and ferric ions accompanied by Cl^-, SO_4^{2-} or CO_3^{2-} and OH^- ions. Ferrite formation through the GR pathway at pH<10 has been shown to result in relatively high residual iron and heavy metal concentrations in solution, as well as inferior sludge settling properties (Perez *et al.*, 1998; Loewenthal *et al.*, 2001).

Ferrite formation at pH>10 showed excellent removal efficiencies (>99%) and good sludge characteristics. Barrado *et al.* (1998) proposed the following reactions for ferrite formation from ferrous at pH>10.5:

1) In the absence of heavy metals and controlled oxidation rate

$$3FeSO_4 + 6NaOH + 0.5O_2 \rightarrow Fe_3O_4 + 3Na_2SO_4 + 3H_2O \quad (1)$$

2) In the presence of heavy metals and controlled oxidation rate

$$xM^{+n} + 3FeSO_4 + 6NaOH + 0.5O_2 \rightarrow M_xFe_{3-x}O_4 + 3Na_2SO_4 + 3H_2O + xFe^{+n} \quad (2)$$

Fe^{+n} represents the total concentration of iron whose precipitation as ferrite is impeded by the other metal cations present in the water. If the pH is maintained at greater than 10 these ferrous

and ferric ions will react again to form magnetite (Fe_3O_4).

The ferrite process is most efficient where the iron to heavy metals ratio is 10-20:1. To achieve this, $FeSO_4$ is added to the solution. The pH is raised to over 10 at 65°C and oxygen is introduced to effect the partial oxidation of ferrous iron. The resulting precipitates are stable and can be easily recovered by magnetic filtration. In addition, as the heavy metals are incorporated into the lattice structure they are not susceptible to leaching from the sludge upon ageing. The major disadvantage of the standard ferrite process is the high temperature, which introduces prohibitive energy costs. The major focus of research in this field is on developing an efficient ambient temperature process (Loewenthal *et al.*, 2001).

1.4.1.2. Adsorption and ion exchange

One of the major drawbacks of treatment systems based on chemical precipitation is their poor efficiency when treating effluents with low metal concentrations. The removal of inorganic pollutants from dilute aqueous media can be achieved by adsorption and ion exchange mechanisms. Adsorption refers to the binding of charged species in solution to reactive groups, of opposite charge, on a solid support. Much of the research in recent years has focussed on the use of cells or sorbents derived from biological material (Kratochvil and Volesky, 1998). A wide range of biosorbents have been tested, including yeast (Brady and Duncan, 1994; Ashkenazy *et al.*, 1997), fungi (Tobin *et al.*, 1990; 1993), algae (Ting *et al.*, 1989; Holan *et al.*, 1993; Leusch and Volesky, 1995; Kratochvil and Volesky, 1998) and the aquatic fern *Azolla* (Zhao and Duncan, 1998; Sanyahumbi *et al.*, 1998). The most important metal binding sites on the surface of biological material are the carboxyl, phosphoryl, amino and thiol groups.

Ion exchange refers to the replacement of toxic heavy metal ions in solution by more benign counter-ions that balance the surface charge of the solid exchanger. The ion exchange resins are derived from both natural (zeolite) and synthetic (synthetic polymers) sources and can be manufactured to contain single or multiple functional groups, depending on the application (Ahmed *et al.*, 1998; Chiarle *et al.*, 2000).

Both biosorbents and ion exchange resins have been successfully utilised in low volume effluent treatment systems. The most common reactor configurations are the packed and fluidised bed reactors, as well as a number of column designs. Their application to high volume effluents such as AMD are limited by reactor design and flow dynamics, with a typical unit containing between 20 and 100kg of biomass (Gadd and White, 1993).

1.4.1.3. Membrane technologies

A number of membrane based technologies have been developed for the treatment of wastewaters polluted with heavy metals. Both electrodialysis and tubular reverse osmosis reactors have demonstrated the ability to desalinate non-scaling mine waters, with the former producing water fit for human consumption. The majority of AMD waters contain high concentrations of sodium and sulphate, leading to scale formation on the membrane surface. There have been some advances in the field with the development of the SPARRO (Slurry Precipitation and Recycle Reverse Osmosis) process, which partially overcomes the scaling problem and reduces operation costs (Juby *et al.*, 1996). The construction and operation costs of membrane reactors, coupled with the volume of polluted water discharges from South African mines limit the large scale application of this technology.

1.4.1.4. Active biological treatment systems

Active biological systems for the treatment of AMD are primarily dependent on the action of sulphate reducing bacteria (SRB). The bacteria are able to utilise sulphate as the terminal electron acceptor to oxidise organic carbon, producing bicarbonate and hydrogen sulphide as by-products. The biochemical pathways involved are complex and will be discussed in greater detail in the relevant chapter of this thesis. A simplified overview of the reaction is shown below:

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \qquad (3)$$

The form of the alkalinity and the sulphide will depend on the pH and aqueous chemistry of the

system. The sulphide reacts with metals in solution to form insoluble metal sulphides and the bicarbonate alkalinity helps to raise the pH of the AMD.

The SRB are grown in purpose built bioreactors under controlled conditions. A number of reactor designs have been tested, including anaerobic filters (Chian and De Walle, 1983), packed bed anaerobic reactors (Maree *et al.*, 1987), mixed systems (Maree and Hill, 1989), fluidised bed systems (van Houten *et al.*, 1994), sequencing batch reactors (Herrera *et al.*, 1991) and upflow anaerobic sludge blanket systems (Barnes *et al.*, 1991). A wide range of simple and complex organic carbon sources have been investigated.

There have been few successful industrial scale applications of this technology, although Shell Research Ltd successfully ran a pilot plant at the Budelco BV zinc refinery in the Netherlands, which was later scaled up to a system capable of treating 7Ml day^{-1}. The system uses a selected, but undefined consortium of sulphate reducing bacteria, with ethanol as the carbon source. The final concentration of all heavy metals was reduced to the ppb range and the sulphate concentration was reduced by over 80% (Barnes *et al.*, 1991).

1.4.1.5. Passive treatment systems

Natural processes commonly ameliorate mine drainage pollution. As contaminated mine drainage flows through receiving systems (streams, rivers and lakes) its toxicity decreases naturally as a result of biological and chemical reactions as well as by dilution with uncontaminated waters. The low pH typical of many mine drainages increases as the water mixes with less acidic water or through dissolution of carbonate minerals. Metals contained in the mine water then precipitate as oxides or hydroxides under the aerobic conditions found in most surface waters. Ferric iron, aluminium and to a lesser extent manganese are the first metals to precipitate out (Hedin *et al.*, 1994).

The passive treatment of mine water developed from pilot scale plants to full scale field implementation during the 1980's. Passive treatment technologies take advantage of natural

chemical and biological processes. Ideally these systems require no addition of chemicals and little or no operation and maintenance inputs. Passive systems depend on processes that are slower than those of active treatments and thus require longer retention times and larger areas to achieve similar results (Hedin *et al.*, 1994).

The goal of passive systems is to enhance natural processes so that they occur within the treatment area and not in the receiving water body. There are two factors which determine if this can be achieved, the kinetics of the contaminant removal processes and the retention time within the system. The retention time for a particular site is often determined by the available land area. However, the kinetics of contaminant removal processes can often be affected by manipulating the environmental conditions that exist within the passive treatment system. Efficient manipulation of contaminant removal processes requires that the nature of the rate-limiting aspects of each removal process be understood (Hedin *et al.*, 1994). The development of passive systems from an experimental concept to full scale field application occurred during the 1990's. While over 200 wetlands have been operating in the Appalachia region of the US, their application has been limited to acid waters with low metal content (Gazea *et al.*, 1996).

1.4.1.5.1. History of passive treatment

The interest in passive systems was sparked by research in the late 1980's which indicated that natural *Sphagnum* wetlands improved the quality of mine drainage without incurring any obvious ecological damage (Wieder and Lang, 1992). A number of experimental wetlands were constructed to mimic the *Sphagnum* moss wetlands. However, *Sphagnum* moss was not readily available, proved difficult to transplant, and had the tendency to accumulate metals to toxic levels within several months (Spratt and Wieder, 1988). Despite these initial setbacks research continued and eventually a design evolved that proved tolerant to years of exposure to contaminated mine drainage and was effective at lowering the concentrations of dissolved metals. They typically consisted of a series of small wetlands (<1 ha) vegetated with cattails (*Typha latifolia*) (Wieder, 1989).

During the development of the most recently applied passive treatment schemes the importance of anaerobic processes in metal removal was identified. In such systems a complex ecosystem is not needed and the treatment cells operate effectively without plants. Pretreatment systems have also been developed which involve contacting the acidic waters with limestone in an anoxic environment before it enters a settling pond or wetland system (Gazea *et al.*, 1996).

1.4.1.5.2. Mechanisms for contaminant removal

A number of physical, chemical and biological processes are known to occur within passive treatment systems to reduce metal concentrations and neutralise the acidity of the influent water. The relative importance of the various processes is difficult to assess due to a lack of accurate quantitative data (Gazea *et al.*, 1996).

The simplest mechanism encountered in a passive wetland system is the dilution of contaminants by inflows of uncontaminated water. Large volume inflows may cause significant changes in the water chemistry that might be mistakenly attributed to biological or chemical processes (Hedin *et al.*, 1994).

After accounting for dilution there are four major processes which can occur in passive systems to reduce the acidity and lower the concentration of metal ions in solution. These are:

- oxidation and hydrolysis
- metal removal by plants, algae and organic substances
- reduction
- limestone addition

1.4.1.5.3. Oxidation and hydrolysis

The mechanism of oxidation of ferrous to ferric iron has been previously covered. This process occurs abiotically, but can also be catalysed by bacteria. The kinetics of both mechanisms are pH

dependent. At pH values of 5 and above the kinetics of oxidation can be described by the following equation:

$$-(d[Fe^{2+}]/dt) = k_1[Fe^{2+}][OH^-]^2{}_pO_2 \quad (4)$$

where $k_1 = 8.0 \times 10^{13}$ min^{-1} atm^{-1} mol^{-2} l^2

${}_pO_2$ = partial pressure of oxygen

From the $[OH^-]^2$ it is clear that for each unit the pH is raised, above 5, the reaction rate increases 100-fold. Under these conditions the most important role of the constructed wetland is to provide a retention time that is sufficient for the dissolved iron to oxidise and precipitate (Gazea *et al.*, 1996).

At lower pHs (pH < 3.5) the kinetics of abiotic oxidation is described by the following equation:

$$-(d[Fe^{2+}]/dt) = k_2[Fe^{2+}]_pO_2 \quad (5)$$

where $k_2 = 1.0 \times 10^{-7}$ min^{-1} atm^{-1}

Under these conditions the oxidation is clearly slow and the contribution of iron oxidising bacteria becomes important. Species such as *Acidothiobaccillus* and *Leptobacillus ferrooxidans* have a pH optimum of between pH 1.5-3.5. They are able to increase the ferrous iron oxidation rate by several orders of magnitude. In experimental aerobic wetland systems the numbers of iron oxidising bacteria has been positively correlated with iron oxidation and removal (Gazea *et al.*, 1996).

As ferrous iron is oxidised to ferric, it is subjected to hydrolysis that results in the rapid precipitation of ferric hydroxide and the release of proton acidity, the mechanisms of which have been discussed previously. Based on the solubility of ferric hydroxide ($K_{sp} = 6.0 \times 10^{-38}$) it is clear that under equilibrium conditions negligible concentrations of dissolved ferric iron exist at pH

values above 3. Natural or constructed aerobic wetlands that receive neutral iron containing waters typically discharge acid water (pH 2-3) as a result of iron oxidation and hydrolysis (Gazea *et al.*, 1996).

Manganese is the second important component of most acid mine drainages that can be removed by oxidation and hydrolysis. Typically manganese is discharged to the surface as the Mn^{2+} ion and for efficient hydroxide precipitation to occur requires the oxidation of the Mn^{2+} to a higher oxidation state. The exact mechanisms occurring in aerobic wetlands have not been fully elucidated, but it has been suggested that Mn^{2+} is oxidised to either the trivalent or tetravalent form, which then precipitates as MnOOH (Hedin *et al.*, 1994). The proposed reaction is shown below:

$$4Mn^{2+} + O_2 + 6H_2O \rightarrow 4MnOOH + 8H^+ \quad (6)$$

Over time the MnOOH is likely to oxidise to the thermodynamically more stable MnO_2. As with iron, the oxidation of manganese is strongly dependent on pH and occurs very slowly at pH values below 8. The process may be accelerated by the action of manganese oxidising bacteria, which are most readily found at pHs of 7 and above (Gazea *et al.*, 1996). The bacteria oxidise Mn^{2+} to MnOOH or the manganate ion (Mn^{4+}).

In alkaline environments manganese can also precipitate in the carbonate form. In the presence of atmospheric oxygen the carbonate may oxidise further to the more stable oxide as shown below:

$$Mn^{2+} + HCO_3^- \rightarrow MnCO_3 + H^+ \quad (7)$$

$$2MnCO_3 + O_2 \rightarrow 2MnO_2 + 2CO_2 \quad (8)$$

Data collected from wetland applications has shown that when a minewater contaminated with both iron and manganese passed through the system the manganese removal was far less effective than the iron and occurred sequentially, not simultaneously (Hedin *et al.*, 1994). There are two

main factors which contribute to this phenomenon. Both mechanisms are pH dependent, but the oxidation of iron occurs more readily and is optimal at lower pH values. The oxidation and hydrolysis of iron releases further proton acidity which makes the conditions for manganese oxidation even less favourable. The second factor is the reduction of oxidised manganese by ferrous iron:

$$MnOOH + Fe^{2+} \rightarrow FeOOH + Mn^{2+} \quad (9)$$

The net result is that manganese removal in passive aerobic systems is a slow and inefficient process, even in situations where ferrous iron concentrations are low (Gazea *et al.*, 1996).

1.4.1.5.4. Metal removal by plants and algae

Emergent vegetation is one of the components of aerobic wetland systems. While accumulation of metals in the plant tissues plays only a minor role in metal removal the presence of plants has a number of associated benefits. Plants are able to diffuse oxygen from their roots, generating localised oxidising zones within the substrate which promote metal removal by oxidation.

Decaying plant material is an important component of a number of other processes which occur in the wetland. It is used as an organic carbon source for sulphate reducing bacteria and contains carboxylic and phenolic acids which are able to complex metal ions from solution. In addition, the presence of emergent vegetation enhances the aesthetics of the treatment system and reduces erosion and dispersal of metal precipitates by wind during dry periods (Gazea *et al.*, 1996).

A considerable amount of research has been conducted on the ability of algae to reduce the concentration of metals in solution. This is achieved by adsorption onto charged functional groups on the cell surface and by accumulation of the metal inside the cell. The mechanisms involved will be discussed in greater detail in chapter three.

1.4.1.5.5. Reduction processes

Bacterial sulphate reduction under anaerobic conditions can be an important factor in determining the final water quality discharged from the passive treatment system. Sulphate reduction occurs in the organic substrate layer, where bacteria such as members of the *Desulfovibrio* species use sulphate to oxidise organic matter and release bicarbonate and hydrogen sulphide (Kalin *et al.*, 1991). The range of species involved, carbon sources utilised and biochemical mechanisms will be discussed in greater detail in chapter five.

Bacterial sulphate reduction is limited to certain environmental conditions. The process is curtailed at pH values below 4 and in the presence of oxidising agents such as O_2, Fe^{3+} and Mn^{4+}. Typically the conditions which favour bacterial sulphate reduction can be maintained in an anoxic wetland environment (Gazea *et al.*, 1996).

The hydrogen sulphide reacts with some dissolved metal ions to form insoluble metal sulphides, which may then precipitate. The removal of dissolved metals in the sulphide form depends on the pH, the solubility of the specific metal sulphide and the concentration of reactants.

While low pH values inhibit the growth and activity of SRB, their activity results in an increase in alkalinity and pH in the surrounding micro-environment. As a result they have been found to be active in sediments below extremely acidic waters (Herlihy *et al.*, 1987). They are also tolerant of relatively high concentrations of toxic heavy metals, with exposure to zinc, lead and nickel at concentrations of 60mg/l not resulting in significant inhibition.

While sulphate reduction is the most important source of alkalinity in recently commissioned wetlands, there is evidence to suggest that in more established wetlands the reduction of ferric iron to ferrous iron is the major source of alkalinity (Vile and Wieder, 1993).

1.4.1.5.6. Limestone addition

The addition of limestone may be employed where the mine drainage has a particularly high acidity. The limestone dissolves to produce calcium and bicarbonate alkalinity, which neutralises acidity and buffers the pH. The solubility of limestone depends on pH, temperature and carbon dioxide concentration. When acid water, pH < 6.4, contacts limestone, the limestone reacts according to the equation:

$$CaCO_3 + 2H^+ \rightarrow Ca^{2+} + H_2CO_3^* \qquad (10)$$

Dissolved carbon dioxide, conventionally noted as $H_2CO_3^*$, is a weak acid and continues to react with limestone, producing calcium and bicarbonate alkalinity which is available for acid neutralisation reactions (Gazea *et al.*, 1996).

In practice, the effectiveness of this process is reduced where the influent water is high in ferrous iron and contacts limestone in an oxidising environment. The resulting formation of ferric hydroxide precipitates leads to coating of the limestone surface and reduces dissolution to almost nil. In anoxic environments, where the iron is maintained in the ferrous form and no precipitate forms at pH < 5.5, limestone addition can be an effective method of reducing acidity (Gazea *et al.*, 1996).

1.4.1.6. Types of passive treatment systems

There are three principal types of passive technologies developed for the treatment of mine discharges. They are:

1. aerobic wetland systems
2. anaerobic organic substrate systems
3. anoxic limestone drains

1.4.1.6.1. Aerobic wetland systems

Aerobic wetlands have been effectively used to treat net alkaline waters, which typically contain enough alkalinity to buffer the acidity produced by metal hydrolysis reactions. The system relies on oxidation reactions and metals precipitate primarily as hydroxides, oxyhydroxides and oxides. The aerobic wetland cells are designed to retard the flow of water long enough for metal oxidation and hydrolysis to occur and for the resulting precipitates to settle. The hydrolysis process releases proton acidity, which retards the oxidation rate. In some cases an amount of crushed limestone is initially added to mediate this effect. Ideally the pH of the wetland is maintained at between 5.5 and 6.5, which enhances the precipitation of iron, manganese and aluminium, the primary constituents of net alkaline minewaters (Younger, 1995; Gazea *et al.*, 1996).

The design is similar to that of a "natural" wetland and consists of basins or channels with a relatively impermeable bottom layer to prevent seepage. This is covered with soil or a similar medium suitable for supporting vegetation. The efficiency of aerobic wetlands for removing iron is limited by dissolved oxygen concentrations. To enhance efficiency, wetlands are often designed with features to enhance aeration, such as steps or waterfalls, followed by quiescent areas. Each aeration step provides sufficient oxygen to remove 50mg/l Fe^{2+}, so where iron concentrations are higher, a series of aeration steps needs to be incorporated (Hedin *et al.*, 1994). The water depth is typically shallow (10-50cm) to further enhance aeration (Younger, 1995), but not consistent throughout the wetland, with deeper (1-2m) areas providing regions for sludge accumulation (Hedin *et al.*, 1994). The length to width ratio is typically over 10 to ensure sufficient residence time. The presence of vegetation helps to regulate the water flow, introduces additional oxygen into the water and sediment, assists in the removal of iron flocs and reduces the erosion potential of the precipitates (Gazea *et al.*, 1996).

1.4.1.6.2. Anaerobic (compost) wetlands

The compost wetland is one of the systems available for the treatment of net acidic mine waters. In these cases the system is required to generate sufficient alkalinity to neutralise excess acidity.

Compost wetlands generate alkalinity through a combination of bacterial activity and limestone dissolution. The SRB require a rich organic substrate in which anaerobic conditions will develop (Hedin *et al.*, 1994; Gazea *et al.*, 1996).

A wide range of organic substrates have been tested and are typically low-cost natural products and wastes, such as horse and cow manure, spent mushroom compost, hay bales, peat, wood chips or sawdust. Some substrates, such as mushroom compost contain limestone, while those that don't are usually supplemented with limestone. The substrate is placed in a non-compacted layer, typically 30-45cm thick. A compost loading of 250-300kg.m^{-2} is usually employed (Hedin *et al.*, 1994). The bacteria reduce sulphate to assist in the oxidation of labile organic carbon. The sulphide which is released reacts with metal ions in solution and the bicarbonate assists in neutralising excess acidity. As the bacterial metabolism is influenced by temperature, the efficiency of the system is affected by seasonal fluctuations in temperature. The loss of efficiency in winter may be reduced by limestone addition and by directing the water flow through the compost layer, rather than over the surface (Younger, 1995; Gazea *et al.*, 1996).

1.4.1.6.3. Anoxic limestone drains (ALDs)

Anoxic limestone drains (ALDs) have become an increasingly popular passive "pre-treatment" technology (Younger, 1995). Essentially these consist of trenches filled with limestone gravel or cobbles (10cm diameter) that are overlayed with a plastic sheet and a layer of clay to exclude oxygen. They are used to raise the pH of net acidic waters by carbonate dissolution prior to metal and/or acidity removal in wetlands (Younger, 1995; Gazea *et al.*, 1996).

ALDs are operated under saturated conditions to further minimise the infiltration of atmospheric oxygen. Their dimensions vary considerably, with most of the early systems consisting of long, narrow (0.6-1m wide) trenches (Hedin *et al.*, 1994), although more recently systems have been installed that are 10-20m wide, without a significant reduction in efficiency.

The mass of limestone required to neutralise a certain discharge for a specific period of time can

be calculated based on the minewater flow rate and the alkalinity-generating performance of the ALD. Research has shown that a 14 hour contact time between the minewater and the limestone is required to achieve a maximum concentration of alkalinity (275-300mg/l). To achieve a contact time of 14 hour approximately 3000kg of limestone is required for each litre per minute of minewater flow. In the above situation the limestone would dissolve at a rate of 160kg per year for each litre per minute of minewater flow (Hedin *et al.*, 1994).

The major problem associated with the use of ALDs is the armouring of the limestone surfaces by iron and aluminium precipitates. If the influent minewater contains over 2mg/l of Fe^{3+} or Al^{3+}, or has a high dissolved oxygen content the efficiency of the ALD system decreases rapidly as a result of armouring, although this is not common in discharges originating from deep level mining, where the vast majority of Fe and Al is in the divalent state (Hedin *et al.*, 1994; Gazea *et al.*, 1996).

The precipitation of metals should ideally not occur within the ALD, so its primary function is to add alkalinity to the water. Therefore they need to be utilised in conjunction with other passive treatment components.

1.4.1.7. Wetland size

The sizing design for wetlands has been developed by Hedin *et al.* (1994) and is based on two key principals:

(i) The pollutant loading which the wetland will receive is calculated as:

$$\text{Loading (g/day)} = Q \text{ (l/min)} \times \text{concentration (mg/l)} \times 1.44 \qquad (11)$$

where Q is the peak flow rate

Where the water is net alkaline, iron is typically the main polluting component. Iron removal

occurs most effectively by oxidation processes in aerobic wetlands. However, where waters are net acidic, iron removal must be accompanied by the neutralisation of total acidity (proton and mineral) in order to generate a satisfactory final effluent. This is best achieved using an anaerobic wetland. Based on this, loadings are determined with reference to iron alone in the case of net alkaline waters and total acidity loadings where net acid waters are to be treated (Hedin *et al.*, 1994; Younger, 1995).

(ii) The minimum size of a constructed wetland is determined by dividing the appropriate loading by an empirical removal rate (RR), which has been determined by the US Bureau of Mines based on existing systems. That is:

$$\text{Wetland size } (m^2) = \text{Loading } (g/d) / \text{RR } (g/d/m^2) \quad (12)$$

Hedin *et al.* (1994) recommended two distinct suites of RR values based on the legal conditions relating to the remediation of the site. Where strict compliance with discharge standards is required a "conservative criterion" (CC) must be used. In this situation a removal rate of 10g of iron per m^2 d^{-1} should be assumed when designing aerobic wetlands receiving net alkaline waters and an acidity removal rate of 3.5g total acidity per m^2 d^{-1} when designing an anaerobic wetland to treat net acidic discharges. The use of these strict compliance criteria will yield large wetland areas and as such will only be popular where legal obligations exist. In many cases the pollution is associated with long abandoned mines, where liability cannot be traced to an existing company. In these cases application of the CC may prove too costly so a second set of RR values, based on "reasonable improvement criteria" (RIC) has been proposed. The RR values are 20 for aerobic wetlands and 7 for anaerobic wetlands, resulting in smaller, less expensive wetlands (Hedin *et al.*, 1994; Younger, 1995).

1.4.1.8. Integrated biological treatment systems

Both active and passive biological systems have been used for the treatment of acid mine drainage, but there are serious constraints to their widespread implementation, particularly in SA. The land

area necessary to treat high volume AMD discharges has restricted the use of passive systems, particularly as the affected areas are typically surrounded by industrial development. The three major factors constraining the active biological treatment approach are the reactor configuration used, the cost of construction and the cost and availability of the carbon source and the electron donor for the microbial reduction processes (Rose *et al.*, 1998).

Research at Rhodes University has focused on the utilisation of cheap or waste carbon sources for sulphate reduction and the integration of SRB and algal based technologies for AMD treatment (Rose *et al.*, 1998; Boshoff, 1999; van Hille *et al.*, 1999). Algal waste stabilisation pond (WSP) technology has been developed over the past 40 years (Mara *et al.*, 1996). While WSPs have been used to treat a diverse range of effluents little attention has focused on their potential to treat AMD.

Rose *et al.* (1998) reported on the treatment of tannery effluent in an Algal Integrated Ponding System (AIPS), similar to the type developed by Oswald (1991). The system consisted of a facultative pond, containing both an anaerobic and an aerobic component. Efficient sulphate reduction and metal precipitation was observed in the anaerobic compartment, while the aerobic cap was responsible for the re-oxidation of excess sulphide, which eliminated potential odour and toxicity concerns. This work led to the consideration of algal ponding as a possible system for the treatment of AMD.

Boshoff (1999) developed an integrated biological system for the treatment of acid mine drainage, utilising tannery effluent or primary sewage sludge as the carbon source for sulphate reduction.

Table 1.2. summarises some of the technologies utilised in metal remediation of waste water.

Table 1.2. Performance characteristics for heavy metal removal/recovery technologies for waste water treatment (Eccles, 1995).

Technology	*Performance Characteristics*				
	pH Change	*Metal Selectivity*	*Influence of Suspended Solids*	*Tolerance of Organic Molecules*	*Concentration of Metal (mg/L) in Treatment*
Activated Carbon	lim. tolerance	moderate	fouled	can be poisoned	<10
Electrochemical	tolerant	moderate	engineered to tolerate	can be accommodated	>10
Ion-Exchange	lim. tolerance	resins can be selective	fouled	can be poisoned	<100
Membrane	lim. tolerance	moderate	fouled	intolerant	>10
Precipitation:					
a) Hydroxide	tolerant	non-selective	tolerant	tolerant	>10
b) Sulphide	lim. tolerance	lim. selection & pH-dependent	tolerant	tolerant	>10
Solvent Extraction	some pH tolerance	metal selective extractants available	fouled	intolerant	>100

(lim.: limited)

Bioremediation represents a group of diverse clean-up strategies which may be employed for waste water treatment (Hamer, 1993). Implementing the use of biological materials for the removal of heavy metals from industrial waste streams may provide an attractive alternative to physico-chemical processes (Sağ *et al.*, 1995). Biological systems may succeed where established competition does not exist or where significant advantages can be found (Eccles, 1995). Such bioremediation also shows promise in terms of fulfilling the requirements of efficiency and cost effectiveness (Volesky, 1999).

Consequently, during the last few years, biotechnology has been receiving increasing attention, opening up a potential for the development of novel and efficient technology in the minerals industry. Two main areas of application for metal recovery have emerged: (1) biological leaching of ores, and (2) selective extraction of metals from dilute aqueous solutions using biological

materials. For metal removal and/or recovery from solution the use of biological methods, known as biosorption, has been thoroughly investigated, and has been suggested as viable alternatives to existing treatment methods.

1.5. Biosorption

Biosorption generally uses a biomass consisting of raw materials obtained from abundant sources, e.g., seaweed (Volesky, 1987), or from industrial wastes, e.g., yeast (Brady *et al.*, 1994) to accumulate metals. Biosorption is defined as the passive sorption and complexation of metal ions by a biomass. Adsorption is a term used to describe metabolic-independent uptake or binding of heavy metals to the biomass, although it is generally difficult to separate physical and chemical processes in such interactions, whereas biosorption is used to describe the non-directed binding that occurs between metals and cellular components of the biomass (de Rome and Gadd, 1991). In contrast, the term bioaccumulation includes all processes responsible for the uptake of metal ions by living cells and this includes biosorptive mechanisms, intracellular accumulation and bioprecipitation mechanisms (Eccles, 1995). Bioaccumulation/biosorption by various forms of biomass, whether of plant or microorganism origin, has been known for some time (Volesky, 1987). Many yeasts, algae and bacteria (Wilhelmi and Duncan, 1995; Hosea *et al.*, 1986; Tzesos, 1985; Özer *et al.*, 1997) are known to be capable of concentrating metal species (toxic or valuable) and thus allow for detoxification or recovery of these metals from industrial solutions and waste waters (Aksu and Açikel, 1999).

Table 1.3 highlights some of the characteristics involved with biosorption and bioaccumulation. The sequestering power of the biomass may be selective for accumulating heavy metals and involves mechanisms ranging from purely physico-chemical interactions such as adsorption to the cell wall, to mechanisms where metal removal depends on the active transport processes of the cell (Fourest and Roux, 1992). However, the disadvantage of bioaccumulation is that once the metal concentration becomes too high or once sufficient metals are adsorbed, the metals disrupt metabolic processes and cause the organism to die. Studies have shown that dead or non-living biomass is able to sequester metals to the same extent or even better than living biomass (Eccles,

1995). This may be more pronounced because of the loss of an "active defense" against metals that may be toxic (Volesky, 1992). Also, nutrient supply is unnecessary and the recovery of the metal is easier by non-destructive treatments which allows for the regeneration of the biomass for subsequent re-use (Gadd, 1988).

Table 1.3. Major characteristics of biosorption and bioaccumulation (Eccles, 1995).

Feature	*Biosorption*	*Bioaccumulation*
Metal Affinity	High under favourable conditions.	In some instances high metal accumulation. Toxicity will affect metal uptake by living cells.
Rate of Metal Uptake	Usually rapid, a few seconds for outer cell wall accumulation.	Slower than biosorption.
Selectivity	Variety of ligands involved, therefore is poor.	Better than biosorption, but less than some chemical technologies.
Temperature Tolerance	Within a modest range.	Inhibited by low temperatures.
Versatility	Metal uptake may be affected by anions or other molecules. Extent of metal uptake is usually pH-dependent.	Requires an energy source. Dependent on ATP-ase activity. Frequently accompanied by the efflux of another metal.

1.5.1. Selection of Biomass

It appears that the biosorption characteristics may vary widely depending on the metal and organism involved (Fourest and Roux, 1992). There are different criteria for the selection of biomass: (1) the type of biomass, (2) the metal species in solution, (3) the preparation of the biomass, (4) the physico-chemical environment, and (5) cost; in many instances the biomass is regarded as waste and is thus considerably cheaper than ion-exchange resins (Eccles, 1995; Volesky, 1992; Volesky, 1999). With respect to its chemical properties, the sorbent should contain functional groups that bind metal ions and the degree of ionisation of the sorbent surface is important. The physical properties of the sorbent should include a specific surface area, pore size and distribution.

1.5.2. Binding Mechanism

Certain types of biomass, even metabolically inactivated or dead cells, can passively bind and accumulate metals from the surrounding aqueous solution. Biosorption does not only depend on the chemical composition of the cell or its components such as the cell wall but also on the external physico-chemical factors and the solution chemistry of the metal. A combination of mechanisms may be involved in biosorption such as complexation, ion-exchange, adsorption and chelation.

A combination of one or various basic metal binding mechanisms may be functional, to varying degrees, in immobilising one or more metallic species on the biosorbent (Volesky, 1987). Metals function as Lewis acids (electron pair acceptors), but depending on the pH, oxidation state and complexation, may exist as metal complexes which may function as Brønsted bases. Changes in oxidation state can profoundly affect steric factors in addition to coordination geometry and coordination numbers. pH changes can also have an effect on the charge of the inorganic complex. Outside the cell, chemical properties can be used to predict interactions between chemical species. Pearson summarised the order of complexation of inorganic ions on the basis of his theory of "hard" and "soft" acids and bases known as the Hard and Soft Acid Base theory (HSAB). According to the HSAB theory, hard acids prefer to bind to hard bases, and soft acids prefer to bind to soft bases (Pearson, 1968). Some examples of hard and soft acids and bases are given in Table 1.4.

Table 1.4. Classification of hard and soft acids and bases (Pearson, 1968; Wood and Wang, 1983).

Hard Acceptor (acid)	*Intermediate*	*Soft Acceptor*
H^+, Na^+, Mg^{2+}, Ca^{2+}, Cr^{3+}, Fe^{3+}	Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}	Ag^+, Au^{3+}, Hg^{2+}, Pd^{2+}, Pt^{2+}
Hard Donor (base)	***Intermediate***	***Soft Donor***
H_2O, OH^-, F^-, Cl^-, SO_4^{2-}, O^{2-}	Br^-, NO_2^-, SO_3^{2-}	CN^-, CO, SCN^-, SH^-, H^-, I^-, $S_2O_3^{2-}$

It should be noted that many of the more reactive metals are soft acids, preferring coordination to bases found in living systems such as thiolate (present in sulphur-containing amino acids). The coordination number of the metal complexes are important for kinetics of binding and stability. Once bound covalently, they are difficult to replace with other competing metal ions (Wood and Wang, 1983). Functional groups such as carbonyls, hydroxides, thiols, carboxyls, phosphates and sulphates can all be active to varying degrees in binding the metal. Ion-exchange can also be responsible for metal sequestering, and microprecipitation on the cell wall of the organism has also been found (Kuyucak and Volesky, 1988b; Volesky, 1987).

1.5.3. Factors Affecting Biosorption

Non-viable, physical adsorption or ion-exchange, occurs at the cell surface and equilibrium between the adsorbed metal on the biomass and the metal in solution is very rapid. This is thought to be a passive uptake. With viable biomass, metal uptake involves the active transport of metal ions across the membrane into the cytoplasm (Özer *et al.*, 1997).

Several factors affect the biosorption of the metal to the biomass, these include: biomass concentration, initial concentration of the metal, temperature and pH. The solution pH affects the solution chemistry of the metals, the activity of the functional groups on the biomass and the competition of the metal ions for binding (Özer *et al.*, 1997; Sağ *et al.*, 1995). Metals are very sensitive to pH changes in adsorption-mediated binding. When pH > pI (the point where the charges are neutral) there is a net negative charge and the functional groups will therefore promote the reaction with positively charged metal complexes. As the pH decreases, the charge on the cell surface is positive, thereby inhibiting the binding of the positively charged metal complex. It is likely that the positive charges will compete with the metal complex for binding and thus the interaction of the metal ions with the biomass is lowered (Sağ *et al.*, 1995). The shape of the adsorption curve gives qualitative information about the adsorption process and the extent of surface coverage of the adsorbate (Faust and Aly, 1987; Tsezos, 1985).

To evaluate the metal uptake by the biosorbent, consideration of equilibrium isotherms is

To evaluate the metal uptake by the biosorbent, consideration of equilibrium isotherms is necessary. The adsorption isotherms are the equilibrium relationship between the concentration of adsorbed material and metal in solution at a given temperature (Özer *et al.*, 1997). At a given temperature a defined amount of sorbed species sequestered by the sorbent will be in equilibrium with the amount of free metal in solution (Eccles, 1995). There are several models which describe the adsorption data (Faust and Aly, 1987).

1.5.4. Equilibrium Isotherms

There are two main equilibrium isotherms to evaluate the performance of a biosorbent in an aqueous system, the Langmuir and Freundlich, of which the latter is the most utilised mathematical description (Faust and Aly, 1987).

1.5.4.1. Freundlich adsorption isotherm

This isotherm is expressed as the following:

$$x/m = KC_e^{1/n}$$

x : the amount of solute adsorbed

m: weight of the adsorbent

C_e: equilibrium concentration of the solute

K and 1/n: constants characteristic of the system.

For linearisation of the data:

$$\log x/m = 1/n \log C_e + \log K$$

By plotting log x/m versus log C_e , a straight line with slope of 1/n and log K as the intercept is obtained. If 1/n is close to 1, then a high adsorptive capacity at high equilibrium concentrations occurs, while $1/n << 1$ indicates that adsorptive capacity is only slightly reduced at lower equilibrium concentrations (Faust and Aly, 1987). If $1/n < 1$ (i.e. $n > 1$) then adsorption is favourable (Özer *et al.*, 1997). Some isotherms are associated with systems where adsorption

does not proceed beyond the monomolecular layer, others involve multilayer formation (Faust and Aly, 1987).

1.5.5. Biosorbent Performance Evaluation

Maximum uptake is an important feature of the sorbent, characterising its performance at high metal concentrations. An isotherm that is steep at low concentrations of sorbate shows a high affinity of the sorbent for a given sorbed species (Eccles, 1995).

In addition to equilibrium studies, the kinetics of the biosorption has to be determined in order to establish the rate of metal uptake and release. Rapid uptake would provide a short solution-contact time and aids the use of much shallower beds of sorbent material. These points are important for quantitative assessment of performance and process design. Biosorption performance depends on the following parameters: temperature, pH, the presence of co-ions and the solution chemistry of the metal (Eccles, 1995).

In considering the biosorption process there are a few points to note regarding the efficiency of the biosorbent to be utilised:

(1) uptake and release of metal should be efficient and rapid,

(2) the active biosorbent should be produced at low cost and be re-usable,

(3) particle size, shape and the mechanic properties of the biosorbent material should be suitable for continuous flow systems, and

(4) the removal of the biosorbent should be cheap, rapid and efficient.

Finally, separation of metal from sorbent should be metal selective, economically feasible and loss of sorbent should be minimal (Eccles, 1995). If the biomass is, however, very inexpensive then combustion of the material may be worthwhile, yielding ash with a high metal concentration (Eccles, 1995).

Many studies have utilised various types of biomass such as chitin, fungi, bacteria, algae as well

as plant material. Reports have shown that shrimp wastes containing chitin are an excellent chelator of copper(II), chromium(III), and nickel(II) demonstrating 95%, 96% and 44-70% removal respectively and compared well with purchased crab chitosan (Chui *et al.*, 1996). While purchased chitosan demonstrated high removal for copper(II), chelators such as EDTA, citrate and tartrate, however, interfered with the adsorption of the metal (Juang *et al.*, 1999).

Numerous studies have demonstrated that algae is an excellent biosorbent for the removal of metals from simulated and waste water solutions. Greene *et al.* (1986b) showed that *Chlorella vulgaris* was able to accumulate uranium(II) from uranium millprocessing streams at an optimum pH of between 4 and 6 (adjusted). The presence of sodium, chlorides, nitrates and sulphates had no effect on the removal of the metal. Non-viable *Chlorella vulgaris, Scenedesmus obliquus* and *Synechocystis sp.*, all exhibited favourable removal rates with copper(II), nickel(II) and chromium(VI) at optimum pH's of 5, 4.5 and 2 respectively. Increased removal occurred with increased metal concentration, up to 250 mg/L (Dönmez *et al.*, 1999). Studies by Volesky's group have demonstrated that the brown algae *Sargassum natans* is capable of removing cadmium(II) (100 mg/g biomass) and lead(II) (220 mg/g biomass) and that the binding of cadmium(II) at pH 4 is an ion exchange process with one metal ion adsorbed per one hydrogen released from the biomass (Volesky, 1992; Yang and Volesky, 1999). Studies with *Datura innoxia* have demonstrated an affinity in the order of copper(II) ~ silver(I) > nickel(II) > cadmium(II) > europium(III) > strontium(II) > barium at pH > 5. The carboxylate groups have been shown to be responsible for most of the binding (Ke *et al.*, 1994). Further studies by Raysan *et al.* (1994) with cadmium(II) and *Datura innoxia* cells in a free and immobilised form, showed that the latter exhibited a higher removal and that two binding sites have been found to exist on the cell walls. The functional groups found to be involved were sulphate, at pH < 3, and single and dual carboxylate groups at pH < 4.

A fungi that has received much attention is *Rhizopus arrhizus*. Tsezos and Volesky (1982a, b) have shown that the biomass is capable of removing radioactive materials such as uranium and thorium from aqueous solution. The biosorbent demonstrated a maximum uptake of 180 mg/g and 120 mg/g biomass of uranium and thorium respectively. Adsorption occurred via binding to the

biomass cell wall. Iron(II) and zinc(II) interfered with the binding of uranium whilst the two metals did not interfere with thorium adsorption. Thorium was found to coordinate to nitrogen sites on the cell wall. It was also found that the uptake of the metal ions, manganese(II), copper(II), zinc(II), cadmium(II), barium, mercury(II), lead(II) and silver(I) was directly related to ionic radii, whilst the molybdenum and vanadate ions were found to be pH-dependent (electrostatic interactions) (Tobin *et al.*, 1984). A study with *Aspergillus niger* demonstrated that it was capable of accumulating 10% of its dry weight as metal. Accumulation was pH-dependent and binding was found to be exclusively by exchange with calcium and magnesium(II) (Akthar *et al.*, 1995).

Higher plant tissues have also been found to be excellent adsorbents of various metals. Studies by Zhao and Duncan (1997a, b, 1998) have demonstrated that *Azolla filiculoides* is an excellent chelator of chromium and nickel. Column studies have shown that the biomass was capable of a maximum uptake of 41.5 mg/g biomass of chromium(VI) at 60% saturation of the biomass at an acidic pH (2.5); whilst chromium(III) exhibited 25 mg/g biomass uptake (Zhao and Duncan, 1997a). Nickel(II) removal in batch studies was found to be 43.3 mg/g biomass at pH 6.5. Whilst in column studies nickel(II) demonstrated 21.6 and 27.7 mg/g biomass uptake, and complete recovery with 0.2 N sulphuric or hydrochloric acid was obtained (Zhao and Duncan, 1998). *Azolla pinnata* (water velvet) was utilised to reclaim mercury(II) from soil. The growth of *Azolla pinnata* was inhibited with the accumulation of increasing concentrations of mercury(II) (Mishra *et al.*, 1987). Another plant material used for recovery of metals from water is the water hyacinth. The roots of the non-viable plant material demonstrated a high level of sorption for copper(II) with a maximum uptake of 20.9 mg/g at pH 4-6. The percentage of copper(II) sorption decreased with increased concentration of copper(II) (Low *et al.*, 1994).

Gold accumulation by various biosorbents has been extensively studied (Table 1.5). Gee and Dudeney (1988) have shown that *Chlorella vulgaris* and *Spirulina platensis* are able to selectively and reversibly adsorb the metal, and once adsorbed, the gold is reduced to gold(I) and gold(0) and crystallised to form hexagonal and triangular laminae. The brown alga, *Sargassum natans*, removed gold(III) at a pH < 3 and the removal was not influenced by the presence of other ions.

A potential for recovery of gold from waste water was shown by the high removal of 98% (Kuyucak and Volesky, 1988a). Another biomaterial utilised in the recovery of gold is a metal recovery agent (MRA) of microbial origin. This MRA is used in the AMT-Bioclaim™ process treatment of waste water. The biosorbent was able to remove 98% of gold from waste water, however, the recovery decreased in the presence of cyanide (Brierley and Vance, 1988).

Table 1.5. Binding capacities of various biosorbents for gold.

Metal	*Biosorbent*	*Maximum Uptake*	*Reference*
Gold(III)	*Sargassum natans*	420 mg/g	Kuyucak and Volesky, 1988a
Gold	MRA	155 mg/g	Brierley and Vance, 1988
Gold(III)	*Chlorella vulgaris*	10% dry weight/ (0.5mmol/g)	Greene *et al.*, 1986a; Darnall *et al.*, 1986
Gold(III)	*Datura innoxia*	>51.8 mg/g	Lujan *et al.*, 1994
	Alfalfa sprouts	>34.2 mg/g	
	Sphagnum peat moss	>30.8 mg/g	
	Cattail stems pH 5	11.6 mg/g	
	Cattail roots pH 5	11.5 mg/g	

Biomass related technologies should not necessarily replace existing treatments but complement them in an integrated optimised process. Appropriate choices of biomass and operational conditions should be used to provide a financially viable treatment. As indicated earlier, one biosorbent receiving attention in selective metal removal and recovery studies is *Azolla filiculoides* and of interest to this study is the potential of this water fern to remove gold and platinum from solution.

1.6. Azolla filiculoides

A native of South America, *Azolla* is a genus of floating aquatic ferns with seven existing species, distributed throughout tropical and temperate regions of the world. The sporophyte of *Azolla* is 10-40 mm in diameter, and is a short branched floating stem bearing root which hangs into the water. The stem and branches are covered with small alternate overlapping leaves (Figure 1.2). Each branch includes a stem with bilobed leaves and adventitious roots. Each bilobed leaf has an upper lobe containing chlorophyll while the lower lobe lacks chlorophyll. The abscission of branches or roots allow the fragmentation of these plants and facilitates vegetative propagation.

Figure 1.2: Several water fern plants floating on the water surface. *Azolla filiculoides* (dark brown overlapping leaves) is represented with duckweed (oval green fronds).

The plant also possesses the ability to utilise atmospheric nitrogen due to a symbiosis with the blue-green algae *Anabaena azollae*, which grows in the cavities of the *Azolla* leaflets (Ashton and Walmsley, 1976; Uheda *et al.*, 1999). This diazotrophic cyanobacterium (Figure 1.3) is associated with the apical meristem of the fern, growing in unison with the fern. The fern is heterosporous, developing mega- and micro-sporangia during the sexual phase of its life-span and the alga maintains its association. No free-living growth form of *Anabeana azollae* has yet been discovered. The fern is heavily dependent on the alga with regards to the nitrogen fixing ability, the alga is able to transfer the nitrogenous compounds to the plant (Ashton and Walmsley, 1976; Samal and Kannaiyan, 1994; Vincenzini *et al.*, 1985).

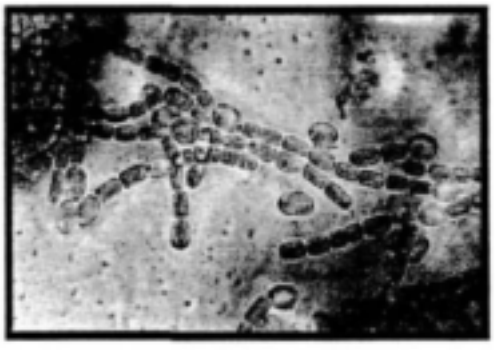


Figure 1.3: Filimentous cyanobacterium *(Anabeana azollae)* from cavities within the leaves of *Azolla filiculoides*. The heterocysts (large oval cells) are responsible for nitrogen fixation ($N_2 \rightarrow NH_3$).

This fern-alga association was shown to be capable of sustaining growth in nitrogen-free media, consequently, the alga allows the fern to colonise water bodies deficient in fixed nitrogen (Asthon and Walmsley, 1976; Samal and Kannaiyan, 1994; Uheda *et al.*, 1999). *Azolla* is of economic importance because of its extensive use in Asia for green manure in rice fields (Uheda *et al.*, 1999)

Since the growth of *Azolla filiculoides* is greatly enhanced by its symbiosis with the blue-green algae *Anabeana azollae*, where water resources are open, the plant forms dense 'mats' which impedes the natural flow of water leading to eutrophication (Figure 1.4).

(A) **(B)**

Figure 1.4: Ponds along the San Dieguito River (San Diego County, California) covered with *Azolla filiculoides*. **(A)**: shows the river covered during the summer months, while **(B)**: shows the river covered with *Azolla filiculoides* during the fall months. The pigment anthocyanin is responsible for the reddish-brown colour.

The proficiency of *Azolla* is such that it is considered a pest, causing interference with the natural aquatic ecosystem, so that indigenous plant and animal life are reduced, and water flow in rivers and canals is restricted. Mechanical, chemical and biological control measures are employed for eradicating the weed, although each has its limitations. Labour intensive mechanical control is only effective in small ponds, while chemical control is considered risky and not totally effective and specialist studies in this field are necessary to reduce possible disastrous effects on the ecosystems. Biological control using insects or pathogens is regarded as a more effective and benign method in controlling weed population. The latter, however, requires stringent tests before the parasite is released to determine its effects in the environment (Anon., 1996). In South Africa, total eradication of *Azolla filiculoides* in some areas is necessary since it limits open waters for recreational and agricultural purposes (Ashton and Walsmley, 1976). Further reasons for eradication of this weed are the reduction in the quality of drinking water, deterioration of aqua biodiversity, clogging of irrigation pumps and reduction of water canals. Recent reports have shown success in the weed being biologically controlled by the frond-feeding weevil (*Stenopelmus rufinasus*) (Hill, 1998).

Interest in aquatic plants as bioindicators arose when analysis of the plant material was able to give an indication of the water environment to which they had been exposed. Various studies have reported uptake of metal ions such as copper, iron, lead and zinc by aquatic plants such *as Azolla pinnata* (water velvet) and *Lemna minor* L. (duckweed) (Jain *et al.*, 1989, 1990; Sarkar and Jana, 1987). Selection of the plant material for metal removal from polluted water will depend on the ease of plant growth and yield of biomass. These conditions are important for evaluation of the plant as a potential biosorbent for metal removal (Jain *et al.*, 1990).

The interest in the use of this plant (*Azolla*) as a biological filter for renovation of waste water has increased. The success of biomass production and the treatment of waste water is provident upon maintaining an adequate year-round plant growth and having high- and low-temperature tolerance, both of which are important factors in the management of macrophytes in various aquaculture applications (Uheda *et al.*, 1999).

Several macrophyte-based treatment systems, such as the water hyacinth *Eichhornia crassipes* (L.), and the sewage treatment systems using duckweed, have reported considerable potential for the removal of pollution from waste water (Jain *et al.*, 1989; Muramoto and Oki, 1983). Similar studies with *Azolla filiculoides* have shown the removal of lead(II), chromium(VI), nickel(II), and copper(II) from aqueous solutions (Sanyahumbi *et al.*, 1998; Zhao and Duncan, 1997a, b, 1998). Mercury was shown to be removed by *Azolla filiculoides* in a concentration and time-dependent manner (Mishra *et al.*, 1987). In other studies, cadmium and uranium were shown to accumulate in the roots (Sela *et al.*, 1988). The main advantage of *Azolla filiculoides* is its high growth rates, and its ability to grow in less than ideal conditions. Periodically, however, the weather conditions are less than ideal, and growth of the plant may be retarded. This could limit its regular supply for large industrial usage. Tel-Or (1995), previously noted that its use in metal adsorption as dry material was more efficient than wet biomass.

In this study, heavy metal uptake by the dried aquatic plant from metal enriched solutions was examined and the performance of the aquatic fern on the removal and recovery of metals, especially gold and platinum, from synthetic solutions as well as mine waste water was evaluated.

1.7. Gold

Man's use of gold predates the period of written history (West, 1975). The word *gold* is anglo-saxon in origin, but its chemical symbol is derived from the Latin word *aurum* (Au) meaning "gold" (Greenwood and Earnshaw, 1989). Gold has been considered a precious metal since ancient times and the search for gold stimulated world exploration and trade. Since ancient times people have recognised and treasured gold for its permanence and beauty. Gold also emerges as an essential industrial metal (electronics) and has a unique status among all commodities as a long term store (West, 1975).

1.7.1. Mining Process

South African gold mining started in the 1870's. Early mining was mainly by placer methods with miners working stream deposits. This process depended on the high density of gold (19.3 $g.cm^{-3}$) compared to sand (2.5 $g.cm^{-3}$). Exploration progressed to underground mining of lode deposits. These are deep narrow veins or reefs which have been difficult to mine because of increased temperature, humidity and rock pressures. Gold is recovered from the ore by cyanidation, amalgamation, flotation, gravity concentration and smelting or a by a combination of these processes. Figure 1.5 represents a summary of more current processes utilised in the mining industry. Gold may be refined by chlorination (Miller process) or electrolysis (Wohlwill process) (West, 1975).

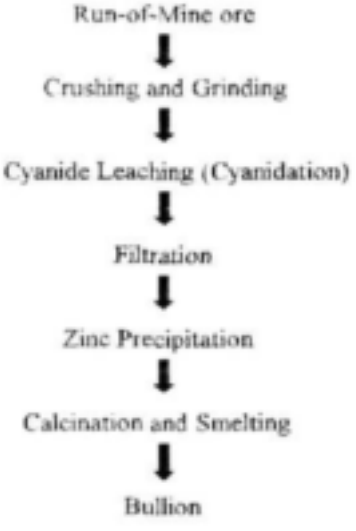


Figure 1.5: Essential steps involved in the cyanide-based extraction process (Woodhouse, 1986).

The method of gold extraction varies from mine to mine and a few variations are described for the recovery process:

(1) *under normal circumstances:* the ore is crushed and ground in order that the ore particles are reduced sufficiently so that gold will be accessible for leaching. Typically the ore is ground to seventy percent less than 200 mesh. Wet-milling is the process of grinding whereby a slurry of finely ground ore in water is produced. The pulp density of forty-five percent w/w solids is then

sent to cyanidation (Woodhouse, 1986). Cyanidation involves adding an alkaline cyanide solution to the pulp in a continuous process through a series of agitated vessels. Oxygen is added to the solution to aid the dissolution process (Woodhouse, 1986);

(2) *in refractory ores:* direct cyanidation is not responsive in that the gold particles are very fine and occluded by various minerals such as sulphides. This is termed refractory because grinding will not remove the gold from the ore and thus is roasted prior to leaching. Since roasting is not always economically viable, pressure oxidation of the milled pulp can be used to oxidise the sulphides and release the gold (Hiskey and Atluri, 1988;Woodhouse, 1986); and

(3) *leaching of gold*: recovering gold from the ore may also involve distributing a weak solution of cyanide over the top of an open mound or levelled heap of ore (heap leaching) and collecting the enriched solutions for gold extraction according to the following process: (this process was developed in 1890 and is still used by many mines due to its simplicity and low cost) (Greenwood and Earnshaw, 1989; West, 1975):

$$4\ Au + 8CN^- + O_2 + 2H_2O \rightarrow 4[Au(CN)_2]^- + 4OH^- \qquad (13)$$

In the presence of a dilute alkaline solution of cyanide, the native gold is rapidly oxidised by dissolved oxygen to form the stable complex $[Au(CN)_2]^-$ ion in aqueous medium (Equation 13). It has since been concluded that most of the gold dissolves according to the following reaction (Equation 14) (Förstner and Wittmann, 1976):

$$2Au + 4CN^- + O_2 + 2H_2O \rightarrow 2[Au(CN)_2]^- + 2OH^- + H_2O_2 \qquad (14)$$

The cyanidation process was pioneered by McArthur and the Forrest brothers, and revolutionised the extractive metallurgy of gold. The tremendous gold output between 1901-1950 correlates to the use of the cyanide process which incorporates two procedures: (1) dilute cyanide concentration which allows the selective dissolution of gold, while chlorination dissolves other impurities, and (2) includes a convenient method for recovering gold from the cyanide solution by precipitation with zinc shavings and the addition of sufficient lead nitrate to enhance the precipitation by forming a zinc-lead couple. Thereafter, the gold slime is added to sulphuric acid

to remove the excess zinc, is refiltered, calcined and finally smelted with borax and silica to produce bullion (Förstner and Wittman, 1976; Hiskey and Atluri, 1988; Woodhouse, 1986). A variation of gold removal utilises the carbon-in-pulp (CIP) method. The aurocyanide solution is adsorbed/recovered from the slurry by activated carbon of a particular size and quality. The carbon collects gold from solution until it contains 300 - 400 ounces of gold per ton of carbon. The gold is then recovered by various processes such as calcination in air or various eluants (alkaline sodium cyanide) or electrowinning. The carbon is reactivated through controlled roasting and made ready for re-use (West, 1975; Woodhouse, 1986).

The main operating problem associated with gold mining is linked to the cyanidation process and the necessity to maintain low tailing effluents and groundwater cyanide levels. The problem, however, dissipates over time due to natural oxidation of the cyanide to a more harmless form. Waste dumps, mill tailings and excavations are increasingly subject to public scrutiny (West, 1975). A considerable effort has been directed towards new and improved reagents for leaching, and in finding new alternative lixiviants that compete with cyanidation. There is a general interest in developing non-toxic environmentally friendly safe substitutes for cyanide. There are a number of reagents that form stable complexes with gold such as thiourea, thiosulphate and halides (Hiskey and Atluri, 1988).

Another process patented by Gold Fields, South Africa, as the BIOX® process, involves using *Thiobacillus ferrooxidans* for refractory ores. These organisms adhere to the ore and slowly oxidise and dissolve away the mineral leaving a porous structure, exposing gold particles, and rendering the material amenable to the cyanidation process. The roasting process prior to cyanidation is now deemed obsolete. The recovery of gold then progresses as per norm. The patent holders have demonstrated that capital and operating costs are much lower than the pressure oxidation and roasting routes. According to Gold Fields, the approach is a technical and economic success.

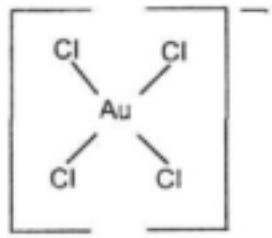
The biggest challenge to the industry arises from the intense exploitation of gold deposits in the past and the near exhaustion of many ores close to the surface, namely the development of better

recovery techniques. Gold-bearing metal scrap is being returned to refiners for recovery (West, 1975). A large number of old abandoned gold mines scattered throughout the world, whose gold extraction processes were primitive compared to today's extraction processes, contain small quantities of gold remaining in their tailing dams and are now similarly being recycled (Woodhouse, 1986). Therefore, the development of a simple yet efficient method for removal of gold from dilute solutions is necessary. This has led to the present biosorption study using *Azolla filiculoides*.

1.7.2. Chemical Properties

In order to understand the mechanism of gold binding or adsorption involved in the biosorption studies it is necessary to understand the chemistry of this noble metal. Gold has an atomic number of 79 and molecular weight of 196.97. It occurs naturally as a single isotope. It has a melting point of 1063°C. Besides being malleable (1 gram being able to be flattened to cover 1 meter square) it is resistant to chemical attack, is ductile, has high electrical and thermal conductivities well as a high reflectivity. All these properties are related to the $d^{10}s^1$ electronic configuration (Greenwood and Earnshaw, 1989; West, 1975). It is also the most electronegative of all metals. The colour of gold is yellow but it may be also obtained in red, blue and violet colloidal forms by the addition of various reducing agents to dilute aqueous solutions of $H[AuCl_4]$. A remarkably stable example is the "Purple of Cassius" (the violet form), which is used as a test for gold(III) as well as a stain for glass and ceramics (Greenwood and Earnshaw, 1989).

The most stable state of gold is the +3 oxidation state which may be obtained by dissolving the metal in *aqua regia* (concentrated hydrochloric acid: nitric acid {3:1}). In general, the dissolution of gold is assisted by the presence of a complexing or coordinating ligand ($Cl^-$of hydrochloric acid) and a strong oxidizing agent such as nitric acid. Gold(III) forms square planar complexes (Greenwood and Earnshaw, 1989):



Gold(III) is able to form mixed complexes of chloride and hydroxide. The first two chlorides are able to be displaced rapidly from the gold(III) chloride complex, while the last chlorides take a longer period. Various mixed species [$AuOHCl_3^-$, $Au(OH)_2Cl_2^-$, and $Au(OH)_3Cl^-$] are able to be formed with $AuCl_4^-$ and $Au(OH)_4^-$. The latter species may be converted to $Au(OH)_3$ (aq), which is unusually stable over a wide range of pH and chloride concentrations. Similarly, gold(III) chloride is converted to $AuCl_3$ at low chloride concentrations (Figure 1.6).

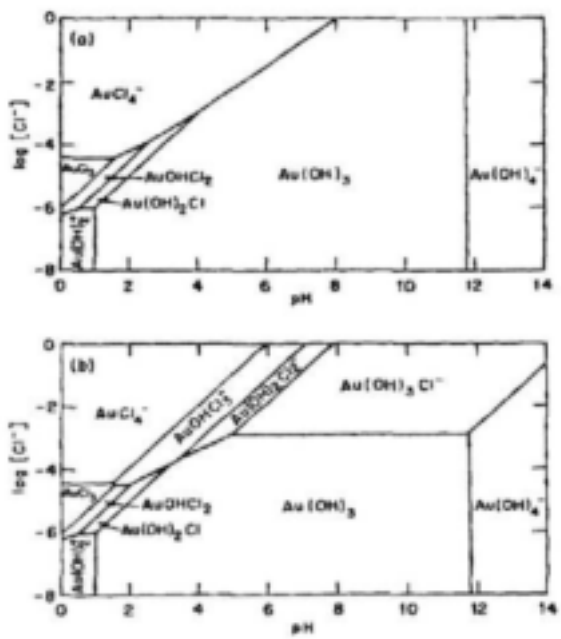
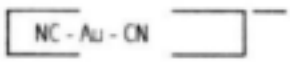


Figure 1.6: Predominance diagram of Au(III)-OH^--Cl^- species. The boundaries indicate conditions under which adjacent species are present in equal concentrations (a) based on calculated equilibrium constants and (b) based on reported equilibrium constants for mixed species (Baes Jr. and Mesmer, 1976).

In the oxidation state +1, gold forms linear two coordinate complexes. These complexes are susceptible to oxidation and disproportionation into Au(III) and Au(0) which renders all its binary

compounds unstable in water except $(AuCN)_2^-$ which is the most stable and consequently most important Au(I) species in the hydrological environment (Greenwood and Earnshaw, 1989).



In terms of Pearson's Hard and Soft Acid Base theory (Table 1.4), gold is classified as a "class-b" or soft acid (Greenwood and Earnshaw, 1989) with Au(I) being softer than Au(III). Such soft metals include metal ions from the right hand side of the transition series and also transition metal complexes with low oxidation states. These form the most stable complexes with ligands such as CO, SCN^- and CN^-. Using the HSAB classification it is possible to predict the relative stabilities of resultant complexes. Soft acids (metals) prefer to react with soft bases, the ligands they prefer are larger and of low positive charge which leads to low electronegativity and high polarisability (Lee, 1991; Pearson, 1968).

Biological studies have shown that an understanding of *in vivo* gold chemistry differs to that of other metals such as copper. The mode of interaction of gold(I) with the thiol groups on some proteins, is highly specific, but other coordination sites may be involved (Brown and Smith, 1980; Coffer *et al.*, 1986; Greene *et al.*, 1986a). Studies have demonstrated that exceptionally stable complexes are formed between gold(I) and L-cysteine and that cysteine will replace the ligands already bound such as cyanide or thiomalate. The interaction of gold(III) with such thiol ligands allows for the possibility of reduction of gold(III) to gold(I) and gold(0). Tetrachloroaurate(III) is thought to react with lysine and histidine side chains of serum albumin and the reaction is thought to involve the initial formation of an ion pair between the negatively charged gold(III) chloride and the positively charged nitrogenous functional groups, followed by the elimination of chloride (Greene *et al.*, 1986a).

1.8. Platinum

Platinum and palladium are rare elements, but they are more abundant than the other platinum group metals (PGM) which include ruthenium, osmium, rhodium and iridium. The PGM metals

occur in sulphidic ores of copper and nickel. Platinum was used for jewellery in the millenium BC in Egypt and also by the Indians from Ecuador and Peru. Today jewellery, accounts for one-third of the platinum market. A large interest is now developing in its use in industry as a three-way catalytic converter in cars. These converters reduce the amount of pollution from the exhaust gases by converting the unleaded exhaust gases of carbon monoxide and oxides of nitrogen into the harmless carbon dioxide and nitrogen (Lee, 1991).

Platinum is also used in the chemical industry (manufacturing of sulphuric acid), the refining of petroleum (reformation of hydrocarbons) and electrical industry (making electrodes). In medical research, platinum has been the main focus in the treatment therapy for cancer utilising Cisplatin and related compounds (Greenwood and Earnshaw, 1989; Lee, 1991; Warshawsky, 1987).

1.8.1. Mining Process

The Bushveld Igneous Complex in South Africa is the largest layered complex in the world. It contains three large suites of intrusive rock of which the Rustenberg suite occupies the western and eastern lobes. The mineralisation is confined to two layers known as the Merensky Reef and UG2 chromatite seam. The Merensky Reef is 200-300 m higher (0.1 to 7 m thick) than the UG2 seam (0.7 to 1.5 m thick) (Cairncross and Dixon, 1999). These two layers contain the largest reserves of platinum in the world. The concentration of PGM's are a thousand times higher than the reserves found in the rest of the world (10 ppm/ton of ore) (Warshawsky, 1987).

The recovery process for platinum group metals (PGM) is closely guarded. The general extraction processes may be summarised as follows: sulphuric acid leaches the base metals out from the anode slimes which contain gold and PGM and leaves gold and PGM "sand". Nitric acid then dissolves the PGM which is further processed to platinum and palladium sponges with rhodium and iridium concentrates. It is important to note that each platinum ore deposit is accompanied by various metals and it is this that differentiates each refining process (Warshawsky, 1987). An alternative process may be found in Figure 1.7.

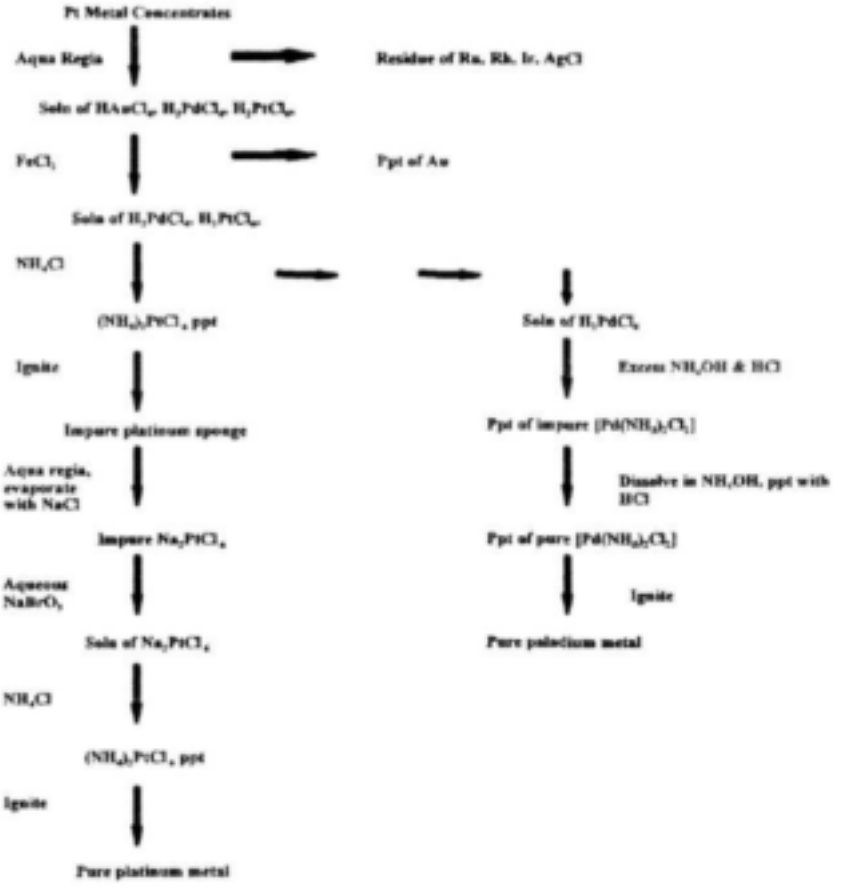


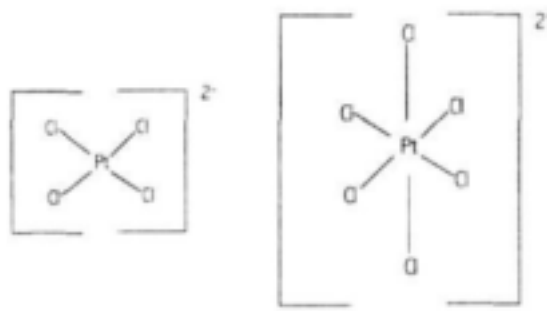
Figure 1.7: Flow-diagram of platinum and palladium extraction (Greenwood and Earnshaw, 1989).

1.8.2. Chemical Properties

Platinum is silvery-white, lustrous, malleable, ductile and thus readily worked. Platinum has a atomic number of 78 and a molecular weight of 195.08. It has a melting point of 1769°C and a boiling point of 4170°C. Platinum is able to form a velvety black powder ($PtO.H_2O$ or PtS) depending on the conditions. Platinum, like gold, dissolves slowly in aqua regia (hydrochloric acid: nitric acid {3:1}) forming chloroplatinic acid $H_2[PtCl_6]$ (Equation 15) (Greenwood and Earnshaw, 1989; Lee, 1991):

$$Pt^0 \xrightarrow{\text{Aqua Regia}} H_2[PtCl_6] \xrightarrow{\text{Heat}} PtCl_4 + 2HCl \qquad (15)$$

Platinum has oxidation states of both +2 and +4 and does not exceed a coordination number of 6. Platinum in the +2 and +4 oxidation state has a strong tendency to form square planar complexes and octahedral complexes respectively and does not form a stable aqua ion (Lee, 1991).



Hydroxy complexes are similarly unstable and are responsible for the acidity of solutions of aqua complexes (Equation 16) (Hartley, 1973). Hydroxy compounds are most stable for the +4 oxidation state.

$$[PtCl_4]^{2-} + H_2O \rightleftharpoons Cl^- + [PtCl_3.H_2O]^- \rightleftharpoons [PtCl_3(OH)]^{2-} + H^+ \qquad (16)$$

In terms of Pearson's Hard and Soft Acid Base theory, the divalent state of platinum shows "class-b" characteristics preferring to bind to CN^- and ligands such as nitrogen or heavy donor atoms rather then oxygen. However, soft platinum(IV) tends to exhibit greater "class-a" character and is very frequently reduced to platinum(II) with soft donors (Greenwood and Earnshaw, 1989; Lee, 1991). The dissolution of platinum occurs with powerful oxidants such as halides, particularly chlorides, and the resulting complexes are stable and highly soluble: $PtCl_6^{2-}$ and $PtCl_4^{2-}$. This is similar to gold complexes in that anionic complexes are formed. Thiourea is a powerful chelating agent used for the removal of platinum, and in the presence of excess thiourea, equilibration leads to the complete substitution of chloro ligands and thus leads to the formation of cationic thiourea complexes of platinum (Warshawsky, 1987).

The medical use of Cisplatin (*cis* isomer of $[Pt(NH_3)_2(Cl)_2]$), as an anti-cancer drug for the treatment of malignant tumours, is highly toxic. The *trans* form has been found to be ineffective. Once injected into the body, the chloride ligands are lost and Pt(II) binds to nitrogen in guanosine (part of the DNA molecule). Cisplatin binds to two different guanosine units and by bridging them the reproduction of the DNA in the tumour cells is disrupted. Tests have shown that the drug is very effective in arresting cancer (Lee, 1991).

The continuous decrease of platinum reserves as well as its importance in the technology field has led to a preliminary investigation to determine the viability of *Azolla filiculoides* in the removal of platinum from synthetic as well as effluent solutions.

1.9. Tannins

Since tannins are able to precipitate metals such as iron, the ability of various tannins to chelate and precipitate gold was explored. Tannins were first described in 1796 as matter in plant tissues which were able to convert hide to leather. The active substances responsible for tanning were found in 1956 to be polyphenolic. Most of the commercially available tannins are obtained from the trees such as wattle, mangrove and quebracho and may be found in the roots, leaves, fruit, bark and wood. Tannins (polyphenols) are characteristic of the chemical defenses of plants and acts as barriers by the astringent taste produced. The tannins are divided into two main broad groups: condensed (proanthocyanidins) and hydrolysable tannins. The condensed tannins have a molecular weight of greater than 20 000 and are composed of polymers which are derived from flavan-3-ols (Figure 1.8). Hydrolysable tannins however have a molecular weight of less than 3000 and are composed of gallic acid or ellagic acid esterified to a sugary moiety (Figure 1.9) (Butler *et al.*, 1984; Ferreira *et al.*, 2000; Haslam, 1989; Haslam and Lilley, 1985; Mole and Waterman, 1987).

Tannins are capable of interacting with proteins to form protein-tannin complexes which tend to be insoluble. This is thought to occur through the phenolic hydroxyl groups which form hydrogen bonds (Haslam, 1989; Kawamoto *et al.*, 1996; Mole and Waterman, 1987).

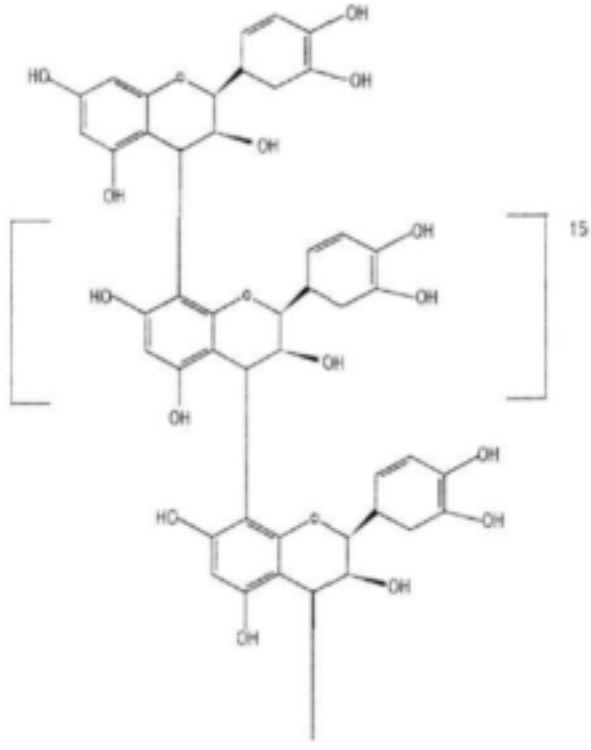


Figure 1.8: Schematic representation of a simple condensed tannin (Sorghum procyanidin).

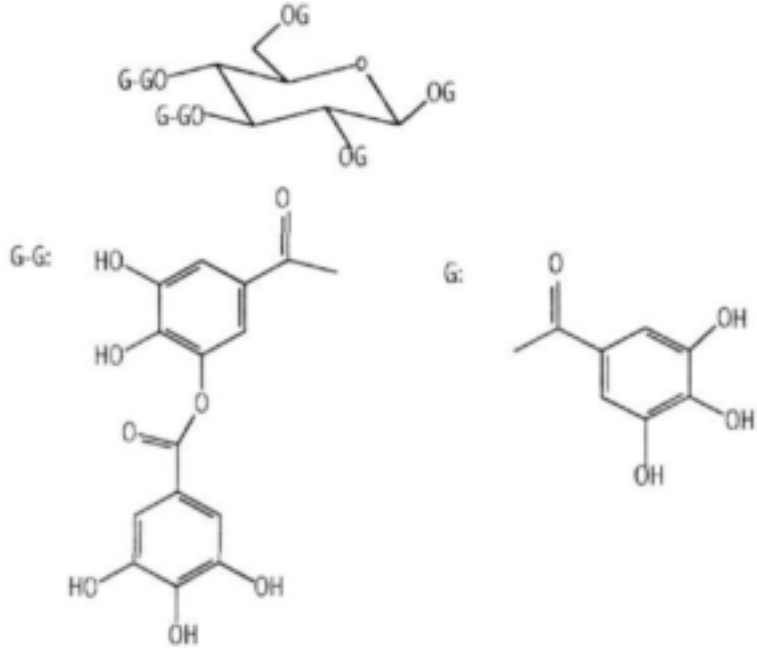


Figure 1.9: Schematic representation of a hydrolysable tannin, gallic acid which is compromised of polygalloyl esters of glucose.

Tannins contain *o*-dihydroxyphenyl chelating functional groups which are able to form stable complexes with metal ions. Tannins are used in industry as micronutrients in foliar sprays as well as hair dye. Leather dyeing involves the interaction of tannins and iron(III) which produces a blue-black colour (McDonald *et al.*, 1996; Randall *et al.*, 1974).

Tannins are also utilised for medicinal purposes in the treatment of illness and disease, e.g., the Bearberry in the northern hemisphere was used for the treatment of bladder and urinary tract infections, while the roots of Agrimony were used as an haemostatic and diarrhetic agent. Tannins have also demonstrated antimicrobial effects for *Aspergillus* and *Fusarium* at a concentration of < 2% w/v (Haslam, 1989; Kawamoto *et al.*, 1996).

Tannins have also been utilised in the remediation of waste water (McDonald *et al.*, 1996; Gosset *et al.*, 1986; Randall *et al.*, 1974). Gosset *et al.* (1986) found that peat was able to remove copper, cadmium, zinc and nickel from solution with a capacity of 200 mM/kg at a pH > 6.7 and an initial concentration of 10 mM. Randall *et al.* (1974) demonstrated that bark from a coastal redwood was able to accumulate metal 10-20% of its dry weight of copper, cadmium, silver, lead, chromium and zinc.

The removal of copper and zinc by low molecular weight phenols at pH 5, was found to be 35% for copper and 27% for zinc. Multiple adjacent hydroxyl groups found in tannins were found to have a high affinity for uranium (Sakaguchi and Nakajima, 1987). Speculation as to the exact mechanism by which this occurs is still ongoing, it may be that the tannins are ion-exchangers and the sites for interaction are the phenolic groups. It has been suggested that a divalent metal ion (M^{2+}) could bind to two adjacent hydroxyl groups releasing two hydrogen ions into solution.

1.10. Lead

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.

1.11. Scope of Investigations

The primary aim of this project was to evaluate the non-viable biomass, *Azolla filiculoides*, as a suitable biosorbent for the removal of gold, platinum and lead from dilute solutions, synthetic as well as waste water solutions.

Preliminary studies on the removal of gold from dilute solutions under varying parameters of pH, temperature, initial gold concentration and biomass concentration in batch reactors was carried out. The effect of the presence of multiple-metals on the binding of gold was also ascertained.

The effect of different flow-rates and initial gold concentrations in a fixed bed column reactor was determined and competition studies with various metals at the established optimal pH of 2 were undertaken. The ability to recover metal bound to the biomass was investigated using a range of eluants to establish a rapid, non-destructive protocol for metal recovery and metal concentration. The re-usability of the biomass for subsequent cycles of adsorption and desorption was established to determine the efficiency of gold removal/recovery over five cycles in a fixed bed column reactor. Modification of *Azolla filiculoides* was investigated in batch studies to attempt to establish which functional groups may be responsible for the binding of gold, and in what manner.

The secondary focus of this project was to evaluate the biosorbent as a candidate for the removal of another precious metal, such as platinum from solution. Preliminary studies included optimisation under batch conditions with varying parameters that involved pH, temperature, initial platinum and biomass concentrations. For column studies, the optimisation studies included a range of flow-rates and initial platinum concentrations.

The third focus involved preliminary studies utilising lead. The effect of parameters such as pH, temperature, biomass and lead concentrations on lead removal from aqueous solution in batch reactors was investigated. Sorption isotherms that were generated were used to determine the maximum lead binding capacity of the *Azolla* biomass. The effect of different flow rates and initial lead concentrations in fixed bed column reactors were also investigated. 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 gold and lead by elution with various eluants. Repeated adsorption- desorption studies were carried out to determine the efficiency of gold and lead removal/recovery over various cycles in fixed bed column reactors.

The effectiveness of the *Azolla* biomass in the remediation of gold and lead from effluent was subsequently investigated.

CHAPTER 2

REMOVAL OF GOLD(III) BY *Azolla filiculoides*: BATCH STUDIES

2.1. INTRODUCTION

A potential critical loss of non-renewable metal resources and the toxicity of metal wastes present a significant opportunity for recovering and recycling metals from waste solutions (Darnall *et al.*, 1986; Kuyucak and Volesky, 1988b). Biomass, such as plant material (Sanyahumbi *et al.*, 1998) and fungi (Fourest and Roux, 1992), have a high potential to bind and concentrate metal ions from aqueous solutions, even when the cells are dead. This phenomenon is termed "biosorption" (Fourest and Roux, 1992; Volesky, 1987). An example of such a biomass is the small aquatic fern, *Azolla filiculoides* which, in South Africa, is regarded as a weed because of its ability to form a dense mat on stream or dam surfaces (Hill, 1998). Biosorbents from natural sources such as *Azolla filiculoides* may possess a sequestering power superior to that of commercially available ion-exchange resins, or the already-in-use activated carbon, and thus enhance the effectiveness and feasibility of the metal recovery process.

Biorecovery of gold has received considerable attention world-wide. Gold in mining effluents is generally in very low concentrations (1 -10 mg/L). In addition, gold behaves very differently from other metals and is not easily removed from solution. Biosorption studies have shown that algae are able to adsorb gold from aqueous solutions under a variety of conditions thereby indicating that biorecovery of gold can be achieved (Greene *et al.*, 1986a). The present study involves the investigation and development of an innovative process for the removal of gold from mining effluent. The advantage of *Azolla filiculoides* as a biomass instead of the more expensive ion-exchange resins currently used is its natural availability and additionally it provides an impetus to harvest the potentially noxious plant from water surfaces.

In this section, batch reactor optimisation studies were performed to determine the optimal biomass and gold concentration, as well as optimal pH and temperature for gold removal by the

non-viable biomass. It is important to note that equilibrium isotherms were not performed with gold(III) due to its high precipitation rate at greater than 40 mg/L gold(III) concentrations. The most prominent metals, other than gold, in the effluent from Mine A (Appendix I) were lead, iron and copper and this prompted an investigation on the competitive binding using synthetic metal solutions.

2.2. Batch Optimisation Studies

2.2.1. Materials and Method

2.2.1.1. Materials

The water fern, *Azolla filiculoides*, was harvested locally from dams around Grahamstown, in the Eastern Cape, South Africa. The plant was thoroughly washed in deionised water (Milli Q, Millipore) and dried at 37°C. The plant material was ground to a constant mesh size 2, and stored in a cool, dry place for subsequent utilisation. All reagents used were of analytical standard and obtained from Saarchem, South Africa. Aqueous gold solutions were prepared from hydrogen tetrachloroaurate(III){$H[AuCl_4]$} and diluted with deionised water until the desired concentration was achieved. All glassware used for experimental purposes was washed in 2.5 M nitric acid and subsequently rinsed with deionised water to remove any possible interferences by other metals. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were used for pH adjustments. Atomic absorption spectrometric standards were prepared from a 1000 mg/L atomic absorption gold solution (Wirsam, South Africa) and diluted with deionised water until the desired concentration was achieved.

2.2.1.2. Method

All experimental work was conducted in duplicate. Biomass (1, 3, 5, 7 and 9 g/L) and gold (2, 4, 6, 8 and 10 mg/L) concentrations were adjusted according to the respective experiment. A volume of 100 mL of a required concentration of gold(III) and biomass was placed in a 300 mL

Erlenmeyer flask and constantly agitated at 200 rpm at room temperature. Aliquots (3 mL) were withdrawn at regular intervals (every five minutes for the first hour, every ten minutes for the second hour and every twenty minutes for the final, third hour) and filtered using cellulose-acetate filters (25 mm diameter, 0.45μM pore size). The filtrate was then analysed for gold using atomic absorption (AA) spectrophotometry (GBC 909AA). The results were expressed as percentage removal of gold(III) from solution. Control experiments used gold(III) solutions in the absence of biomass to exclude the possibility of gold precipitation. In the pH study, the pH was adjusted every half-hour. In the temperature study, the flasks were shaken in a thermostatically-controlled incubator (Labcon, South Africa).

2.2.2. Results and Discussion

Gold removal by the biomass was found to be rapid with the majority being removed within the first 20 minutes. The exception was in the pH studies.

2.2.2.1. Effect of biomass concentration

The percentage removal of gold(III) at 5 mg/L, at biomass concentrations of 1 to 9 g/L (intervals of 2 g/L) demonstrated that the optimum concentration was 5 g/L with 100% removal within 20 minutes (Figure 2.1). For convenience, this concentration of biomass, 5 g/L, was utilised for all further experiments. Gold(III) removal decreases progressively with higher biomass concentrations but significant removal (95%) was still observed at the highest biomass concentration examined.

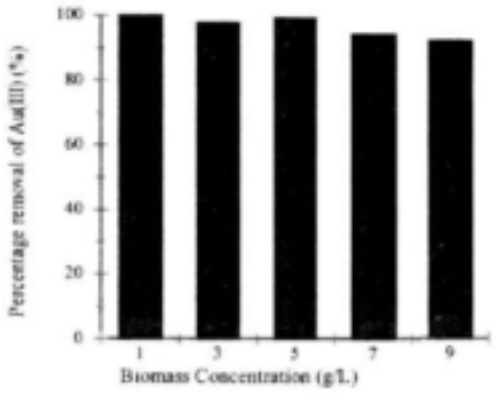


Figure 2.1: The effect of biomass concentrations on the adsorptive capacity of *Azolla filiculoides* with an initial gold concentration of 5 mg/L and pH of 2. The experiment was carried out at room temperature and agitated at a speed of 200 rpm.

2.2.2.2. Effect of initial concentration of hydrogen tetrachloroaurate(III)

Due to the low concentration of gold found in effluents (1-10 mg/L), it was decided to use typical concentrations of 2, 4, 6, 8 and 10 mg/L of gold(III). Results show that a removal of 86%, 95%, 94%, 98% and 99% were achieved respectively (Figure 2.2). A concentration of 8 mg/L rather than 10 mg/L gold(III) was employed in further studies since similar removal kinetics were obtained and because the lower value was more typical of the dilute solutions obtained in waste water.

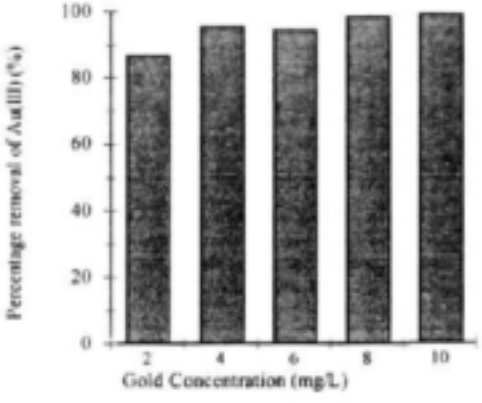


Figure 2.2: The effect of initial gold concentrations on the adsorptive capacity of *Azolla filiculoides* at a biomass concentration of 5 g/L and pH of 2. The experiment was conducted at room temperature and agitated at a speed of 200 rpm.

2.2.2.3. Effect of pH

pH studies showed a substantial pH sensitivity in the binding capacity of gold(III) to the biomass, with optimal removal at pH 2. The adsorption mechanism of gold(III) seems to be ionic rather than covalent hence the dependence of gold binding on pH (Gee and Dudeney, 1988; Greene *et al.*, 1987). The results in Figure 2.3 indicate that the gold(III) complex is in the anionic $[AuCl_4]^-$ form shown by its preference to bind at pH 2 (Cotton and Wilkinson, 1980; Greene *et al.*, 1987). The negatively charged complex may bind to the positively charged functional groups on the surface of the biomass (Greene *et al.*, 1987). pH also affects the protonation of the functional groups on the biomass as well as the metal chemistry. At pH's 3 and 4 incomplete functional group protonation probably results in decreased gold(III) uptake, while at pH 5 and 6 maximum uptake is achieved after 180 minutes suggesting a slow equilibrium between $[AuCl_4]^-$ and gold(III) hydroxy species.

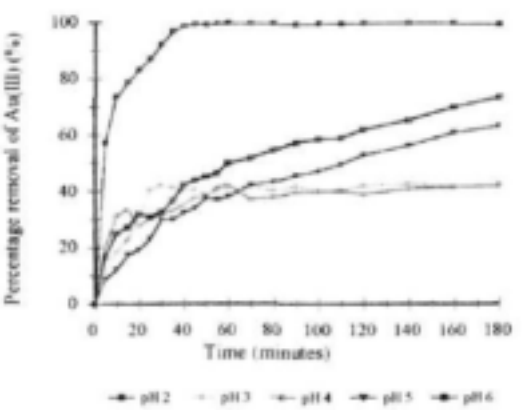


Figure 2.3: The effect of pH on the adsorptive capacity of *Azolla filiculoides* at an initial gold concentration of 8 mg/L and biomass concentration of 5 g/L. The experiment was conducted at room temperature and agitated at 200 rpm.

2.2.2.4. Effect of temperature

Contrary to viable biomass studies (Kuyucak and Volesky, 1988a), variation in temperature with non-viable biomass from 10-50°C, had little effect on the percentage removal of gold at pH 2,

with 100 % removal for all temperatures investigated (Figure 2.4). The process of adsorption may be energy-independent under the experimental conditions investigated.

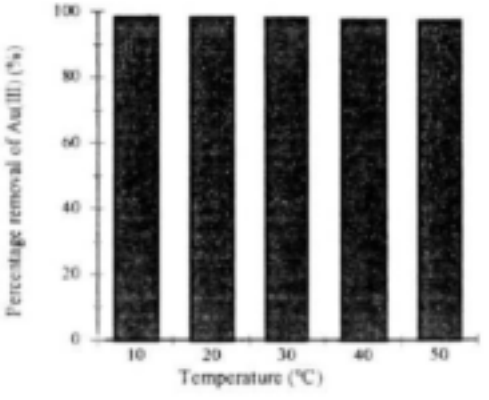


Figure 2.4: Effect of temperature on the adsorptive capacity of *Azolla filiculoides* at an initial gold concentration of 8 mg/L, biomass concentration of 5 g/L and pH 2. The experiment was conducted at room temperature and agitated at 200 rpm.

2.3. The Competitive Effect of Various Metals on the Adsorption of Gold(III)

The aim of the study was to determine if metals present in waste water interfered with the gold(III) adsorptive capacity of the plant material. A pH of 2 was utilised since it was established to be optimal for the metal. Although previous experiments expressed the concentration of the metal in the form of mg/L, for comparison of the various binding characteristics of each metal, concentration in the form of moles/L or M was utilised. To understand the binding characteristics, equimolar concentrations of each metal was employed. Differing molar ratios of metal to metal were utilised to determine whether competitive effects occurred. For this reason concentrations of the metal equivalent to the effluent were used (actual metal concentrations found in gold effluent from Mine A (Appendix I)).

2.3.1. Materials and Methods

2.3.1.1. Materials

Azolla filiculoides and reagents were obtained and prepared as described in Section 2.2.1.1. All reagents used were of analytical standard and obtained from Saarchem, South Africa. Aqueous lead, iron, copper and gold solutions were prepared from lead(II), iron(III) and copper(II) chlorides and hydrogen tetrachloroaurate(III) solution respectively. All metal solutions were diluted with deionised water (Milli Q, Millipore). Atomic absorption spectrometric standards were prepared from 1000 mg/L lead, 1000 mg/L iron, 1000 mg/L copper and 1000 mg/L gold atomic absorption solutions (Wirsam, South Africa) and diluted with deionised water.

2.3.1.2. Method

All experimental work was conducted in duplicate. All metal solutions of varying concentrations prepared were verified using the AA spectrophotometer. Firstly, each metal was investigated individually at equimolar concentrations (50μM), and secondly, at concentrations simulating individual effluent concentrations. The effluent concentrations were as follows: 25 μM for lead,10 μM for iron, 200 μM for copper, and 5 μM for gold. The final section of this experiment involved preparing a single solution of the metals at an equimolar concentration (50μM) and another solution at concentrations simulating the effluent.

A volume of 100 mL at a biomass concentration (5 g/L) and specific concentration of metal at pH 2, was placed in 300 mL Erlenmeyer flasks and constantly agitated at 200 rpm at room temperature. The pH was adjusted to 2 with HCl and NaOH every 30 minutes over a period of three hours. Aliquots (3 mL) were withdrawn and filtered using cellulose-acetate filters (25 mm diameter, 0.45μM pore size). The flasks were agitated in a thermostatically-controlled incubator. The filtrate was then analysed for lead, iron, copper, and gold using AA spectrophotometry (GBC 909AA). Control experiments involved individually studying each metal in the absence of the biomass at equimolar and effluent concentrations and as a single mixed metal solution. The results

were expressed as percentage removal of lead, iron, copper and gold or metal from solution.

2.3.2. Results and Discussion

The initial concentration of metal solutions were kept constant (50μM) to determine the binding mechanism of gold(III) to the biomass and similarly for lead(II), iron(III) and copper(II).

2.3.2.1. Removal of effluent metals and the effect of an equimolar metal concentration on the biosorptive capacity of Azolla filiculoides for gold(III)

Lead(II) exhibited a 50% removal from solution (Figure 2.5) remaining more or less constant for 3 hours. The removal of iron(III) (Figure 2.6) from solution exhibited a maximum of 30-40% removal within 30 minutes. Iron(III) and lead(II) chloride may form cationic ions in solution at this particular pH, thus the binding of these two metals to the biomass may not favoured at pH 2 since the biomass surface is likely to be positively charged (Volesky, 1990). Optimum pH's for lead, copper and iron were found to be 4.5, 5.5 and 3 respectively (data not shown). Removal values of 50% and 30-40% for lead and iron respectively, may either indicate that some negative charges do occur on the biomass surface at this pH, or that the binding of the metals to the biomass may not be simply electrostatic.

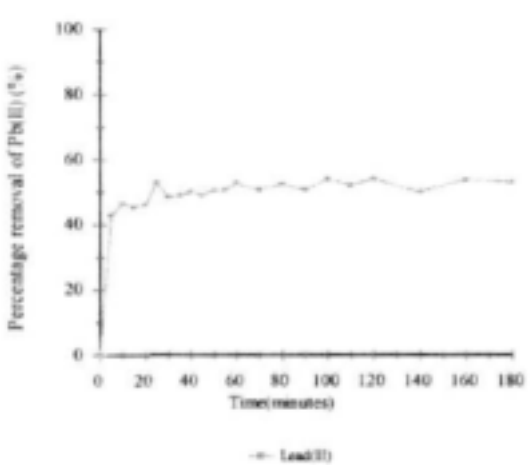


Figure 2.5: Removal of lead(II) (50 μM) from aqueous solution at pH 2. The following parameters were utilised: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was kept constant throughout the experiment.

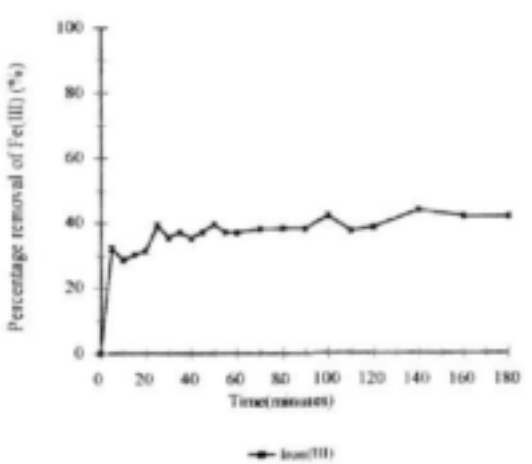


Figure 2.6: Removal of iron(III) (50 μM) from aqueous solution at pH 2. The following parameters were utilised: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was kept constant throughout the experiment.

The biomass performed poorly with copper(II) chloride, showing approximately 10% removal (Figure 2.7), while the binding of the gold(III) was rapid with 100% removal occurring within 40 minutes (Figure 2.8). The chemistry of gold is considerably different from the other metals. The hydrogen tetrachloroaurate(III) complex is anionic, $[AuCl_4]^-$, thus the positively charged biomass, *Azolla filiculoides,* is extremely conducive for the binding of gold(III). The binding of the metal to the biomass may be due to the surface charge of the biomass and/or due to the complex chemistry of the metal, both of which may play a large role in the adsorption process.

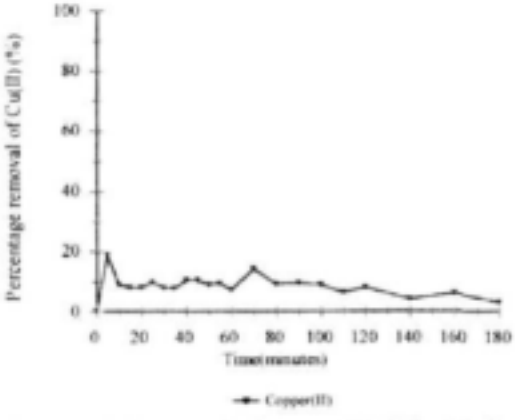


Figure 2.7: Removal of copper(II) (50 μM) from aqueous solution at pH 2. The following parameters were utilised: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was kept constant throughout the experiment.

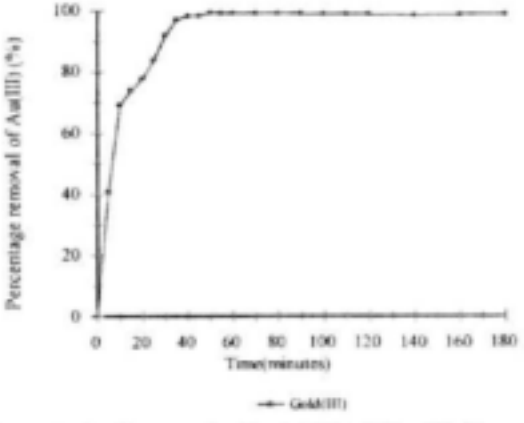


Figure 2.8: Removal of gold(III) (50 μM) from aqueous solution at pH 2. The following parameters were utilised: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was kept constant throughout the experiment.

A further study was performed to ascertain the competitive uptake of the metals lead(II), iron(III), copper(II) and gold(III) on the adsorptive capacity of *Azolla filiculoides*. A summary of the individual metal studies (Figures 2.5-2.8) is represented in Figure 2.9a. It was necessary to present a summary of the individual experiments to compare the uptake values of all four metals in a single solution at the same concentration of 50μM (Figure 2.9b).

Lead(II) displayed a modest decline of about 10% (Figure 2.9b) compared to the individual metal study where maximum removal of between 40 and 50% occurred. Iron(III) showed a dramatic 30% decrease in removal when compared to the individual study, with 5-10% removal occurring eventually decreasing to 0% when the study was concluded. Copper(II) also showed a modest

decline initially with 10% removal at 25 minutes and gradually decreasing over the three hour period to 0%. In Figure 2.9b 100% of the gold(III) in solution was removed. The results indicate that total gold(III) extraction is not affected by the presence of other metals, however, there is a significant effect on the removal rate, which suggests a more complicated mechanism of uptake. Since the metals are all cationic complexes whereas gold(III) is anionic at this pH, lead(II) and copper(II) may compete with iron(III) for similar binding sites on the biomass.

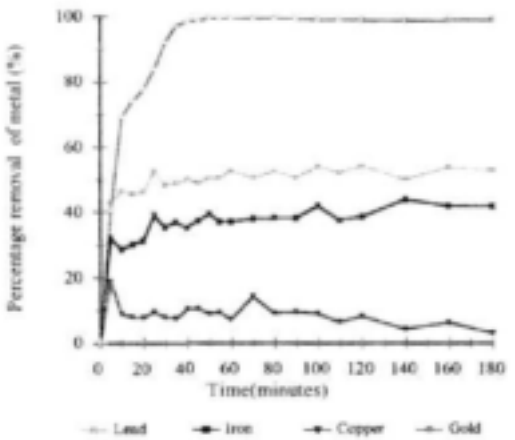


Figure 2.9a: Removal of various metals from aqueous solutions (individual metal studies). Final concentration of all four metals was 50 µM. Parameters included: biomass concentration of 5 g/L, room temperature and agitation speed of 200 rpm. A pH of 2 was maintained throughout the experiment.

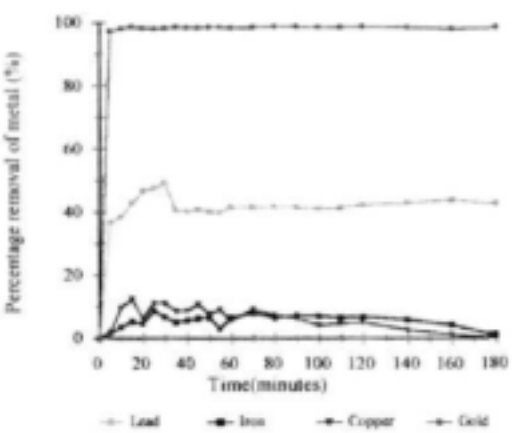


Figure 2.9b: Removal of various metals from an aqueous solution (mixed metal study). Final concentration of all four metals was 50 µM. Parameters included: biomass concentration of 5 g/L, room temperature and agitation speed of 200 rpm. A pH of 2 was maintained throughout the experiment.

2.3.2.2. Removal of effluent metals and the effect of effluent concentrations on the biosorptive capacity of Azolla filiculoides for gold(III)

The main purpose of this study was to establish whether the selected metals at simulated effluent concentrations would influence the binding capacity of the biomass for the uptake of gold and similarly for lead, iron and copper. The effluent concentrations were found to be: 25 µM for lead, 10 µM for iron, 200 µM for copper and 5 µM for gold.

The rapid uptake of a metal is desirable since this allows a short contact time to occur between the solution and biosorbent. Lead(II) at 25 µM exhibited a more rapid response and higher removal rate with 60% removal after 10 minutes (Figure 2.10) than in the individual equimolar

study (Figure 2.5). Extraction of 30% of 10 μM iron(III) from solution occurred within 15 minutes (Figure 2.11). The low removal rate may be explained by the fact that while the biomass contains sites of positive as well as negative charges, a higher proportion of the former is more likely at this pH.

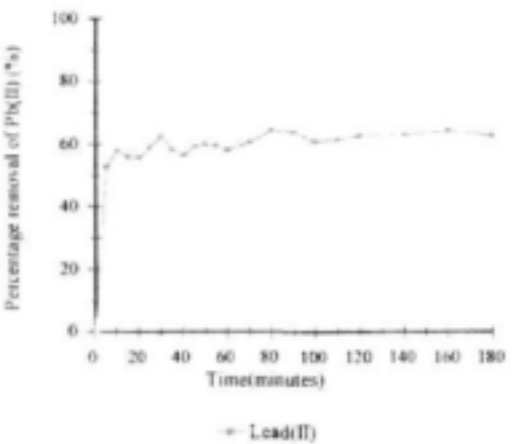


Figure 2.10: Removal of lead (25 μM) from aqueous solution at pH 2. Variables include: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was maintained throughout the experiment.

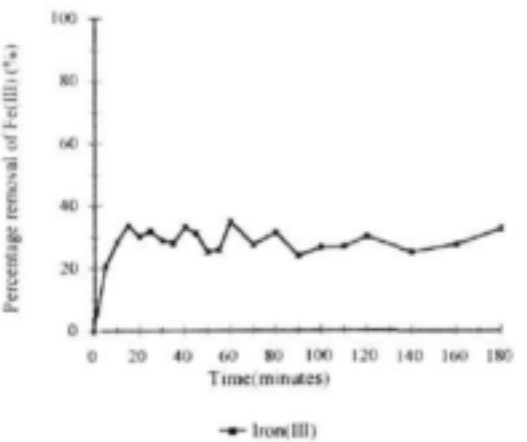


Figure 2.11: Removal of iron (10 μM) from aqueous solution at pH 2. Variables include: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was maintained throughout the experiment.

However, the efficacy of the biomass was poor in the removal of copper(II) at 200 μM showing an initial removal of 20% within the first 5 minutes and gradually decreasing to 0% over the three hour period (Figure 2.12). A removal of 90% of gold(III) (5 μM) from solution is achieved within 20 minutes (Figure 2.13) at its optimum pH of 2. A slight decrease in the percentage binding as compared to Figure 2.8 may be attributed to the low concentration of gold(III) employed in this experiment.

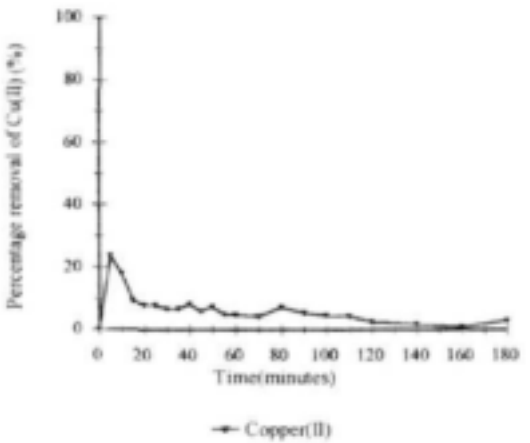


Figure 2.12: Removal of copper (200 µM) from aqueous solution at pH 2. Variables include: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was maintained throughout the experiment.

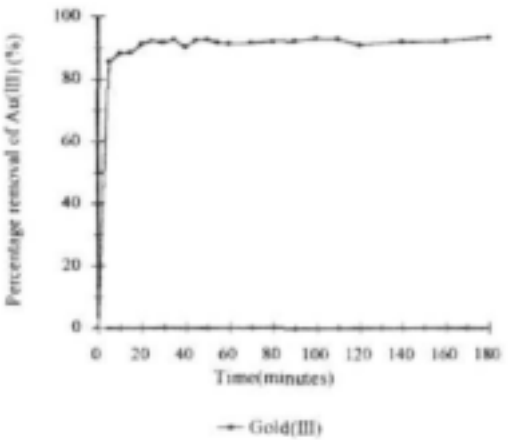


Figure 2.13: Removal of gold (5 µM) from aqueous solution at pH 2. Variables include: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was maintained throughout the experiment.

When comparing the individual metal studies at equimolar concentrations and concentrations simulating the effluent, the percentage removal rate is only slightly altered. A comparison of the binding characteristics of each of the metals individually and a mixed metal solution containing all four metals was carried out to determine whether each metal competed with each other during the adsorption process. Figure 2.14a represents a summary of the individual metal studies (Figures 2.10-2.13).

The synthetic metal solution containing all four metals at effluent concentrations demonstrated that the removal of gold(III) (90%) from the mixed metal solution corresponds to the individual metal study, thus indicating no interference occurred with the binding of the metal. Lead(II) with 50-60% removal showed a slight decrease of about 5%, iron(III) having a maximum removal of 30% in the individual and mixed metal study but the latter eventually decreasing to 15% at the end of the three hour incubation period. Finally the removal of copper(II) from solution showed an initial response of 20% removal in the individual metal study and 15% in the mixed metal study, but both had a similar response in that the removal eventually decreased to 0%. It can therefore be deduced that the metals investigated at effluent concentrations had no appreciable effect on each other and their adsorptive characteristics.

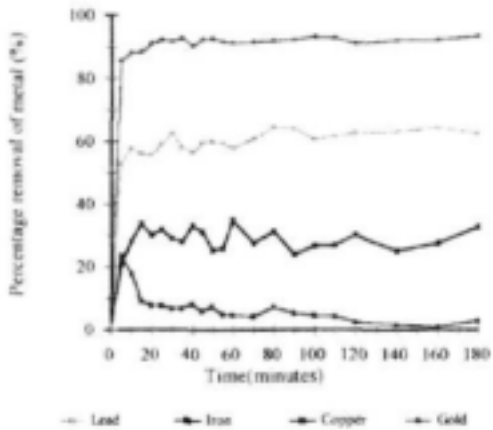


Figure 2.14a: Removal of various metals from aqueous solutions (individual metal studies). Initial effluent concentrations were: lead(II) (25 μM), iron(III) (10 μM), copper(II) (200 μM), and gold(III) (5 μM). The following conditions applied: biomass concentration: 5g/L, room temperature and agitation speed of 200 rpm. A pH of 2 was maintained throughout the experiment.

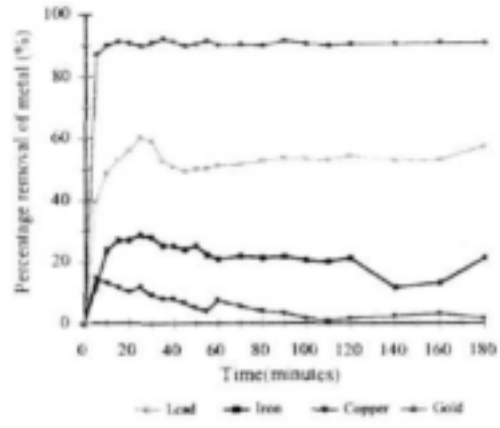


Figure 2.14b: Removal of various metals from an aqueous solution (mixed metal study). Initial effluent concentrations were: lead(II) (25 μM), iron(III) (10 μM), copper(II) (200 μM), and gold(III) (5 μM). The following conditions applied: biomass concentration: 5g/L, room temperature and agitation speed of 200 rpm. A pH of 2 was maintained throughout the experiment.

2.4. Effect of Ligands on the Adsorption of Gold(III)

The effect of a range of ligands is important to understand the binding and reaction mechanism of gold(III) and *Azolla*. This has been subdivided to investigate the interference of halides and hard, borderline and soft bases.

2.4.1. Effect of Halides

The chemical characteristics of gold(III) in the presence of various halides was studied. Gold is able to form complexes with ligands with various degrees of affinity, this in turn may affect the binding of gold(III) to the biomass.

2.4.1.1. Materials and Method

2.4.1.1.1. Materials

Azolla filiculoides and reagents were obtained and prepared according to Section 2.2.1.1. Sodium bromide, sodium chloride and sodium iodide were obtained from Saarchem, South Africa. Atomic absorption standards were prepared from a gold atomic absorption standard solution (1000 mg/L) (Wirsam, South Africa) and diluted with deionised water.

2.4.1.1.2. Method

All experiments were conducted in duplicate. A biomass concentration and gold(III) concentration of 5 g/L and 40 μM (pH 2) were utilised respectively. The pH was adjusted to 2 every 30 minutes. A stock solution (1 M) of sodium bromide, sodium chloride or sodium iodide was prepared and the various concentrations of halides were made up to the following concentrations with deionised water: 10 μM, 100 μM, 1 mM or 10 mM depending on the experiment. The halide, of a specific concentration, was added to a gold(III) (40 μM) solution in a final volume of 100 mL. The solution containing the metal and the halide was added to the biomass in an Erlenmeyer flask (300 mL). The mixture was agitated for a period of three hours at a speed of 200 rpm and adjusted to pH 2 every 30 minutes. A sample (5 mL) was removed at the end of the incubation period and filtered (cellulose-acetate, 25 mm diameter, 0.45 μM pore size). No halides were added to the control experiments. The results were analysed for the gold concentration utilising an AA spectrophotometer. The results were expressed as percentage inhibition of gold(III) uptake from solution.

2.4.1.2. Results and Discussion

As described in Chapter 1, the HSAB theory has been utilised to try and elucidate the binding mechanism of gold(III). Because of gold's "class-b" characteristics, i.e., soft acid, the strength of coordination to halide ligands increases in the order of chloride, bromide and iodide. Gold(III)

is able to form square planar complexes and so the chloride ligand is replaced with bromide, for example, but less so than with iodide (Greene *et al.*, 1986a; Greenwood and Earnshaw, 1989). Figure 2.15 demonstrates that increasing concentrations of the bromide and iodide ligand diminishes the uptake of gold(III) from solution by 13% and 25% at 10 mM respectively, however, chloride has no effect. The effect of inhibition of gold(III) uptake occurs according to the affinity series in the following order: $Cl^- < Br^- < I^-$.

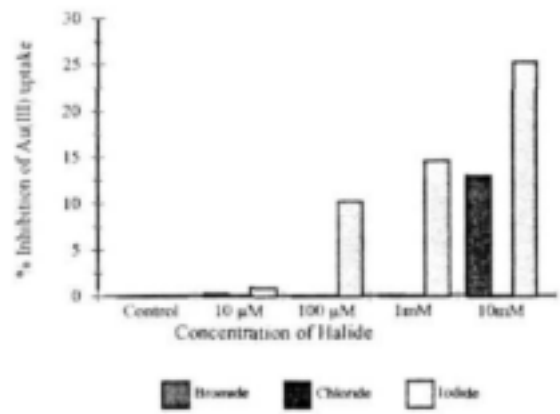


Figure 2.15: The effect of competing halides bromide, chloride and iodide at various concentrations on the removal of gold(III) (40 µM) at pH 2 from solution. The following parameters were utilised: biomass concentration: 5 g/L, agitation speed of 200 rpm and room temperature.

Bromide and iodide have a higher affinity for gold and these less labile ligands may interfere with the secondary coordination of gold to the biomass. This view is supported by the 2 (log formation constant) values in Table 2.1. The halide sensitivity indicates that the binding mechanism of gold(III) to the biomass is complex, possibly involving an initial ionic interaction between the biomass and the anionic species $[AuCl_4]^-$, followed by some degree of breaking of the gold-halide coordinated bond on solvolysis, coordination to the biomass or oxidation of Au(III) to Au(I).

Table 2.1. Formation constants for gold(I) and gold(III) complexes (Greene *et al.*, 1986a).

Species (AuL_n: n = 2 or 4)	*Log Formation Constant*
$AuCl_2^-$	$k_1 = 12.15,\ k_2 = 7.79,\ 2_2 = 19.94$
$AuBr_2^-$	$k_1 = 11.98,\ k_2 = 8.41,\ 2_2 = 20.39$
AuI_2^-	$k_1 = 17.1,\ k_2 = 6.7,\ 2_2 = 23.8$
$Au(CN)_2^-$	$2_2 = 33.7$
$Au(NH_3)_2^+$	$k_1 = 10.14,\ \ k_2 = 8.0,\ 2_2 = 18.14$
$Au(SCN_2H_4)_2^+$	$2_2 = 21.3$
$AuCl_4^-$	$k_1 = 9.26,\ k_2 = 8.31,\ k_3 = 7.31,\ k_4 = 6.16,\ 2_4 = 26$
$AuBr_4^-$	$2_4 = 32$
$Au(CN)_4^-$	$2_4 = 56$
$Au(NH_3)_4^{3+}$	$2_4 = 30$

k = stepwise formation constant, 2 = repesents the overall formation constant ($2_n = k_1k_2k_3....k_n$)

2.4.2. Effect of Bases

In aqueous solutions, such as waste water, the presence of anions may affect the binding of gold(III) to the biomass. Various anions (bases) with soft, borderline and hard (HSAB) characteristics were investigated to determine whether particular anions affected the removal of gold(III) from solution.

2.4.2.1. Materials and Method

2.4.2.1.1. Materials

Azolla filiculoides and reagents were obtained and prepared according to Section 2.2.1.1. Mercaptoethanol, sodium sulphate and sodium sulphite were obtained from Saarchem, South Africa. Atomic absorption standards were prepared from gold atomic absorption standards (1000 mg/L) (Wirsam, South Africa) and diluted with deionised water.

2.4.2.1.2. Method

All experiments were conducted in duplicate. A biomass concentration (5 g/L) and gold(III) concentration (40 μM, pH 2) was utilised and the pH adjusted to 2 every 30 minutes. A stock solution of 1 M of the anions (SO_4^{2-} and SO_3^{2-}) were made from their sodium salts and diluted with deionised water (Milli Q, Millipore) to produce a final concentration of 10 μM, 100 μM, 1 mM and 10 mM. Mercaptoethanol was diluted with deionised water until the desired concentration was achieved. Appropriate volumes of gold(III), Na_2SO_4, Na_2SO_3 and mercaptoethanol were made up to produce a final volume of 100 mL, this was added to the biomass in an Erlenmeyer flask (300 mL) and agitated at 200 rpm for 3 hours. A sample (5 mL) was removed at the end of a three hour incubation period, filtered (cellulose-acetate, 25 mm diameter, 0.45 μM pore size) and analysed for gold utilising an AA spectrophotometer. No bases were added to the control experiments. The results were expressed as percentage inhibition of gold(III) uptake from solution.

2.4.2.2. Results and Discussion

An attempt to relate the HSAB characteristics of the anion and the uptake of gold(III) was undertaken. Pearson (1968) proposed that the differential behaviour of certain groups of bases (ligands) were attributed to their differing polarisability. The HSAB theory is able to predict that the bonds formed between hard acids (class-a) and hard ligands is ionic (pH-dependent), while the bond formed between soft acids and soft bases is more covalent in nature (pH-independent) (Avery and Tobin, 1993; Brady and Tobin, 1995; Greenwood and Earnshaw, 1989). Soft acids (class-b) exhibit the following preferential sequence for metal-binding donor atoms: S > N > O (Brady and Tobin, 1995).

The results in Figure 2.16 demonstrate that mercaptoethanol, as a soft base, had no effect on the removal of gold(III) from solution. It is likely that the low pH (2) hampers the electron donor ability of the mercaptoethanol. Darnall *et al.* (1986) support these results when it was determined that pH 5 was optimal for gold-mercaptoethanol interaction.

Sulphate results in 33% inhibition of gold(III) removal at 10 μM, gradually increasing to 60% at 10 mM. Since sulphate is known to be a hard base (Table 1.4) and thus has no tendency to bind to gold(III), the results obtained suggest that the long residence time (3 hours) of the base in contact with the biomass increased the likelihood that sulphate occupied potential binding sites on the biomass for gold adsorption.

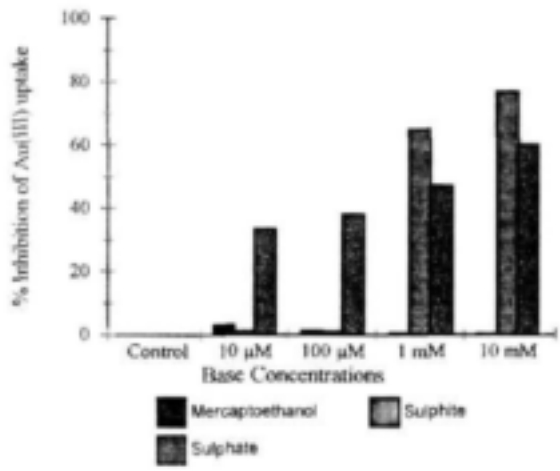


Figure 2.16: Effect of the bases mercaptoethanol, SO_4^{2-} and SO_3^{2-} at various concentrations on the uptake of gold(III) (40 μM) at pH 2 from solution. The following parameters were utilised: biomass concentration: 5g/L, room temperature, agitation speed of 200 rpm.

Figure 2.16 also shows that the percentage of inhibition of gold uptake with sulphite increased from 1.14% at 10 μM to 76.9% at 10 mM. Sulphite is a borderline base and thus has a tendency to bind either to soft or hard acids depending on the environment. The base may complex with $[AuCl_4]^-$ and the resultant complex may not be as conducive for binding to the biomass at pH 2 as $[AuCl_4]^-$. Another factor which may have influenced gold binding was sulphite binding to potential sites on the biomass.

2.5. SUMMARY

Waste water from the mining industry is extremely complex containing various compounds which are able to influence metal adsorption. It is necessary to take all of these factors into account before *Azolla* can be considered a viable treatment method. It is necessary to establish the binding characteristics of the metal to determine its potential interaction with the biomass under various conditions.

From the batch studies performed with hydrogen tetrachloroaurate(III) the following optimal conditions were found: a biomass concentration of 5 g/L and an initial gold(III) concentration of 8 -10 mg/L. It can also be deduced that the gold(III) is in the anionic form, $[AuCl_4]^-$ as shown by its preference to bind to the biomass at pH 2, and that the process of adsorption is temperature independent.

Individual metal studies at an equimolar concentration (50 μM), and at a constant pH of 2, showed the following removal: lead(II) 50%, iron(III) 30%, copper(II) 10%, and gold(III) 100%. To determine whether competition between the metals of interest occurred, a mixed metal solution containing all four metals was investigated. No interference occurred in the removal of gold(III), while lead(II) and copper(II) removal was slightly lowered and iron(III) revealed a significant decrease of between 30% and 40% compared to the individual studies. In the synthetic effluent studies in which all the metals were adjusted to final concentrations similar to that of the effluent, the individual metal studies indicated that the percentage removal of lead(II) was 60%, iron(III) 30%, copper(II) initially 20% gradually decreasing to 0% and gold(III) 90%. Comparing the individual to the mixed metal results suggests no significant competition between metals. Gold(III) binding was not affected by the presence of copper(II) even though a 40-fold excess existed.

HSAB theory dictates that gold(III) should bind strongly to soft bases. Two series of hard, borderline and soft bases were investigated: Cl^-, Br^-, I^- and SO_4^{2-}, SO_3^{2-} and $S_2O_3^{2-}$ (thiosulphate), respectively. *Soft bases*: iodide inhibited gold(III) uptake by 25% at 10 mM and thiosulphate

precipitated the gold(III) on preparation and thus could not be studied (results not shown). Mercaptoethanol was used as a replacement but showed no inhibition of gold(III) adsorption, most likely due to the unfavourable pH. *Borderline bases:* bromide inhibited gold(III) uptake by 13% and sulphite 77% at 10 mM. *Hard bases*: chloride showed no gold(III) inhibition at all concentrations studied but sulphate reduced gold(III) uptake by 60% at 10 mM. The possibility that anions bind to cations, potentially inhibiting gold(III) binding sites on the *Azolla filiculoides* and hinder uptake in this manner cannot be excluded, although chloride showed no interference. The results cannot be applied directly to the HSAB theory and thus are not conclusive. The metal utilised, pH and ligand are specific to a particular system and thus must be studied individually.

The results indicate that the biomass under the optimal conditions achieves 100% gold(III) removal from solution, strongly supporting the possible application of *Azolla filiculoides* biomass in the bioremediation of gold. The adsorption profiles of lead, iron, copper and gold are significantly distinct and this contrast in binding characteristics enhances the feasibility for selective removal and recovery of each metal.

CHAPTER 3

REMOVAL OF GOLD(III)BY *Azolla filiculoides*: COLUMN STUDIES

3.1. INTRODUCTION

Due to the lowering of world-wide reserves of precious primary metal-bearing ore, the recovery in particular of gold and platinum from waste water has received widespread attention. Currently, expensive methods for the recovery of these metals are being utilised such as ion-exchange and activated carbon. The need for the development of economically viable and efficient recovery processes for the removal of metals from waste solutions prompted an investigation into the recovery of gold using biological materials, such as plant material (Edyvean *et al.*, 1997; Kuyucak and Volesky, 1988a, b; Volesky, 1990).

Materials of biological origin demonstrate various degrees of affinity for different metals and species, and it is this selectivity which allows for the development of biosorbents for the recovery of specific metal(s) (Kuyucak and Volesky, 1988a; Volesky, 1990). The plant of interest in this study was *Azolla filiculoides*. Batch studies (Chapter 2) have shown that gold(III) is rapidly accumulated by the non-viable plant material at low pH (100% removal within 20 minutes at dilute concentrations of 2-10 mg/L). Batch studies are usually limited to a low volume treatment, thus it was necessary to investigate a column system for the removal and recovery of gold(III) and various other metals on a larger scale. The utilisation of *Azolla filiculoides* satisfied the following requirements: (a) low cost and re-usability, (b) the necessary particle size, shape and mechanical strength to endure continuous-flow conditions, and (c) rapid metal uptake (Banks, 1997; Garnham, 1997; Volesky, 1990; this study).

There are various types of reactors which can be used for the removal of metal ions from solution: *conventional stirred tank reactors* (CSTR), in which biosorbents are kept in a homogenous suspension under well-mixed conditions, are operated in batch or continuous-flow mode and are

mainly used for activated-sludge systems; and *packed-bed reactors* (PBR) in which the bed is in contact with the aqueous phase either in an up-flow or down-flow mode. The sorption kinetics of the metals onto the non-viable biomass is especially suited to this particular reactor (Banks, 1997; Volesky, 1990).

A continuous-flow system of the packed-bed reactor type was investigated for the treatment of metal-containing solutions in this study (Figure 3.1).

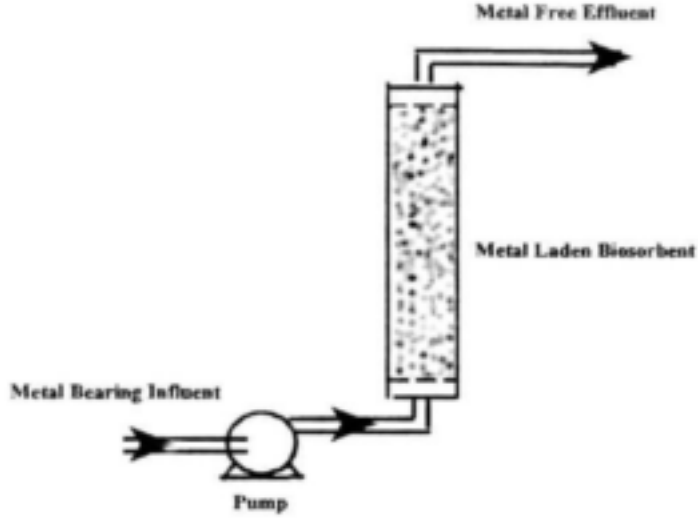


Figure 3.1: Schematic representation of a typical packed bed reactor with an up-flow mode commonly utilised in biosorption studies.

The initial part of this chapter investigated the effects of flow rate and initial gold(III) concentration on the removal of gold(III) from solution. Once the optimal conditions were established the removal of various metal-containing solutions was examined to determine whether lead(II), iron(III), copper(II) and gold(III) influenced the binding characteristics of *Azolla filiculoides*.

3.2. Optimisation Studies

3.2.1. Materials and Method

3.2.1.1. Materials

Azolla filiculoides and reagents were prepared and obtained as described in Section 2.2.1.1. The plant material, however, was not ground but rather left in its natural state. Aqueous gold(III) solutions were prepared from hydrogen tetrachloroaurate(III) {$H[AuCl_4]$} which was obtained from Saarchem, South Africa. Atomic absorption standards were prepared from gold atomic absorption solutions (1000 mg/L) (Wirsam, South Africa) and diluted with deionised water.

3.2.1.2. Method

All experimental work was conducted in duplicate. Gold(III) solutions (5 - 80 mg/L) (1 litre) were pumped through a packed column in an up-flow mode containing 5 g of whole *Azolla filiculoides* in a bed volume of 49 mL (utilising the following formula: $V_T = \pi r^2 h$, r = 1.55 cm , h = 10 cm). The solution was pumped at a desired flow-rate depending on the study (5 to 20 mL/min). Samples were then collected at regular intervals (every 20 mL for the first 100 mL, thereafter every 100 mL) using a fraction collector (Pharmacia, South Africa) and analysed for gold using an AA spectrophotometer. The results were expressed as percentage removal of gold(III) from solution (final concentration relative to initial gold concentration). Changes in pH were recorded for each fraction.

3.2.2. Results and Discussion

3.2.2.1. Effect of flow-rate on the adsorption of gold(III) by Azolla filiculoides

The use of whole *Azolla filiculoides* rather than ground-up biomass was a more realistic approach in column studies since the natural form of *Azolla* has better physical characteristics which may

allow for re-usability, efficiency and is better suited to a continuous-flow mode in column studies.

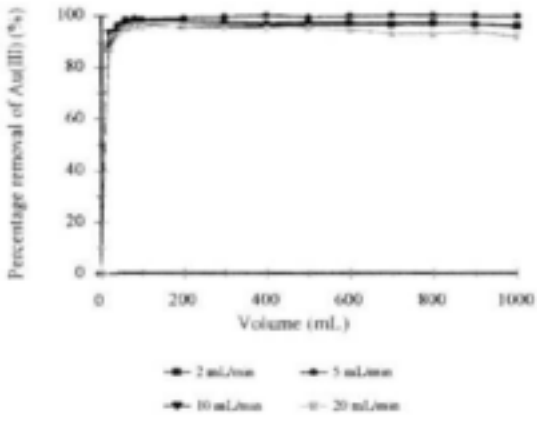


Figure 3.2a: The effect of various flow-rates on the adsorption of gold(III) from solution. Parameters utilised were as follows: pH 2, biomass concentration: 5 g/L, initial gold(III) concentration: 5 mg/L, bed-volume: 49 mL and room temperature.

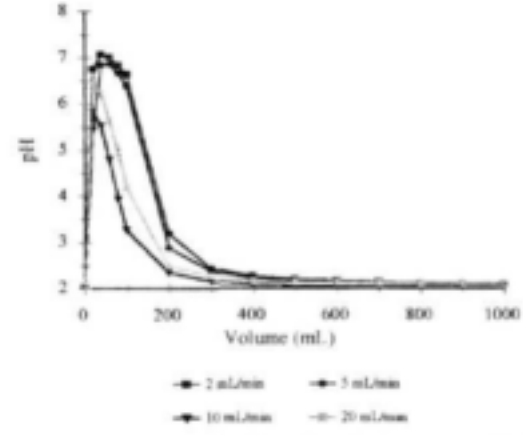


Figure 3.2b: pH profile of a 5 mg/L gold(III) solution at various flow-rates. Parameters utilised were as follows: pH 2, biomass concentration: 5 g/L, gold(III) concentration: 5 mg/L, bed-volume: 49 mL and room temperature.

Figure 3.2a shows that increasing the flow-rate from 2 mL/min to 20 mL/min had no marked effect since 96% and 92% removal from solution occurred respectively. The column retention times varied from approximately 24.5 to 2.5 minutes at 2 mL and 20 mL respectively. For all subsequent studies a flow-rate of 5 mL/min was utilised since a removal rate of 99% was achieved. Maximum removal of gold(III) occurred within 80 mL at all flow-rates thus indicating that the biomass has a high affinity for the metal. Other studies have shown that gold(III) is able to be adsorbed by algal biomass such as *Saragassum natans* (Kuyucak and Volesky, 1988a), *Spirulina platensis* and *Chlorella vulgaris* (Darnall *et al.*, 1986; Gee and Dudeney, 1988; Greene *et al.*, 1987; Hosea *et al.*, 1986) with high efficiency and thus supports the results obtained in this study. It is necessary to take into consideration that the adsorptive characteristics do differ according to the biomass used. It is interesting to note that while the pH initially increases to pH 7 and rapidly decreases to the influent pH of 2 (Figure 3.2b), the adsorption of gold(III) is not significantly influenced. The apparent pH-independence of gold(III) removal might suggest that the binding is covalent. However, gold is partially or completely hydrolysed in the alkaline region yielding species from $[AuCl_3OH]^-$, $[AuCl_2(OH)_2]^-$, $[AuCl(OH)_3]^-$ to $[Au(OH)_4]^-$ (Figure 1.5) (Karamushka *et al.*, 1995; Kuyucak and Volesky, 1988a). Gold(III) present in the medium thus

remains in the form of an anionic complex, even though the ligand changes when the pH of the solution varies.

3.2.2.2. Effect of initial gold(III) concentration on the adsorption of gold(III) by Azolla filiculoides

The batch studies (Chapter 2) have shown that the optimal pH for gold(III) binding is 2. For this reason it was decided to utilise an initial pH of 2. Studies have also shown that maximum adsorption of *Azolla filiculoides* for hydrogen tetrachloroaurate(III) is 120 mg Au/g biomass (data not shown).

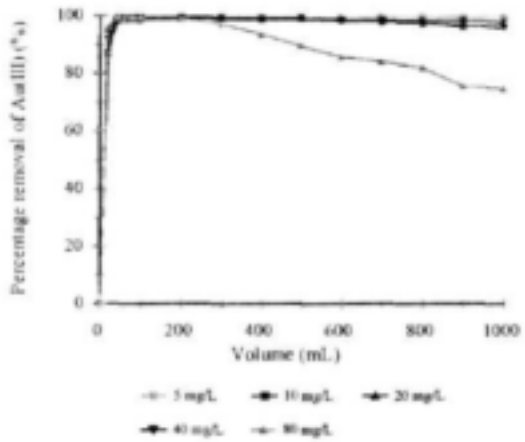


Figure 3.3a: The effect of various initial gold(III) concentrations on the adsorption of gold(III) from solution. The parameters were as follows: initial pH of 2, flow-rate at 5 mL/min, biomass concentration: 5 g/L, room temperature and bed volume: 49 mL.

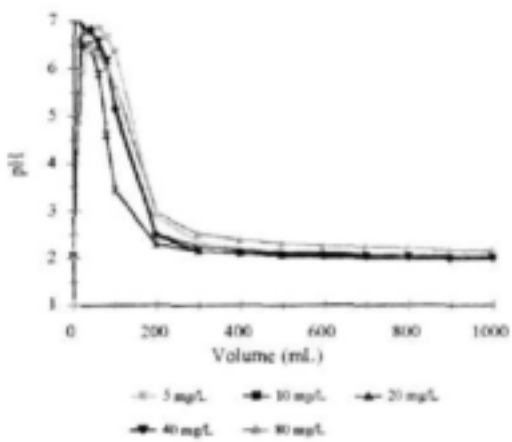


Figure 3.3b: pH profile of various gold(III) concentrations. The parameters were as follows: initial pH of 2, flow-rate at 5 mL/min, biomass concentration: 5 g/L, room temperature and bed volume: 49 mL.

Maximum removal of all initial gold(III) concentrations occurred at 60 mL (Figure 3.3a). At an initial gold(III) concentration of 80 mg/L a gradual reduction in metal removal from solution occurred which was due to precipitation in the form of the purple coloured colloidal gold (Purple of Cassius) rather than the saturation of the biomass. The formation of the precipitate at the highest concentration, 80 mg/L, suggests that at lower gold(III) concentrations an equilibrium between soluble and insoluble species may exist. At higher concentrations of gold(III) the equilibrium may shift so that precipitation may be favoured. Gold(III) removal was again not influenced by changes in pH (Figure 3.3b).

The tannin, anthocyanin (Figure 3.4), present on the leaves of *Azolla filiculoides* (Teixeira *et al.*, 1994) during summer and autumn may be responsible for gold precipitation. Laboratory studies have shown that tannins react with various metals and are capable of forming insoluble metal complexes with a concomitant reduction of the metal, such an example is a condensed tannin with ferric chloride ($FeCl_3$) (McDonald *et al.*, 1996; Okuda *et al.*, 1982). Most tannins contain *o*-dihydroxyphenyl chelating functional groups and form stable complexes with many metal ions which are able to precipitate out of solution (McDonald *et al.*, 1996) (Figure 3.4).

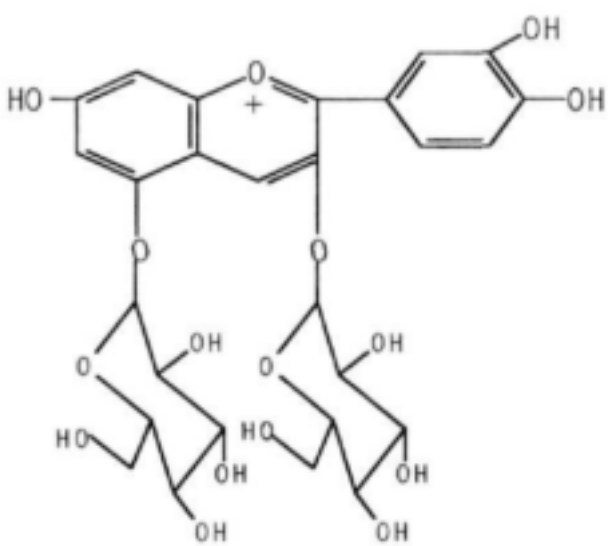


Figure 3.4: Schematic representation of the constituents of a condensed tannin, anthocyanin (Bickley, 1999).

It is thus suspected that the binding and the precipitation of the gold(III) complex occurs through the hydroxy (OH) groups of condensed tannins (Figure 3.5).

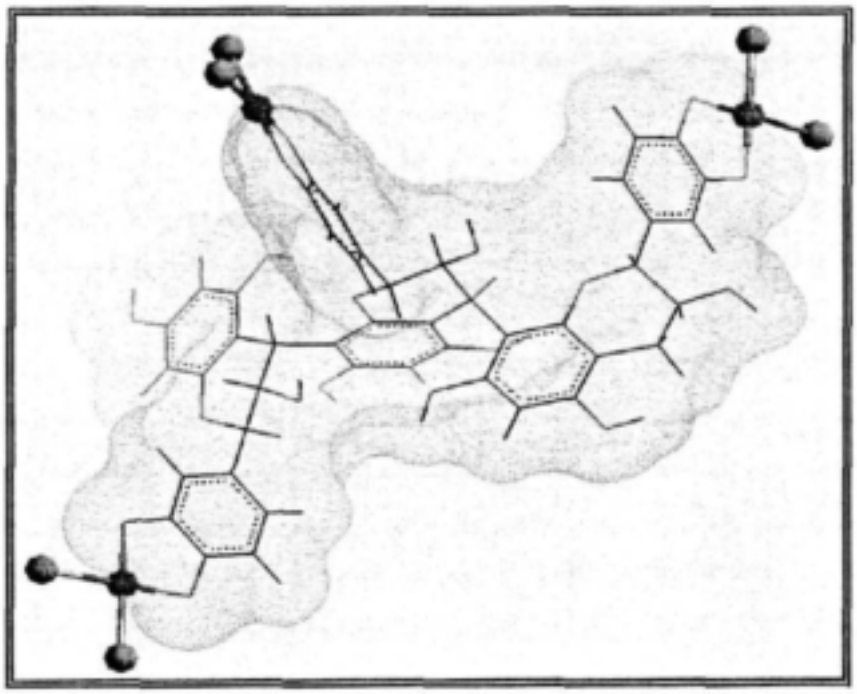


Figure 3.5: Minimised-energy conformation modelling of hydrogen tetrachloroaurate(III) and a typical condensed tannin. The Cerius II™ modelling programme with molecular mechanics (MM) and universal force field (UFF) was utilised.

This provides a plausible explanation for the reaction observed between the condensed tannin(s) and hydrogen tetrachloroaurate{$H[AuCl_4]$}which forms an insoluble purple metal complex (Purple of Cassius). The distinct colour of the gold-tannin complex is indicative of colloidal gold (the specific colour is dependent on the reducing agent used) (Greenwood and Earnshaw, 1989).

3.3. The Competitive Effect of Various Metals on the Adsorption of gold(III)

The aim of these experiments was to determine if the presence of other metals in solution, at equimolar and simulated effluent concentrations, interfered with the adsorptive capacity of the plant material for gold(III) and similarly for, lead(II), iron(III) and copper(II) at pH 2 (optimal for gold(III) adsorption).

3.3.1. Materials and Method

3.3.1.1. Materials

The biomass *Azolla filiculoides* and reagents were obtained and prepared as described as in Section 3.2.1.1. All reagents used were of analytical grade and obtained from Saarchem, South Africa. Lead, iron, copper and gold solutions were prepared from lead(II) chloride, iron(III) chloride, copper(II) chloride and hydrogen tetrachloroaurate(III) respectively, and diluted with deionised water until the final desired concentration was achieved. Atomic absorption spectrometric standards were prepared from 1000 mg/L lead, 1000 mg/L iron, 1000 mg/L copper, and 1000 mg/L gold atomic absorption solutions (Wirsam, South Africa) and diluted with deionised water.

3.3.1.2. Method

All experimental work was conducted in duplicate. All metal solutions of varying concentrations prepared were verified using the AA spectrophotometer. The metal solutions were prepared and investigated individually under two separate experimental conditions. Firstly, all metals were investigated individually at an equimolar (50μM) concentration, and secondly individually, at concentrations simulating effluent concentrations. Effluent concentrations were as follows: 25 μM for lead,10 μM iron, 200 μM for copper and 5 μM for gold. The final section of this study involved preparing a single solution of the metals at an equimolar concentration and and another solution at concentrations simulating the effluent.

All the solutions were prepared at an initial pH of 2. A solution (1 litre) was pumped through a packed column in an up-flow mode containing 5 g of whole *Azolla filiculoides* in a bed volume of 49 mL. Samples were then collected at regular intervals (every 20 mL for the first 100 mL, thereafter every 100 mL) using a fraction collector (Pharmacia, South Africa) and analysed for lead, iron, copper, gold using an AA spectrophotometer. The results were expressed as percentage removal of metal from solution (final concentration relative to the initial metal concentration). Changes in pH were recorded for each fraction.

3.3.2. Results and Discussion

The primary aim of this study was to determine the adsorption characteristics of the four primary effluent metals individually and as a mixed-mixed solution at an equimolar and simulated effluent concentrations.

3.3.2.1. Removal of effluent metals and the effect of an equimolar metal concentration on the biosorptive capacity of Azolla filiculoides for gold(III).

In his Hard and Soft Acid and Base theory, Pearson (1968), described the preferential binding of cations to ligands. In short, soft acids (cation) preferentially bind to the soft bases (ligand), such examples would be gold(III) and a sulphur or nitrogen ligand, respectively. Likewise, a hard acid (cation) preferentially binds to a hard base (ligand), for example copper and oxygen or chlorine respectively. The binding characteristics of soft acids to soft bases tends to be covalent and pH-independent whereas the binding characteristics of hard acids to hard bases tends to be ionic or electrostatic and pH-dependent (Greene *et al.*, 1987).

Figure 3.6 demonstrates the borderline coordination behaviour of lead(II). As the pH increases rapidly within the first 20 mL fraction from pH 2 to 6 and decreases again to the influent pH, the binding to *Azolla* is reflected by the percentage removal of lead(II) from solution and is minimally affected over the wide pH range investigated between the 0-200 mL fraction. Thereafter, lead(II) adsorption is affected by the presence of hydrogen ions in the surrounding medium. The latter trend demonstrates the initial soft acid nature of the metal suggesting that its initial binding may be covalent, however the binding is then transferred to pH-dependent (ionic) binding once the pH returns to the influent pH of 2.

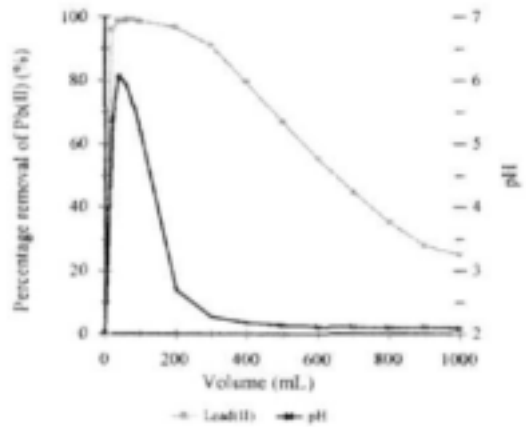


Figure 3.6: Removal of lead(II) (50 μM) from aqueous solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate of 5 mL/min and bed-volume: 49 mL.

The removal of iron(III) increased with an increase in pH, demonstrating 78% removal at pH 6.2, and the removal rapidly followed the pH decrease to the influent pH of 2 (Figure 3.7). Iron(III) is a hard acid, preferentially binding to hard bases or ligands. The complex could thus be affected by the ions present in the surrounding aqueous medium, i.e., competition may exist between the metal complex and H^+ for binding sites on the surface of the biomass.

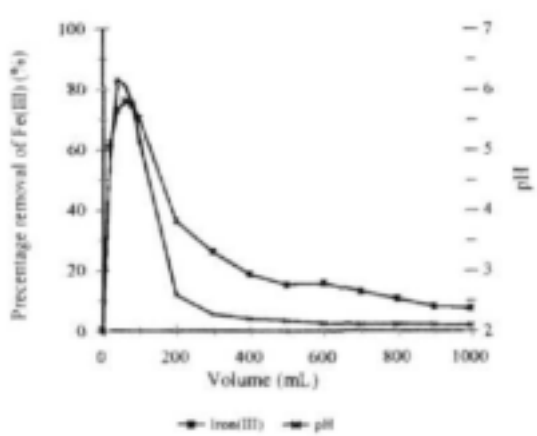


Figure 3.7: Removal of iron(III) (50 μM) from aqueous solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate of 5 mL/min and bed-volume: 49 mL.

Copper(II) is a borderline acid (but is considered harder than lead(II)) preferring to bind to hard or soft bases to form complexes. These metal complexes also have the tendency to be affected by pH and thus the binding of the metal complex to biomass may be characterised by the acidity or basicity of the surrounding medium. The harder borderline acid nature of the metal is clearly demonstrated in Figure 3.8. As the pH increases so does the maximum removal of the metal increase reaching 87 percent at pH 6. When the pH gradually decreases to 2, the removal of copper(II) decreases to 20 percent. This would suggest that the binding site for copper(II) on the *Azolla* is most likely an oxygen donor.

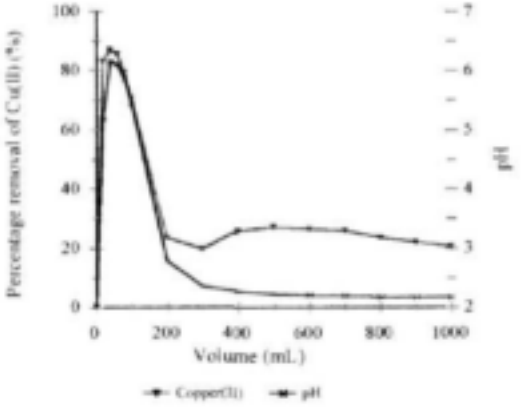


Figure 3.8: Removal of copper(II) (50 μM) from aqueous solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate of 5 mL/min and bed-volume: 49 mL.

Gold(III) is a soft acid, and thus has a tendency to form complexes with soft bases. There is a minor pH-dependence in the first 20 mL as *Azolla* sites are protonated. A removal of 100% occurs as the pH increases sharply from 2 to 6.5 (Figure 3.9). As the pH decreases back to the influent pH of 2, over a small volume range (200 mL), the removal of gold(III) from solution remains constant at 100%.

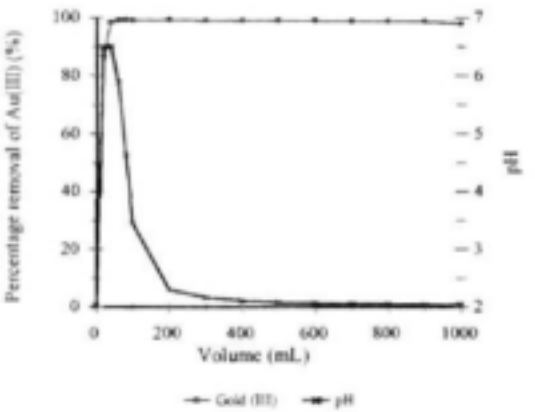


Figure 3.9: Removal of gold(III) (50 μM) from aqueous solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate of 5 mL/min and bed-volume: 49 mL.

The competitive influence of each of the cations on gold(III) removal in a mixed-metal solution was then investigated. Figure 3.10a represents the results of the individual metal studies (Figures 3.6-3.9) in a single graph, while Figure 3.10b, shows the mixed metal solution of lead(II), iron(III), copper(II) and gold(III) all at an equimolar concentration of 50μM (pH 2). It is interesting to note that the comparison between the individual and the presence other metals in the solution demonstrated no effect on gold(III) removal whereas there is clear competition between the borderline cations, lead(II) and copper(II) (Figure 3.10b) in favour of the harder cation, iron(III). The low pH is also an important factor in the low removal rate.

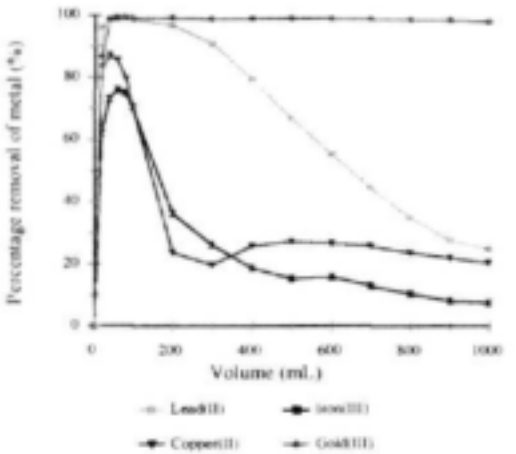


Figure 3.10a: Removal of various metals from aqueous solutions (individual metal studies) at a 50 μM concentration and an influent pH of 2. Parameters utilised were as follows: biomass concentration: 5 g/L, flow-rate: 5 mL/min, room temperature, bed-volume of 49 mL.

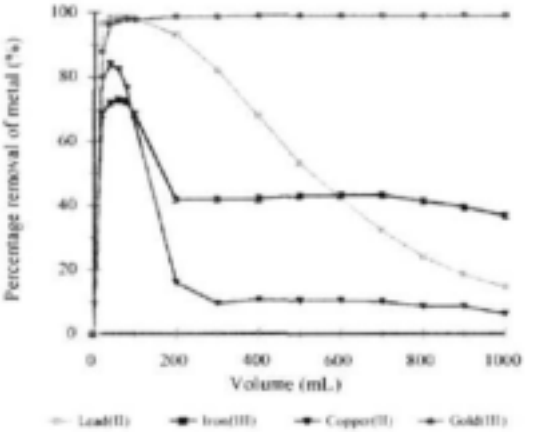


Figure 3.10b: Removal of various metals from a mixed metal solution at a 50 μM concentration and an influent pH of 2. Parameters utilised were as follows: biomass concentration: 5 g/L, flow-rate: 5 mL/min, room temperature, bed-volume of 49 mL.

3.3.2.2. Removal of effluent metals and the effect of effluent concentrations on the biosorptive capacity of Azolla filiculoides for gold(III)

The aim of this study was to determine whether the cations at effluent concentrations would exhibit different removal characteristics as compared with the equimolar concentration studies. The four primary metals that were found in the effluent were: lead, iron, copper and gold at concentrations of: 25 μM, 10 μM, 200 μM and 5 μM respectively.

At a concentration of 25 μM, lead(II) showed the same response (Figure 3.11) as observed in the earlier experiments (Figure 3.6), with 100 percent removal occurring within the first 20 mL fraction as the pH increased to 6, followed by a gradual decrease in removal to 50 percent as the pH decreased to 2. Further explanation for this is provided in Section 3.3.2.1.

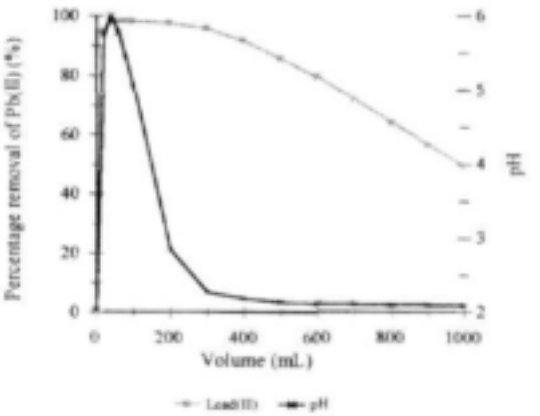


Figure 3.11: Removal of lead(II) (25 μM) from solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

The adsorption of iron(III) at 10 μM by *Azolla filiculoides* demonstrated a maximum removal of 72% (Figure 3.12) and is comparable to the equimolar concentration study which also exhibited 78% extraction (Figure 3.7). The results obtained for this study are explained in Section 3.3.2.1.

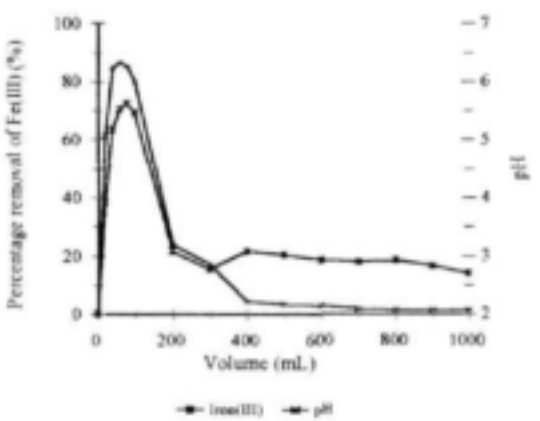


Figure 3.12: Removal of iron(III) (10 μM) from solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

Figure 3.13 represents the removal of copper(II) at 200 μM from solution at an initial pH of 2. The removal of the cation from solution mimics the change in pH. As the pH increases to 5.5,

82% removal occurs, this is followed with an immediate decrease in pH and removal to 2 and 10% respectively. Further explanation of the results obtained in Figure 3.13 is provided in Section 3.3.2.1. It is noted that after 1 L had been passed through the column the percentage copper(II) removed (10%) was less than that for the 50 μM solution (Figure 3.8).

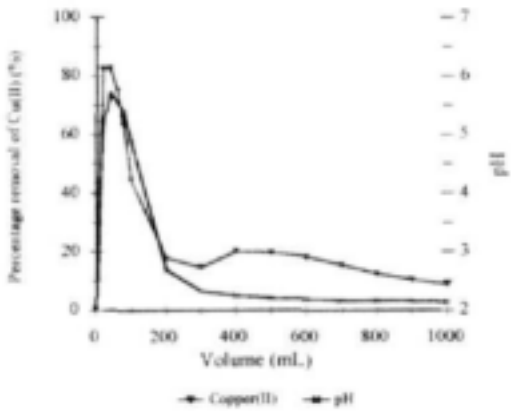


Figure 3.13: Removal of copper(II) (200 μM) from solution accompanied with a pH profile. Parameters were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

Gold(III) (5μM) displays 100 percent removal from solution within the 100 mL fraction (Figure 3.14). The results obtained is comparable to Figure 3.9 and is described further in Section 3.3.2.1.

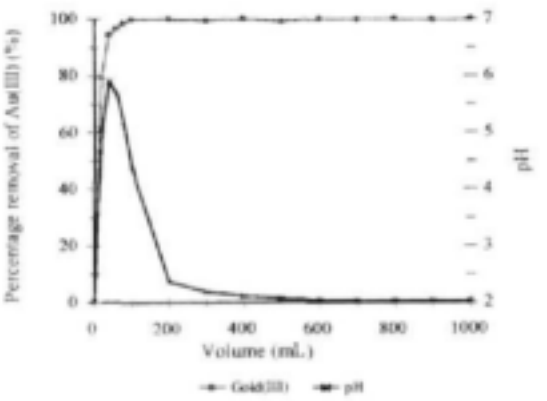


Figure 3.14: Removal of gold(III) (5 μM) from solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

A comparison of metal removal by individual solutions at effluent concentrations 25, 10, 200 and 5 μM for lead(II), iron(III), copper(II) and gold(III) respectively (Figure 3.15a) with a mixed metal solution at the same concentrations (Figure 3.15b), showed that both the individual metal study and the mixed-metal solution for lead(II) demonstrated a maximum removal of 98%, however at the end of the experiment a difference of 25% was found. Iron(III) showed a marked response in that at the individual metal study a maximum of 72% removal (80 mL) was observed while the cation in the mixed-metal solution at the corresponding volume exhibited a 32% removal. Copper(II) removal compared well with the individual metal and mixed-metal solutions. Gold(III) showed a decrease of 10% in removal rate as compared with the single metal study indicating some competition for binding to the biomass.

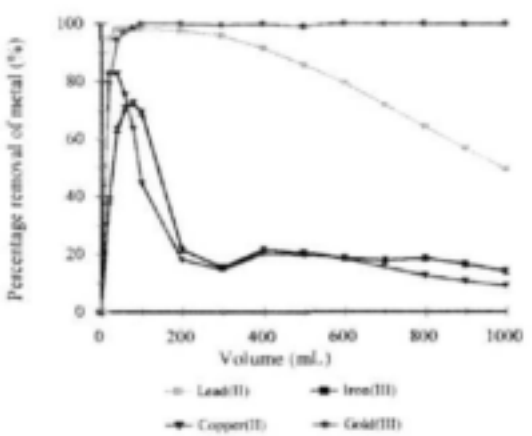


Figure 3.15a: Removal of various metals: lead(II) (25 μM), iron(III) (10 μM), copper(II) (200 μM) and gold(III) (5 μM) from solutions (individual metal studies) at an influent pH of 2. Parameters included: room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

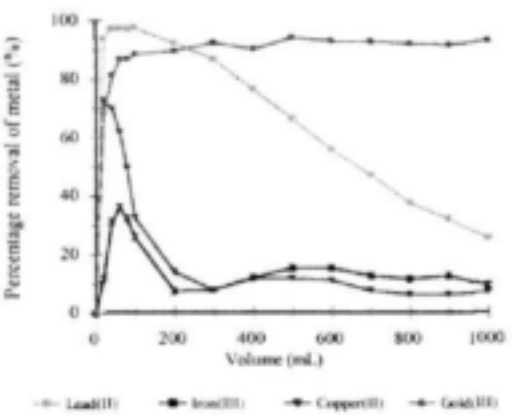


Figure 3.15b: Removal of various metals: lead(II) (25 μM), iron(III) (10 μM), copper(II) (200 μM) and gold(III) (5 μM) from solution at an influent pH of 2. Parameters included: room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

3.4. Effect of Ligands on the Adsorption of Gold(III)

Results in Chapter 2 (Figure 2.15) indicated that the largest inhibitive effect occurred at a 10 mM halide concentration. It was thus decided to utilise this concentration in the column studies.

3.4.1. Effect of Halides

3.4.1.1. Materials and Method

3.4.1.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 3.2.1.1. Aqueous gold(III) solutions were prepared from hydrogen tetrachloroaurate(III) {$H[AuCl_4]$}, sodium bromide, sodium chloride and sodium bromide were obtained from Saarchem, South Africa. Atomic absorption standards were prepared from a gold atomic absorption solution (1000 mg/L) (Wirsam, South Africa). All solutions were diluted with deionised water.

3.4.1.1.2. Method

All experimental work was conducted in duplicate and at room temperature. A final biomass concentration (5 g/L) and a solution containing the desired gold(III) (40 μM) and ligand (10 mM) concentration at pH 2 was prepared. The solution (1 litre) was then pumped through a packed column in an up-flow mode containing 5 g of whole *Azolla filiculoides* in a bed volume of 49 mL. The solution was pumped at a flow-rate of 5 mL/min. Samples were then collected at regular intervals (every 20 mL for the first 100 mL, thereafter every 100 mL) using a fraction collector (Pharmacia, South Africa) and analysed for gold using an AA spectrophotometer. The results were expressed as percentage removal (final concentration relative to initial concentration) of gold(III) from solution.

3.4.1.2. Results and Discussion

The results in Figure 3.16 showed that the addition of chloride had no effect on the removal of gold(III) from solution (100% removal). With bromide and iodide, an initial 95% removal of gold(III) was found with a gradual decrease to 85% and 65% respectively at the conclusion of the study. The affinity of the halides for gold(III) with increasing coordination is as follows: chloride < bromide < iodide. The results correspond closely to the data obtained in the batch studies (Chapter 2, Figure 2.15). A more detailed explanation of these results with regards to the HSAB theory may be found in Section 2.4.1.2.

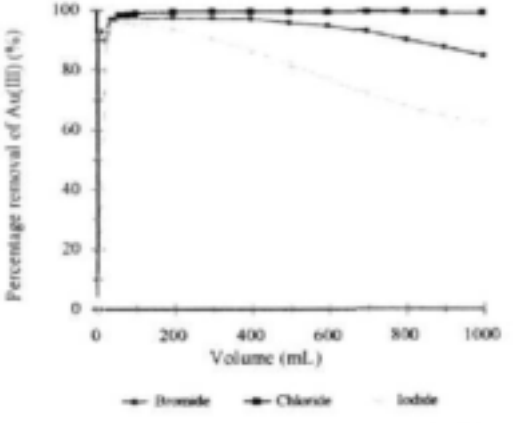


Figure 3.16: The effect of competing halides: bromide, chloride and iodide at 10 mM on the uptake of gold(III) (40 μM) from solution at pH 2. The following parameters were utilised: biomass concentration of 5 g/L, flow-rate of 5 mL/min and room temperature.

3.4.2. Effect of Bases

3.4.2.1. Materials and Method

3.4.2.1.1. Materials

The plant material and reagents were prepared and obtained as described in Section 3.2.1.1. Aqueous gold(III) solutions were prepared from hydrogen tetrachloroaurate(III) {$H[AuCl_4]$}, mercaptoethanol, sodium sulphite and sodium sulphate were obtained from Saarchem, South Africa. Atomic absorption standards were prepared from gold atomic absorption solutions (1000 mg/L) (Wirsam, South Africa). All solutions were diluted with deionised water.

3.4.2.1.2. Method

All experimental work was conducted in duplicate and at room temperature. A final biomass concentration (5 g/L) and a solution containing the desired gold(III) (40 μM) and base (10 mM) concentration at pH 2 was prepared. The solution (1 litre) was then pumped through a packed column in an up-flow mode containing 5 g of whole *Azolla filiculoides* in a bed volume of 49 mL. The solution was pumped at a flow-rate of 5 mL/min. Samples were then collected at regular intervals (every 20 mL for the first 100 mL, thereafter every 100 mL) using a fraction collector (Pharmacia, South Africa) and analysed for gold using an AA spectrophotometer. The results were expressed as percentage removal (final concentration relative to initial concentration) of gold(III) from solution.

3.4.2.2. Results and Discussion

Gold(III) adsorption is pH-independent and its binding characteristics follow that of a soft acid binding preferentially to soft bases. Mercaptoethanol demonstrated no significant effect on the removal of gold(III) from solution (Figure 3.17). Sulphate, as a hard base, suggests that binding of the anion to gold(III) is unfavourable and thus interference with the adsorption of gold(III) due to the anion should not occur. In contrast to the batch study, sulphate has very little effect on the adsorption of gold, thus suggesting that the shorter residence time, of approximately 10 minutes versus 3 hours, may have played a role in the increased removal of gold(III) from solution. Sulphite exhibited the largest effect, with 70% inhibition. Sulphite, as a borderline base, may bind

to gold with the resultant complex being unfavourable for binding, also sulphite may bind to the biomass with a resultant chemical transformation occurring so that conditions now prevents gold(III) adsorption. A more detailed explanation of the HSAB theory may be found in Section 2.4.2.2.

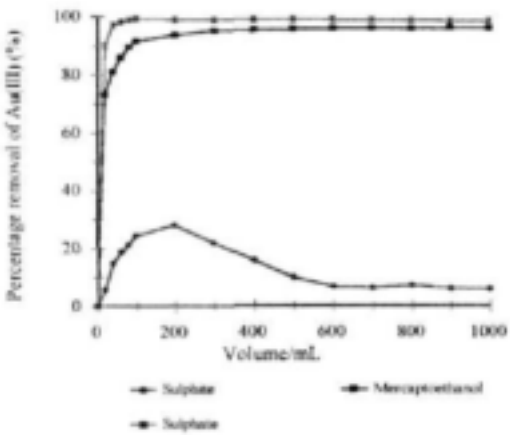


Figure 3.17: The effect of various bases mercaptoethanol, SO_3^{2-} and SO_4^{2-} at 10 mM on the uptake of gold(III) (40 μM) at pH 2 from solution. The following parameters were utilised: biomass concentration: 5 g/L, flow-rate of 5 mL/min and room temperature.

3.5. SUMMARY

The data described in this chapter has demonstrated the suitability of *Azolla filiculoides* for use in the adsorption of gold(III) utilising a packed-bed column. The mechanical strength, particle size and large surface area of the plant material is particularly suited for its utilisation in a continuous-flow mode. The plant material is able to adsorb gold(III) from solution with 92-100% removal at various flow-rates ranging from 2-20 mL/min respectively. The adsorption of gold(III) at the optimal flow-rate of 5 mL/min (100% removal) at pH 2 demonstrated that dilute gold(III) concentrations are able to be adsorbed. Gold(III) binding to tannins present on the plant material may occur. Molecular modelling, using the Cerius II molecular mechanics programme and employing the Universal Force Field, of the gold(III) complex to a condensed tannin shows binding to occur through the hydroxy groups. Many metal complexes and tannins form insoluble complexes (McDonald *et al.*, 1996). It is probable that gold(III) follows a similar pattern with the tannin present on the plant material.

In the equimolar (50 μM) metal study (pH 2), the individual metal studies exhibited the following responses: a maximum removal of 100% for lead(II), 78% for iron(III), 84% for copper(II) and 100% for gold(III). To determine whether these metals interfered with gold(III) binding to the biomass, a synthetic mixed metal solution containing all four metals at an equimolar concentration was passed through the column. No interference was observed for gold, but competition did occur amongst the other metals investigated, which highlights the distinctive chemistry and adsorption profile for each of the metals investigated. When the individual metal studies were adjusted to final concentrations found in effluent, lead(II) (25 μM) showed a maximum removal of 98%, iron(III) (10 μM) 72%, copper(II) (200 μM) 82% and finally gold(III) (5 μM) 100%. When all the metals were placed in a single solution at effluent concentrations, a decrease in the removal of all the metals occurred. Lead(II) uptake decreased by 25% at the conclusion of the study. Iron(III) showed a decrease in maximum removal of approximately 40% when compared with the single metal experiment. Copper(II) and gold(III) exhibited similar responses in that their removal decreased by 10% in the mixed metal solution. The different behaviour of gold(III) and lead(II) removal at an equimolar and 1:5 molar ratio concentration suggests that a borderline acid can

minimally affect the efficiency of gold recovery depending on their relative concentrations.

The series of hard, borderline and soft bases displayed similar results to the batch studies with the exception of sulphate. *Soft bases*: iodide exhibited 65% removal, while mercaptoethanol had no significant effect on the removal of gold(III). *Borderline bases*: bromide and sulphite showed 85% and 30% removal respectively. *Hard bases*: chloride and sulphate both demonstrated a 100% removal of gold(III) from solution.

It has thus been demonstrated that *Azolla filiculoides* has potential as a biosorbent for its utilisation in the removal of gold(III), as well as lead(II), iron(III) and copper(II) from solution. The unique binding characteristics of each metal allows for selective pH-controlled removal and possible recovery of each metal while gold(III) is retained on the biomass. Finally, uptake is rapid especially with the metal of interest, gold(III). It seems that most of the criteria with regards to the utilisation of the biosorbent in removal of the metal(s) can be met. Further investigation was consequently undertaken to adsorb the metals of interest, namely lead(II), iron(III), copper(II) and gold(III), onto the plant material and to selectively recover them from the biomass.

CHAPTER 4

DESORPTION STUDIES

4.1. INTRODUCTION

One of the primary concerns for any metal bioremediation process design is the expedience with which the metals are sequestered and the relative ease with which the metals are able to be recovered. Those systems which allow for non-destructive recovery as well as efficient biomass regeneration are the most attractive. It is also important that each biomass chosen should have a certain degree of selectivity and it should be noted that temperature as well as pH play a role in the binding characteristics of the metal and biosorbent (Darnall *et al.*, 1986; Volesky, 1990).

If the metal complex binds preferentially at pH 2, for example, this may indicate binding to protonated *Azolla* groups such as amines and thus the anionic complex is assumed to interact with the biosorbent as the concentration of positive charges increase (low pH's) (Crist *et al.*, 1981). Metals whose binding is pH-dependent allows control of pH for selective metal ion separation (Darnall *et al.*, 1988). However if the binding characteristics exhibit pH-independent binding, this indicates that the binding of the metal to the biomass may be stronger and thus an alternative separation method may be required (Banks, 1997; Volesky, 1990). The latter exhibits characteristic properties of a more covalent nature (Crist *et al.*, 1981). Gold and platinum are classified according to Pearson's classification as "soft" (Pearson, 1968). "Soft" metals (acids) bind to soft ligands (bases) such as amine or thiol groups and thus are minimally influenced by ionic interactions and pH. The stronger binding nature of gold or platinum suggests that a complexing agent or ligand is necessary to enhance the recovery of the metal from the biomass, e.g. acidic thiourea (Hosea *et al.*, 1986). Another important factor to take into account is the volume of desorbent in proportion to the volume of solution treated. A minimal amount of desorbent used allows for a more concentrated metal solution for recovery (Banks,1997; Garnham, 1997; Volesky, 1990). An eluant capable of achieving a high desorption efficiency for the metal of interest is necessary in order for the process to be feasible.

Studies have demonstrated that gold(III) is able to interact with a high affinity with various forms of biomass such as *Chlorella vulgaris* and *Azolla filiculoides* (Hosea *et al.*, 1986; Antunes *et al.*, 2001). Gee and Dudeney (1988), Hosea *et al.* (1986) and Kuyucak and Volesky (1988a) revealed that upon binding of gold, biomolecules present on the biomass surface may be responsible for the reduction of gold(III) to gold(I) and in some cases reduction to gold(0). Hosea *et al.* (1986) have demonstrated that once gold(III) is bound to the biomass, the metal is reduced to a linear biomass-Au(I)-Cl complex. Thiourea, is known as a strong complexing agent and thus forms stable soluble complexes with Au(I) and it is this property that makes thiourea an attractive prospect for the removal of gold from biosorbents. At low pH's, the gold chloride complex $[AuCl_4]^-$, is able to be reduced to Au(I) and Au(0) (Kuyucak and Volesky, 1988a). The elution of gold with thiourea can be enhanced through the addition of an oxidant. It is thought to involve a redox phenomenon (Kuyucak and Volesky, 1989b) which is capable of reversing the initial binding of the metal to the biomass, by changing the oxidation state of the metal and thus readily solubilising the gold. Kuyucak and Volesky (1989a) further suggest that the addition of the oxidant to the desorption solution enhances the equilibrium solution capacity and the rate of elution.

If the biosorbent used is not considered for recycling or re-use for any apparent reason, especially if it is cheap and in abundant supply, the material may be ashed or combusted as an alternative process. Ashing produces a high metal concentrate which also allows for disposal of the spent biomass once biosorption and the recovery of the metal has been completed (Volesky, 1990).

It has already been established that *Azolla filiculoides*, the biosorbent of interest, is available in abundance and that harvesting the material is economical. The plant material has a high affinity for gold(III), demonstrating a rapid 100% uptake in batch and column studies (Chapters 2 and 3 respectively). In this chapter, the desorption profiles of seven desorbents were investigated under batch and column conditions to determine which of these were most suitable. The most promising desorbent was then studied using several oxidants in an attempt to enhance the rate of desorption.

Thiourea is well known as a suitable alternative to cyanide leaching of gold and is also less toxic. Thus preliminary studies were carried out with thiourea to establish whether this agent was able to elute gold from the biomass. Gold, once bound to the biomass, may change its oxidation state to +1 or 0 and various studies have shown that thiourea forms stable complexes with Au(I) according to the following equation (Gee and Dudeney, 1988; Groenewald, 1976; Hiskey and Atluri, 1988; Hosea *et al.*, 1986; Kuyucak and Volesky, 1989a):

$$Au^{+} + 2CS(NH_2)_2 \rightleftharpoons [Au\{CS(NH_2)_2\}_2]^{+} \quad (17)$$

It is important to note that the oxidation state of gold plays a role in thiourea being able to solubilise gold. Thus for thiourea to form the Au(I)-complex, the metal must be either an ion or be present with an oxidising agent. The oxidising agent present is able to oxidise gold in the 0 oxidation state to +1 and thus form a stable thiourea complex (Hosea *et al.*, 1986; Kuyucak and Volesky, 1989a).

4.2. Batch Desorption Studies

4.2.1. Ratio of Adsorbent to Desorbent

The volume of desorbent required is crucial to lower the disposal volume (especially for toxic and precious metals), the reduced volume concentrates the metal and allows for an enhanced metal recovery and regeneration of the biomass for successive cycles. This study utilised various oxidising agents (ammonium peroxodisulphate and perchloric acid) in combination with thiourea in an endeavor to increase the elution of gold from the biomass in a concentrated solution. An attempt was also made to ascertain whether the speciation of gold had changed over a incubated period of 1 and 24 hours, this was monitored by the elution of gold from the biomass.

4.2.1.1. Materials and Method

4.2.1.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 2.2.1.1. Perchloric acid (60%) was obtained from Saarchem, South Africa. Thiourea and ammonium peroxodisulphate were obtained from Merck, Germany. All solutions required were dissolved in deionised water unless specifically indicated. Thiourea was dissolved in deionised water and acidified with hydrochloric acid until the desired pH was achieved.

4.2.1.1.2. Method

All experimental work was conducted in duplicate. A biomass concentration of 5 g/L and gold(III) concentration of 5 mg/L at pH 2 was utilised. Samples (100 mL) placed in 300 mL Erlenmeyer flasks were constantly agitated at 200 rpm at room temperature. After one hour, once adsorption was complete, the biomass was filtered (cellulose-acetate, 50 mm diameter, 0.45 μM pore size) and placed back in the Erlenmeyer flask. Various desorbents {thiourea (0.1M, pH1.7), ammonium peroxodisulphate (0.06 M) and thiourea (0.1M), pH 2, or 2% perchloric acid in combination with 8% thiourea and 0.5% 0.1 M HCl, pH 1.4} were utilised depending on the experiment. The desorbent (25, 50 or 100 mL) was added to the flask immediately after adsorption and agitated for a period of 4 hours and a sample (5 mL) removed every hour.

A second study was performed to determine whether the oxidation state of gold had changed once adsorption had occurred. After adsorption (1 hour) the biomass was filtered and placed back in the Erlenmeyer flasks, sealed and left to stand for a period of 1 and 24 hours at room temperature. Once the incubation period was complete, desorption was carried out as normal, with thiourea (0.1M, pH1.7), ammonium peroxodisulphate (0.06 M) and thiourea (0.1M), pH 2, or perchloric acid (2%) in combination with thiourea (8%) and 0.1 M HCl (0.5%) (pH 1.4) with an equal volume of adsorbent to desorbent. Samples from the adsorption and desorption stage were analysed for gold using AA spectrophotometry. The results were expressed as percentage

gold adsorbed on/or removed from the biomass after one and four hours for adsorption and desorption respectively.

4.2.1.2. Results and Discussion

4.2.1.2.1. 0.1 M Thiourea

Gold recovery requires a strong complexing agent, such as thiourea at pH 1.5-2.5. Various studies have shown that optimal gold recovery from the biomass *Chlorella vulgaris* and *Spirulina platentis* occurred with thiourea (Gee and Dudeney, 1988; Hosea *et al.*, 1986). Figure 4.1a demonstrates that a volume of 25 mL removes a maximum of 15% of gold from the biomass, whilst 50 and 100 mL exhibit 28% and 37% removal respectively. Thus a ratio of 3:1of adsorbent to desorbent is not satisfactory, whilst a 1:1 ratio produces maximum desorbing conditions for this particular eluant. A difference of 5% removal by the desorbent was demonstrated between the incubation of the biomass 1 and 24 hours after adsorption (Figure 4.1b). However, an increase of 10% in the desorption of gold from the biomass was found on standing after 1 hour as compared with immediate desorption after adsorption occurred. This suggests that within the hour a small change in the oxidation state, i.e. to +1, may have resulted.

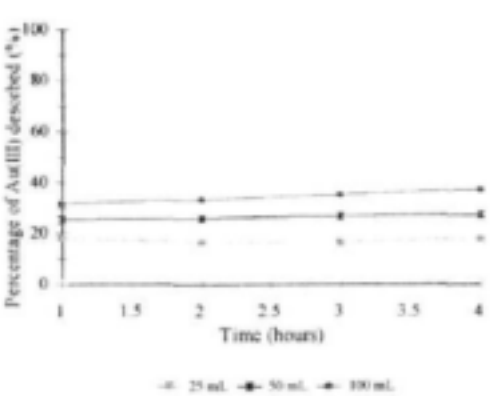


Figure 4.1a: Desorption of gold from *Azolla filiculoides* with various volumes of 0.1 M thiourea at pH 1.7. Desorption followed immediately once adsorption was complete. Parameters utilised included: room temperature, agitation speed of 200 rpm.

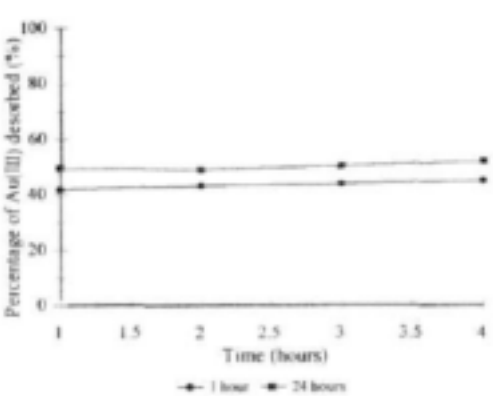


Figure 4.1b: The effect of incubating the plant material for 1 and 24 hours, in the absence of a desorbent. Desorption then proceeded as in Figure 4.1a utilising a ratio of adsorbent:desorbent of 1:1.

4.2.1.2.2. Thiourea (8%), Perchloric acid (2%) and 0.1M HCl (0.5%)

Alternative oxidants such as ammonium ferric sulphate and ferric chloride in combination with thiourea have previously been utilised (Kuyucak and Volesky, 1988a, b, 1989a). Darnall *et al.* (1988) utilised this particular desorbent to elute gold from resin and was thus explored in this study. Results in Figure 4.2a reveal that a ratio of adsorbent/desorbent (3:1) gave 48% removal of gold from the biomass, whilst ratios of 2:1 and 1:1 had 65% and 72% removal respectively. The results obtained in this study when compared with the results of thiourea (0.1 M) indicate that an oxidising agent increases the removal of gold from the biomass.

The incubation of *Azolla* for 1 and 24 hours demonstrated a 7% variation, again implying that the oxidation state of gold remained unchanged (Figure 4.2b).

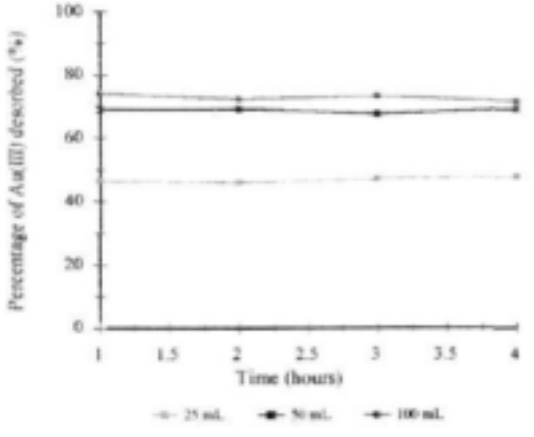


Figure 4.2a: Desorption of gold from *Azolla filiculoides* with various volumes of 8% thiourea, 2% perchloric acid, 0.5% 0.1M HCl at pH 1.4. Desorption followed immediately once adsorption was complete. Parameters utilised included: room temperature, agitation speed of 200 rpm.

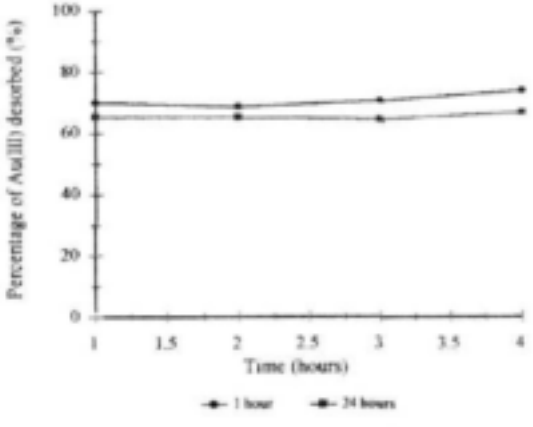


Figure 4.2b: The effect of incubating the plant material for 1 and 24 hours in the absence of a desorbent. Desorption then proceeded as in Figure 4.2a utilising a ratio of adsorbent:desorbent of 1:1.

4.2.1.2.3. 0.1 M Thiourea and 0.06 M Ammonium Peroxodisulphate

Thiourea in combination with an oxidant was utilised in an attempt to increase the elution of gold from the biomass. Ammonium peroxodisulphate is a vigorous oxidising agent (Greenwood and Earnshaw, 1989) and it was for this reason that this particular compound was utilised at a similar concentration to perchloric acid. The results in Figure 4.3a demonstrated that at a ratio of 3:1 of adsorbent to desorbent, displayed 36.7% removal, while a ratio of 2:1 and 1:1 exhibited 48% and 61% removal respectively. A 1:1 ratio again gives maximum gold desorption. The volume of desorbent is obviously critical since a large difference occurs between the different ratios for the removal of gold from the biomass. These results also indicate that an oxidising agent substantially increases the elution of gold from the biomass.

A difference of 3% between 1 and 24 hour incubation exists, demonstrating that over the period investigated there is little change in the oxidation state of gold once bound to the biomass (Figure 4.3b).

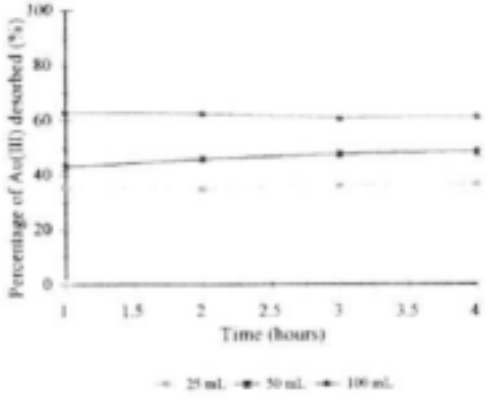


Figure 4.3a: Desorption of gold from *Azolla filiculoides* with various volumes of 0.1 M thiourea and ammonium peroxodisulphate (0.06 M) at pH 2. Desorption followed immediately once adsorption was complete. Parameters utilised included: room temperature, agitation speed of 200 rpm.

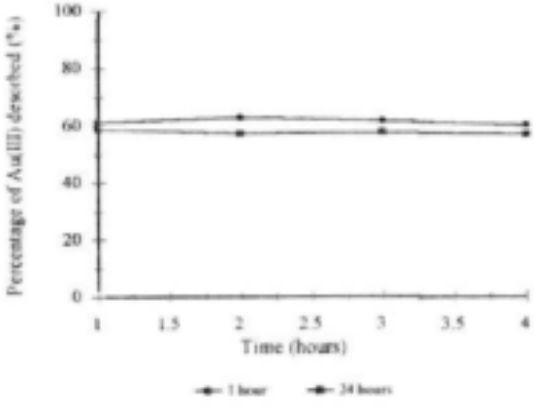


Figure 4.3b: The effect of incubating the plant material for 1 and 24 hours in the absence of a desorbent. Desorption then proceeded as in Figure 4.3a utilising a ratio of adsorbent:desorbent of 1:1.

4.2.2. Oxygen-, Air- and Nitrogen - Assisted Desorption

4.2.2.1. Materials and Method

4.2.2.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 4.2.1.1.1. Cylinders of nitrogen, air and oxygen were obtained from Afrox, South Africa.

4.2.2.1.2. Method

All experimental work was conducted in duplicate. The adsorption process was followed as described in Section 4.2.1.1.2. An equal volume of desorbent (100 mL) and acidic thiourea (0.1 M) was added to an air-tight Erlenmeyer flask and allowed to agitate for a period of 3 hours, with oxygen, nitrogen or air being bubbled at a constant rate. Samples (5 mL) were removed utilising an air-tight syringe every hour. Once the studies were complete, samples from the adsorption and desorption stages were analysed for gold using AA spectrophotometry. The results were expressed as percentage desorption of gold from the biomass.

4.2.2.2. Results and Discussion

The adsorption stage of this experiment demonstrated 93-98% gold uptake from solution. The addition of oxidants to the eluant solution has been shown to enhance the elution capacity and rate (Kuyucak and Volesky, 1989a, Section 4.2.1). The results in Figure 4.4 demonstrates that nitrogen-assisted desorption gives a maximum desorption of 81% of gold from the biomass, whilst air- and oxygen-assisted desorption showed 86% and 97% desorption respectively. Maximum removal of all gas-assisted desorptions occurred within 1 hour of desorption commencing. As demonstrated earlier, although thiourea is capable of limited desorption of gold, these results indicated that the presence of an oxidising agent, such as oxygen and to a lesser extent air, facilitates an enhanced removal of gold from the biomass.

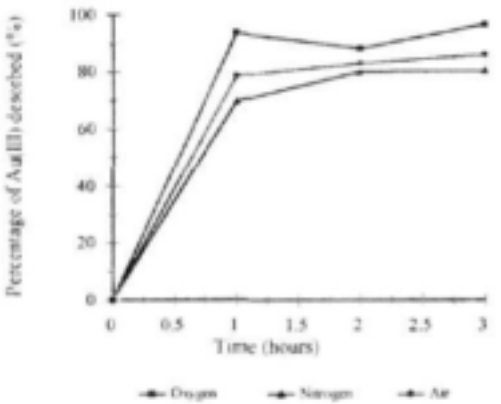


Figure 4.4: Desorption of gold utilising oxygen, air and nitrogen in combination with thiourea. The following parameters were utilised: **Adsorption**: initial gold(III) concentration: 5 mg/L, biomass concentration: 5 g/L, pH 2, agitation speed of 200 rpm and room temperature. **Desorption**: all gases were bubbled at a constant rate, volume ratio of adsorbent/desorbent: 1:1.

4.3. Column Desorption Studies

The initial batch experiments were undertaken to determine whether thiourea was capable of removing gold from the biomass. The batch studies demonstrated that gold bound to the biomass is probably in the +1 oxidation state, and the amount of gold in the +1 oxidation state changes very little over 24 hours, shown by the small increase in elution with thiourea (Section 4.2.1.2.1). The oxidation state of gold(III) probably changes upon binding to the biomass to gold(I) which is favourable for thiourea complexation. However gold(I) may reduce to gold(0) over time, thus the presence of an oxidising agent enhances the elution of gold from the biomass (Sections 4.2.1.2.2 and 4.2.1.2.3) due to oxidation of gold(0) to gold(I). Since batch systems are only applicable to low volumes, and as column processes would ultimately be more feasible for treatment on a larger scale, a comprehensive study of the possible oxidation states of bound gold in a column process was studied utilising various desorbents from mineral acids to complexing agents such as thiourea. The desorbents were thus compared to determine which would ultimately be utilised, for its superior complexing ability.

4.3.1. Initial Desorption Studies

4.3.1.1. Materials and Method

4.3.1.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 3.2.1.1. All reagents used were of analytical grade and obtained from Saarchem, South Africa, except for thiourea and ammonium peroxodisulphate which was obtained from Merck, Germany.

4.3.1.1.2. Method

All experimental work was conducted in duplicate. Gold(III) solutions prepared from hydrogen tetrachloroaurate(III) were dissolved in deionised water to achieve the desired final concentration of 5 mg/L. A solution (1 litre) was then pumped (in an up-flow mode) through a packed column containing 5 g of whole *Azolla filiculoides* (bed volume of 49 mL). The solution was pumped at an initial flow-rate of 5 mL/min (Chapter 3, Section 3.2.2.1). Samples were collected at regular intervals (every 20 mL for the first 100 mL, thereafter 100 mL) using a fraction collector (Pharmacia, South Africa) and analysed for gold using an AA spectrophotometer. A maximum desorbent volume of 200 mL (a five fold concentration factor) was selected as a cutoff since a larger volume for disposal or concentration of metal would be uneconomical. The desorbent was also pumped through the column in an up-flow mode at a flow-rate of 5 mL/min and fractions (20 mL) collected by the fraction collector. The results were expressed either as percentage removal or as mg Au desorbed once a volume of 200 mL of desorbent had passed through the column.

4.3.1.2. Results and Discussion

Figure 4.5 shows a typical adsorption and pH profile for gold(III) (with 100% removal from solution within 40mL). As described in Chapter 3, the interaction appears to be initially ionic (this is very rapid) followed by covalent binding, this may be accompanied with a change in the

oxidation state of gold(III) to gold(I) or (0). With covalent binding the adsorption process is pH-independent and thus unaffected by ionic and electrostatic interactions in the surrounding medium. Since the binding to the biomass is covalent, a strong elutant is necessary.

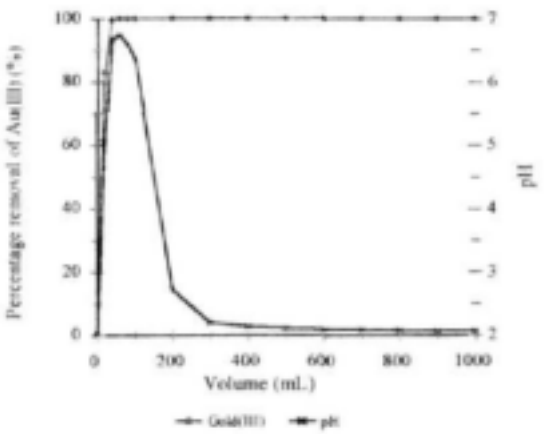


Figure 4.5: Typical adsorption and pH profile obtained when gold(III) is removed from solution by *Azolla filiculoides*. The parameters utilised were: initial gold(III) concentration of 5 mg/L, biomass concentration of 5 g/L, pH 2, flow-rate of 5 mL/min and room temperature.

4.3.1.2.1. 0.1 M Nitric acid (HNO_3)

Concentrated solutions of nitric acid are strongly oxidising and is able to solubilise most metals except for gold and platinum. Thus a more aggressive agent is generally necessary to solubilise these two metals (Greenwood and Earnshaw, 1989). Results obtained from this study demonstrate that nitric acid on its own as an eluant does not desorb any gold. Although a strong oxidising agent, the dissolution of gold generally requires a complexing ligand, such as free chlorine (Greenwood and Earnshaw, 1989), which is not present in the above desorbent. The failure of the acid to elute the gold suggests that adsorbed gold is not principally an acid-soluble complex such as gold(III) oxide (Kuyucak and Volesky, 1989a).

4.3.1.2.2. 0.1 M Sulphuric acid (H_2SO_4)

As one of the cheapest bulk mineral acids, sulphuric acid has a high dielectric constant and electrical conductivity and this results in self-dissociation. In the liquid phase, at least seven defined species exist such as: HSO_4^-, $H_3SO_4^+$, H_3O^+, $HS_2O_7^-$, $H_2S_2O_7$ and H_2O. Sulphuric acid forms salts with many metals which are often very stable. The sulphate ion is tetrahedral and thus is capable of acting as a bridging or chelating ligand (Greenwood and Earnshaw, 1989). Sulphuric acid's complexing ability is not sufficient for binding to gold and for elution to occur (Figures 4.6a and b). Thus a total of only 0.122 mg of gold (2.44%) was removed from the biomass under the given conditions, with maximum removal occurring within 20 mL.

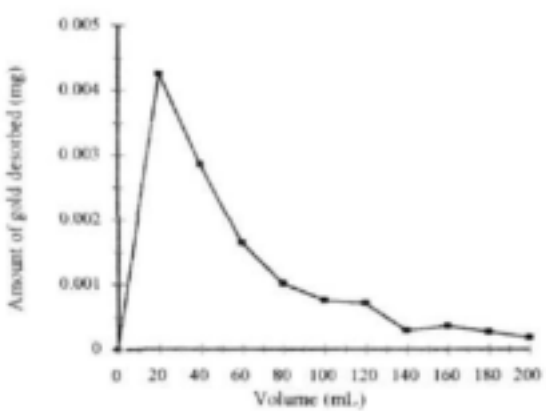


Figure 4.6a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M H_2SO_4. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

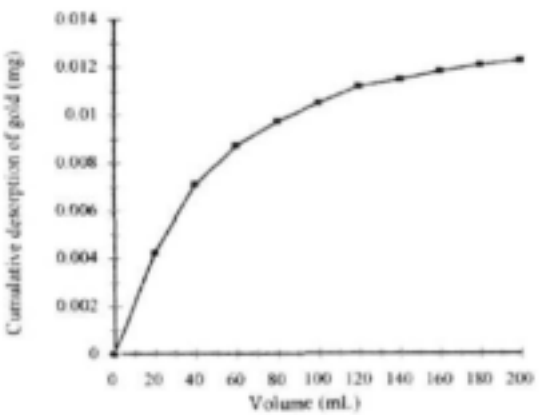


Figure 4.6b: Cumulative desorption of gold utilising 0.1 M H_2SO_4. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.3. 0.1 M Ethylenediamminetetraacetic acid (EDTA)

Known for its ability to strongly chelate metals from solution, EDTA's ability to chelate gold from the biomass has previously been investigated (Kuyucak and Volesky, 1989a). EDTA contains four donor oxygen atoms and two donor nitrogen atoms in each molecule and has the ability to form six co-ordinate complexes with most metals in solution at the correct pH (Lee, 1991). It has been suggested that its ability to sequester metals is purely as a result of physico-chemical adsorption

of EDTA to the metal (Kuyucak and Volesky, 1989a). In the present study results in Figures 4.7a and 4.7b suggest that a physico-chemical adsorption of gold may have not occurred but rather another type of binding since only 0.005 mg (0.1%) of gold was removed from the biomass. Maximum removal occurred within 40 mL. EDTA as a hard base will preferentially bind to cations which are hard acids and not a soft acid such as gold.

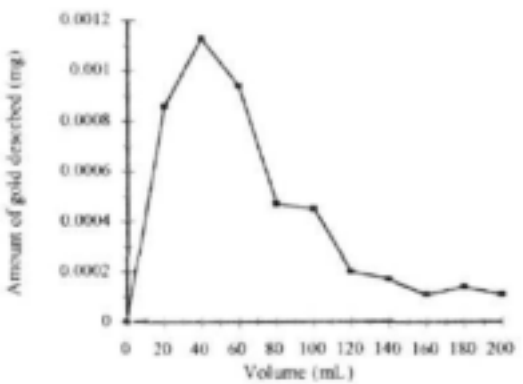


Figure 4.7a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M EDTA. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

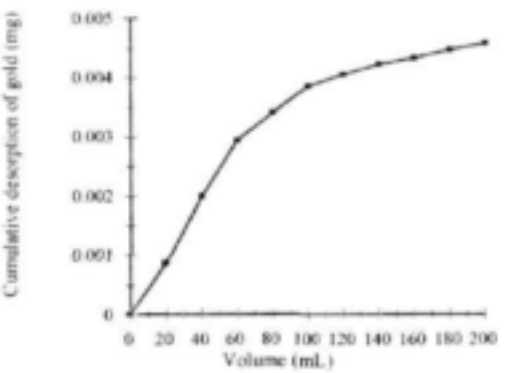


Figure 4.7b: Cumulative desorption of gold utilising 0.1 M EDTA. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.4. 0.5 M Mercaptoethanol

Thiols, as soft base ligands, readily form covalent bonds with soft acid metals such as gold and platinum and these functional groups may be present on the biomass surface. These groups also play an important role in the selective chelating or complexing of other metals of interest. It has been found that two adjacent sulphydral groups are important in chelating metals (Sharma *et al.*, 1987). Mercaptoethanol ($SHCH_2CH_2OH$) is able to remove silver and gold from *Chlorella vulgaris* (Darnall *et al.*, 1986). Thus the following study investigated the ability of mercaptoethanol to elute gold from the biomass. Results in Figures 4.8a and 4.8b show that maximum removal had been achieved by 180 mL with a total of 0.149 mg (2.97%) of gold eluted by the desorbent respectively. This suggests that the presence of one sulphydral group in the elutant may not be sufficient to strongly complex the metal, or alternatively that the reaction

kinetics are slow as is observed with thiourea.

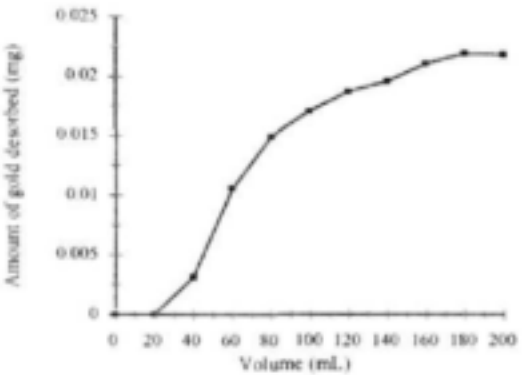


Figure 4.8a: Amount of gold desorbed for every 20 mL fraction utilising 0.5 M mercaptoethanol. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

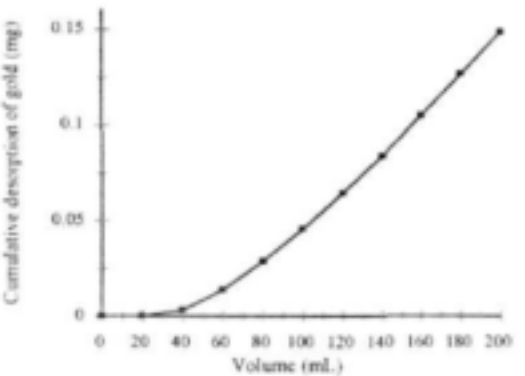


Figure 4.8b: Cumulative desorption of gold utilising 0.5 M mercaptoethanol. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.5. 0.1 M Potassium hydroxide (KOH)

Greenwood and Earnshaw (1989) and Kuyucak and Volesky (1989a) indicated that gold oxide, Au_2O_3 (the only known oxide of gold), is soluble in concentrated alkaline solutions and may form salts of the $[Au(OH)_4]^-$ ion. It was therefore decided to study whether the gold(III) upon binding to the plant material changed speciation to an oxide. Results in Figures 4.9a and 4.9b demonstrate that a maximum removal utilising KOH occurred within 40 mL and 0.188 mg (3.76%) of gold was removed in total, suggesting that no significant oxidation of gold had occurred. The physical appearance and structure of the plant material changed dramatically when using KOH as an elutant, the accompanying elution solution changed to dark brown and the plant material acquired a paste-like appearance which suggested that the integrity of the biomass was destroyed.

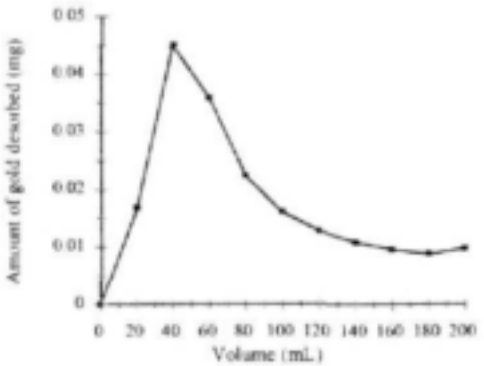


Figure 4.9a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M KOH. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

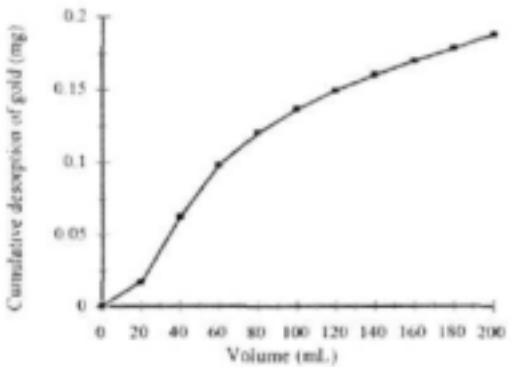


Figure 4.9b: Cumulative desorption of gold utilising 0.1 M KOH. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.6. 0.1 M Potassium bromide (KBr) and 20% Ethanol (EtOH)

Potassium bromide and ethanol are currently used in some mining industries to remove gold adsorbed onto activated charcoal. Potassium bromide has a high affinity for gold (Chapters 2 and 3) and ethanol has been found to markedly increase the desorption of gold (Heinen *et al.*, 1976). An investigation into the removal of gold from plant material by Kuyucak and Volesky (1988a, 1989a) utilising this particular elutant showed a 35% elution in batch studies of *Sargassum natans*. In the present study using KBr and ethanol as an eluant for gold, maximum removal occurred within 40-60 mL (Figure 4.10a), with only a total of 0.427 mg (8.54%) gold removed from the biomass (Figure 4.10b).

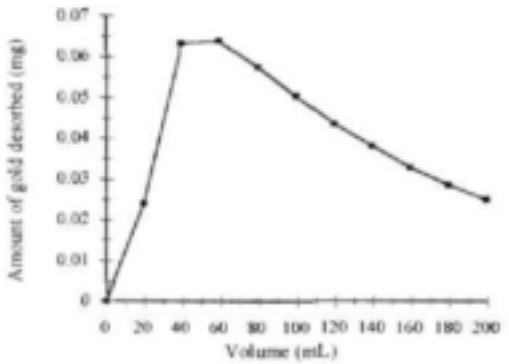


Figure 4.10a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M KBr + 20% EtOH. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

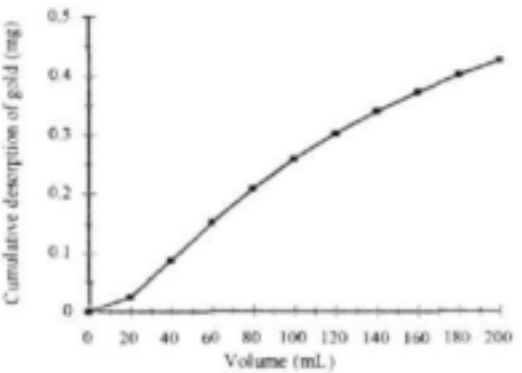


Figure 4.10b: Cumulative desorption of gold utilising 0.1 M KBr + 20% EtOH. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.7. 0.1 M Thiourea

Various studies have shown that acidic thiourea (pH ± 1.5), $(NH_2)_2CS$, forms stable complexes with Au(I) which allows acidic leaching of the gold from ores and concentrates (Hosea *et al.*, 1986; Gee and Dudeney, 1988; Kuyucak and Volesky, 1989a). As previously discussed, the oxidation state of gold plays an important role for thiourea to solubilise gold and the gold(I)-thiourea complex ion is the only known soluble thiourea species of the aurous ion (Hiskey and Atluri, 1988; Hosea *et al.*, 1986; Kuyucak and Volesky, 1989a) but the reaction kinetics are enhanced in the presence of oxidising agents. A slow, steep rise in the removal of gold from the biomass occurred and a maximum had not been achieved by the end of the desorption (200 mL), a cumulative removal of 0.824 mg (16.5%) (Figures 4.11a and 4.11b respectively) was found. Although a strong chelating agent of gold, its reaction kinetics are indeed slow. It may be possible that a slower flow-rate or increased volume of thiourea for desorption may be necessary to increase the recovery of gold from the biomass.

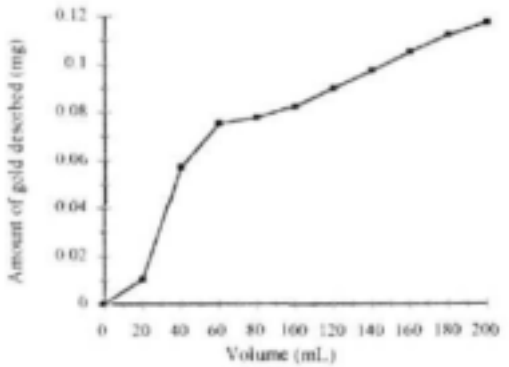


Figure 4.11a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

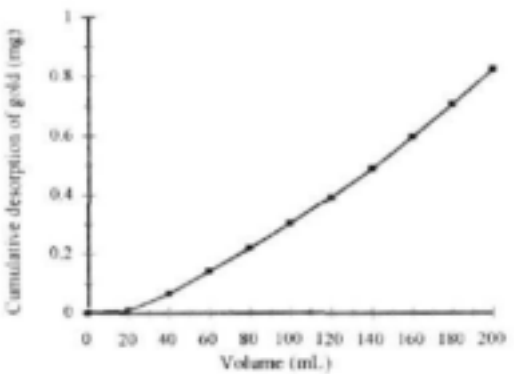


Figure 4.11b: Cumulative desorption of gold utilising 0.1 M thiourea. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.8. 0.1 M Thiourea in combination with 0.02 M Ammonium ferric sulphate $NH_4Fe(SO_4)_2$ or 0.02 M Ammonium ferrous sulphate $(NH_4)_2Fe(SO_4)_2$

Due to the slow kinetics associated with thiourea desorption it was decided to compare the removal of gold utilising ferric ammonium sulphate and ferrous ammonium sulphate in combination with thiourea. Studies by Kuyucak and Volesky (1988a, 1989a, b) indicate that ferric ammonium sulphate, as an oxidising agent, is a very efficient elutant. Again thiourea was utilised as the complexing agent for gold. Ferric ammonium sulphate with thiourea gave a maximum removal at 160 mL and a total of 2.309 mg (46.2%) gold was removed from the biomass (Figures 4.12a and 4.12b respectively). Ferrous ammonium sulphate is not regarded as an oxidising agent and it was therefore used for comparative purposes. The results show a maximum removal at 60 mL and a decrease in efficiency of removal with 0.070 mg (1.4%) of the gold being removed (Figures 4.13a and 4.13b). The results reflect that a thirty fold increase of gold recovery was aided by the addition of an oxidant.

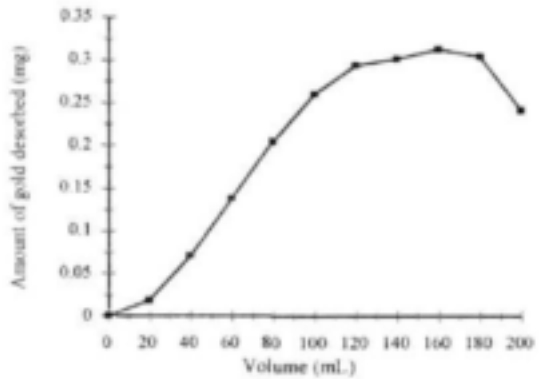


Figure 4.12a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.02 M $NH_4Fe(SO_4)_2$. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

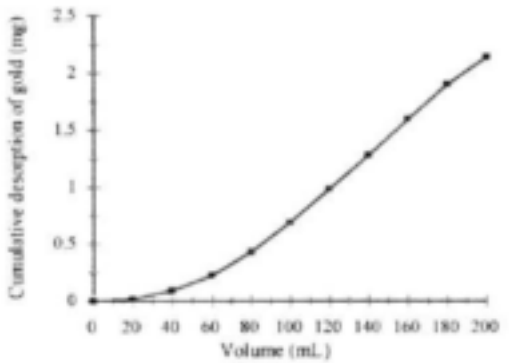


Figure 4.12b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.02 M $NH_4Fe(SO_4)_2$ Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

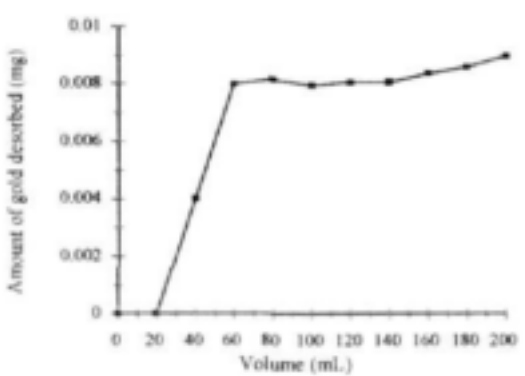


Figure 4.13a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.02 M $(NH_4)_2Fe(SO_4)_2$. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

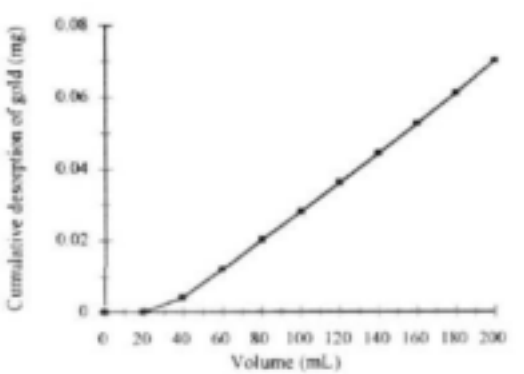


Figure 4.13b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.02 M $(NH_4)_2Fe(SO_4)_2$ Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.9. 0.1 M Thiourea and 0.06 M Ammonium peroxodisulphate at pH 1.46 (adjusted) and 2.46 (non-adjusted)

Another strong oxidising agent is amonium peroxodisulphate and this was used in combination with thiourea. A further objective of this experiment was as a comparison between an optimally pH adjusted and non-adjusted solution. A pH of 1.5-2.5 for thiourea was utilised by Gee and Dudeney (1988) as optimal for the recovery gold from algae such as *Chlorella vulgaris* and *Spirulina platensis*. Thus the choice of pH 1.46 (Section 4.2.1.2.1) was utilised as a comparison with no pH adjustment (pH 2.46). As seen from Figure 4.14a maximum removal occurred at 100 mL at pH 1.46 while Figure 4.15a demonstrates maximum removal within 80 mL at pH 2.46. Elution at pH 1.46 resulted in a total of 3.322 mg of gold (64.5%) being removed (Figure 4.14b), while a total of 3.769 mg (75.4%) of gold is removed at pH 2.46 (Figure 4.15b). This indicates that the higher pH is optimal with respect to the removal of gold with this particular oxidant indicating that an oxidant and pH play an important role in the elution of the metal from the biomass. Since the unadjusted pH of the thiourea solution (2.46) study showed better recovery, the pH was left unadjusted for subsequent desorption studies.

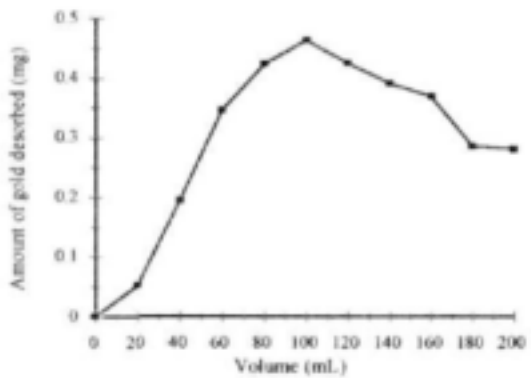


Figure 4.14a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.06 M $NH_4S_2O_8$ at pH 1.46. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

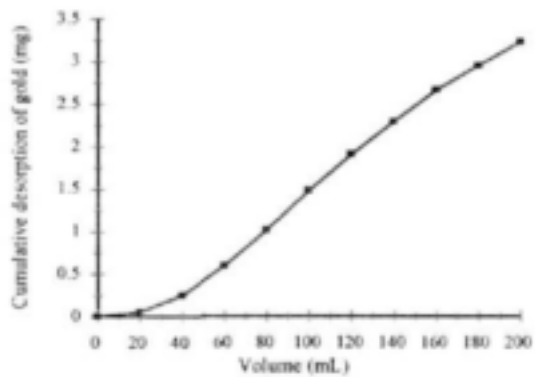


Figure 4.14b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.06 M $NH_4S_2O_8$ at pH 1.46. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

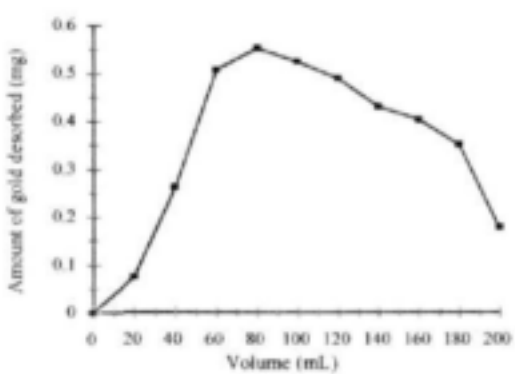


Figure 4.15a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.06 M $NH_4S_2O_8$ at pH 2.46 Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

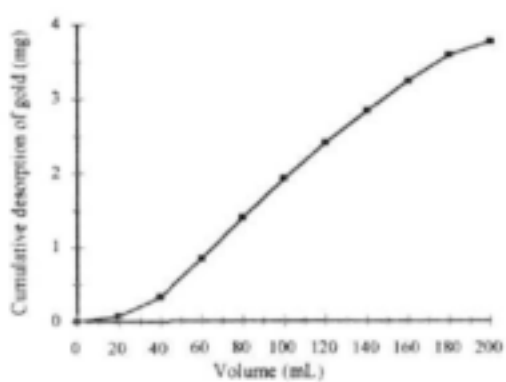


Figure 4.15b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.06 M $NH_4S_2O_8$ at pH 2.46. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.10. 0.1 M Thiourea and 0.02 M Ferric chloride ($FeCl_3$)

Kuyucak and Volesky (1989a) found that $FeCl_3$, as an oxidant, in combination with thiourea enhanced the rate of elution. A maximum removal occurred at the 140 mL fraction and a total of 3.456 mg (69%) gold was removed from the biomass within the elution volume studied (Figures 4.16a and 4.16b respectively). Compared with the previous study, utilising thiourea (0.1 M, pH 1.5), the results indicate that the presence of this oxidant increases the elution of gold from the biomass by 52.5%.

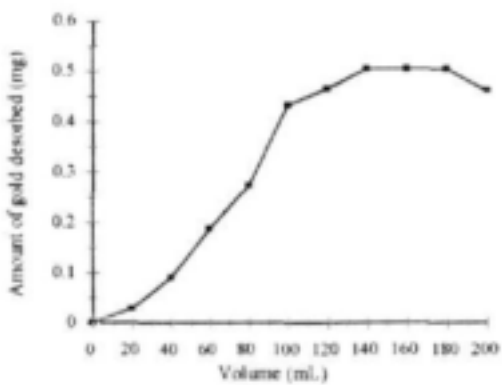


Figure 4.16a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.02 M $FeCl_3$. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

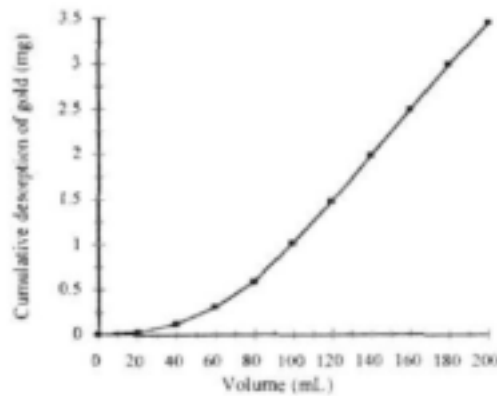


Figure 4.16b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.02 M $FeCl_3$. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.11. Thiourea (8%), Perchloric acid (2%) ($HClO_4$) and 0.1 M HCl (0.5%)

Perchloric acid is an extremely powerful oxdising agent which reacts with most organic materials and rapidly oxidises silver and gold (Greenwood and Earnshaw, 1989). Darnall *et al.* (1988) utilised this desorbent to elute gold from resin. Since a combination of thiourea and oxidant increased the removal rate of gold from the biomass by up to four fold the use of perchloric acid was included. Maximum removal rate was found after 100-120 mL eluant (Figure 4.17a), while the results in Figure 4.17b indicate a total removal of 5.00 mg (100%) of gold from the biomass occurred. The slight discrepancy in the bell shaped curve not reaching zero at 200 mL, may be due to inherent insensitivity of the atomic absorption spectrophotometer at such dilute

concentrations of gold. This data shows that the use of a powerful oxidising agent gives a rate of oxidation of the metal which is sufficient for almost complete solubilisation to occur within the 200 mL volume of eluant used.

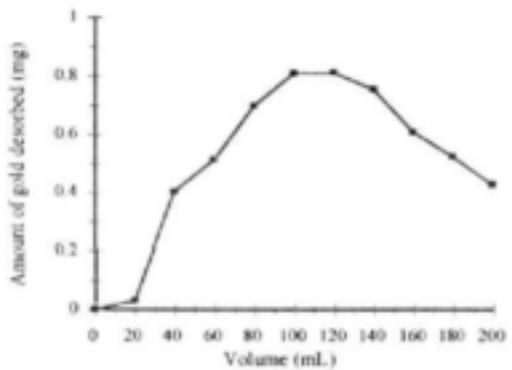


Figure 4.17a: Amount of gold desorbed for every 20 mL fraction utilising 8% thiourea, 2% $HClO_4$ and 0.5% 0.1 M HCl. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

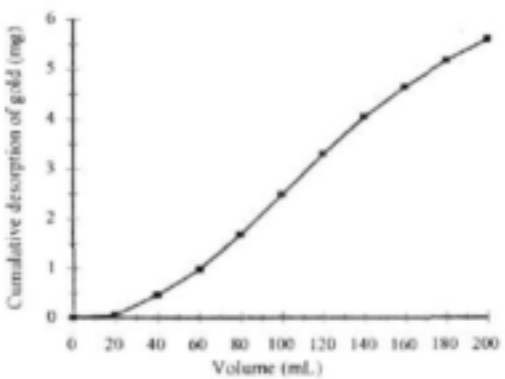


Figure 4.17b: Cumulative desorption of gold utilising 8% thiourea, 2% $HClO_4$ and 0.5% 0.1 M HCl. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.3.1.2.12. Thiourea (8%), 0.06 M Nitric acid, and 0.1 M HCl (0.5%)

A comparison of nitric acid as an alternative to perchloric acid at the same concentration was studied. Nitric acid is also known to be a strong oxidising agent as well as a more economical alternative to perchloric acid. While nitric acid on its own did not result in significant gold desorption used in combination with thiourea (Figure 4.18a) a significant removal occurred at a maximum of 140 mL. A total of 2.863 mg (56.5%) of gold was removed from the biomass within the 200 mL (Figure 4.18b) of desorbent. The study indicates that although nitric acid is more economical, it is not as strong an oxidising agent as perchloric acid, and therefore not as efficient.

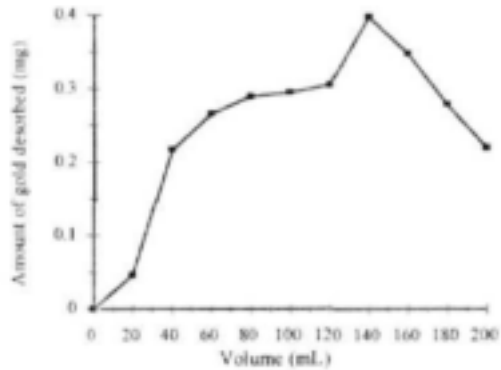


Figure 4.18a: Amount of gold desorbed for every 20 mL fraction utilising 8% thiourea, 0.06 M HNO_3 and 0.5% 0.1 M HCl. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

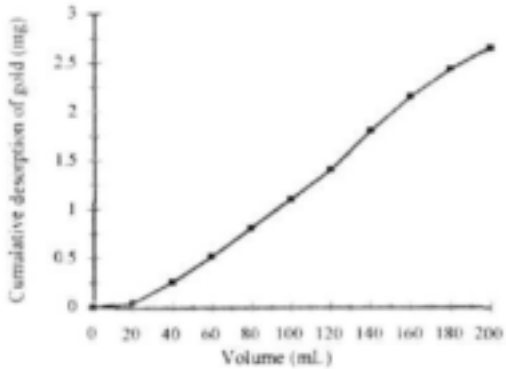


Figure 4.18b: Cumulative desorption of gold utilising 8% thiourea, 0.06 M HNO_3 and 0.5% 0.1 M HCl. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

4.4. SUMMARY

Most biosorption processes employ either packed- or fixed-bed reactors utilising an up- or down-flow modes. The process whereby a metal-laden solution is immobilised on a non-viable biosorbent of choice followed by elution of the metal in a concentrated solution is very similar to the ion-exchange process. If the process of adsorption is electrostatic the desorption of the metals then becomes a simple matter allowing for the use of mineral acids which are economical. The choice of the recovery solution is important in that it should be non-destructive and allow for regeneration of the biomass to be utilised in multiple adsorption/desorption cycles without loss of efficiency. A critical factor for removal of metals is the volume of elutant to the volume of solution treated. The smaller volume of elutant allows for a more concentrated solution, lowering disposal costs, as well as multiple re-use of the biosorbent, and further recovery (Banks, 1997; Garnham, 1997; Volesky, 1990).

Various batch studies were conducted in an attempt to understand the binding and desorption characteristics of gold. To lower the disposal volume, an investigation into the ratios of adsorbent to desorbent were undertaken utilising acidic thiourea, thiourea in combination with oxidants such as ammonium peroxodisulphate and perchloric acid. Of the ratios investigated (3:1, 2:1 and 1:1) the 1:1 ratio was the most effective in the removal of gold from the biomass and it was found that the oxidising agents enhanced the process. Since gold(III) has been known to undergo a change in speciation over time, this possibility was investigated. Incubating the biomass with gold(III) for a period of 1 and 24 hours, followed with desorption, indicated that the gold speciation had not changed significantly.

Although thiourea is a strong complexing agent, enhancement of its ability to desorb gold in combination with oxygen, air and nitrogen was demonstrated. Maximum removal of gold(III) from solution of greater than 93% was achieved, whilst desorption results indicated that thiourea in the presence of an oxygen, air or nitrogen had the following removal of 97%, 86% and 81% respectively. Thus purging the gold bearing biomass with air or oxygen may be important to elute gold from the biomass.

In subsequent column studies, the amount of gold recovered from the biomass for the various desorbents used is summarised in Table 4.1 below.

Table 4.1. Summary of the elutants utilised for the desorption of gold from *Azolla filiculoides*.

Desorbents	*Gold Recovered Utilising 200 mL Eluant(%)*
0.1 M HNO_3	0
0.1 M H_2SO4	2.60
0.1 M EDTA	0.10
0.5 M Mercaptoethanol	2.97
0.1 M KOH	3.76
0.1 M KBr and 20% EtOH	8.54
0.1 M Thiourea	16.50
0.1 M Thiourea and 0.02 M $(NH_4)_2Fe(SO_4)_2$	1.40
0.1 M Thiourea and 0.02 M $NH_4Fe(SO_4)_2$	46.20
0.1 M Thiourea and 0.02 M $NH_4S_2O_8$ pH 1.46 (adj.)	64.50
0.1 M Thiourea and 0.02 M $NH_4S_2O_8$ pH 2.46	75.40
0.1 M Thiourea and 0.02 M $FeCl_3$	69.00
8% Thiourea, 2% $HClO_4$, 0.5% 0.1 M HCl	100.00
8% Thiourea, 0.06 HNO_3 , 0.5% 0.1 M HCl	56.50

adj.: adjusted

The low gold recovery utilising acid desorbents, HNO_3 and H_2SO_4, demonstrated that the final state of the gold bound to the biomass is not simply an acid soluble complex. The low recovery (0.1%) obtained using EDTA is mainly due to its characteristic hard base property which will not be compatible for complexing with gold which is a soft acid. Mercaptoethanol with the presence of only one thiol donor site does not result in enhanced removal of gold (2.97%) most probably due to slow kinetics. Potassium hydroxide, as an elutant, was marginally better with 3.76% removal thus indicating that a small portion of the gold bound may be in the form of a gold oxide complex (Au_2O_3). Potassium bromide and 20% ethanol showed that KBr is a weak chelator of

gold with a removal of 8.54% from the biomass. A removal of 16.5% of gold with thiourea (0.1M) at pH 1.5 indicates that the gold complexed with this particular elutant is in the form of +1 oxidation state. The utilisation of oxidants to increase the desorption of gold from the biomass was more successful. Oxidising agents such as ammonium ferric sulphate, ammonium peroxodisulphate, ferric chloride, perchloric acid and nitric acid all increased the elution of gold from the biomass substantially. Bound gold is oxidised from gold(0) to gold(I) through the interaction with the oxidant and the +1 oxidation state is then optimal for thiourea complexation. This suggests that the majority of gold is bound in the final state as gold(0). The elutants thiourea (8%), perchloric acid (2%) and 0.1 M HCl (0.5%) were the most promising in the removal of gold from the biomass and were thus utilised for subsequent studies.

The utilisation of thiourea in combination with various oxidants, especially perchloric acid, seems to be a viable option since it is able to achieve a high efficiency in the recovery of gold from the biomass. Additional considerations such as cost analysis of ashing of the plant material versus desorption of gold from the biomass would determine the process ultimately utilised.

CHAPTER 5

THE REMOVAL OF GOLD FROM SOLUTION BY THE VIABLE MICROBIAL BIOMASS *Phomas sp.*

5.1. INTRODUCTION

Various types of biomass such as fungi, algae, bacteria, and yeast have been utilized for metal ion sorption (Akthar *et al.*,1995). Microorganisms may bind metals via mechanisms which include bioaccumulation and/or adsorption generally known as bisorption. Adsorption of metals from solution may be achieved by use of dead biomass, inactivated through heat or chemical treatment (Akthar *et al.*,1995; White and Gadd, 1990). The microbial removal and bioaccumulation of metals has received a great deal of interest over the last decade. Fungi have been shown to have considerable capacity to absorb toxic metals such as radionucleoides (thorium and uranium) (White and Gadd, 1990; Treen-Sears *et al.*, 1984; Gadd, 1990), silver, and precious metal ore leachings (Akthar *et al.*,1995). The mechanism of uptake varies and this is largely due to the many morphological fungal types (filamentous or unicellular) (White and Gadd,1990).

Functional groups present on the extracellular polysaccharides on the cell surface of bacterial cells have also shown to accumulate metals and the extent is dependent on the changes in medium composition and excretion of metabolites which can act as metal chelators (Akthar *et al.*, 1995; Gadd,1990; Norberg and Rydin, 1984; Treen-Sears *et al.*,1984; White and Gadd, 1990). The uptake of metals is slow via bioaccumulation although greater amounts of metal can be accumulated. Once inside the cell, metal ions may preferentially be located within specific organelles and/or bind to proteins such as metallothionein (Gadd,1990). Removal of metals through precipitation due to the interaction of metals with excreted polysaccharides or enzymes may also take place (Gadd, 1990; Akthar *et al.*, 1995; Norberg and Rydin, 1984; White and Gadd, 1990). To minimise costs most of the microbial biomass used for biosorption are obtained from industrial waster processes and fermentations (Yetis *et al.*, 1998).The fungus utilized in this study was isolated from natural plant material and has been identified as *Phoma sp.*. It is

reportedly a common indoor air allergen and is commonly found on various plant parts and in soil. Morphological characteristics of this fungus include the production of dark colonies resulting from microscopic dark fruiting structures called pycnidia which harbor one-celled spores. Since the fungus was easily available, the feasibility of its utilisation in gold(III) removal from solution was considered. Investigation into the various parameters affecting uptake such as pH, temperature, biomass and gold concentration were examined. It is proposed that microbe-based technologies may have an important role in environmental protection from metal contamination.

5.2. Materials and Method

5.2.1.1. Materials

5.2.1.1.1. Isolation of the fungi

The fungus was isolated from *Azolla filiculoides* and subsequently purified by subculturing and was identified by the Agricultural Research Council, South Africa. Stock plate cultures were grown up on potato dextrose agar for a period of four to five days at 25°C and stored at 5°C until required. *Phoma sp.* was cultured in rotatory shaking incubator at 25°C using soya peptone (10 g/L), sucrose (10 g/L), ammonium sulphate (1 g/L) and dipotassium hydrogen orthophosphate (2 g/L) at pH 6.5. The biomass was filtered and utilized for subsequent experiments.

5.2.1.1.2. Sorption experiments

All experiments were performed in duplicate. All chemical reagents were of analytical grade (Saarchem, South Africa). Hydrogen tetrachloroaurate(III) solutions were prepared using distilled water. pH adjustments were made using NaOH and HCl.

5.2.1.2. Method

All experiments were conducted in duplicate. Growth curves for the fungus was obtained. Once the optimal growth period was established, the utilization of viable or non-viable (heat-treated) biomass in gold(III) uptake studies was investigated. The sorption experiments were performed by suspending the biomass and gold(III) at the required concentrations in an Erlenmeyer flask (300 mL) and shaking at 200 rpm for a period of three hours. Samples (3 mL) were removed at regular intervals and the gold concentration was measured using an atomic absorption spectrophotometer (GBC 909AA).

In another study, isolated *Phoma sp.*, was filtered (cellulose-acetate, 50 mm, 0.45 µM pore size) and placed in solutions of gold(III) at 10, 80 mg/L for a period of 24 hours, thereafter, the samples were prepared for transmission electron microscopy. The detection of gold in the fungus was determined using Energy Dispersive X-ray Spectroscopy (EDX, 120kV Phillips EM 420, coupled to EDAX DX-4 energy dispersive X-ray system).

5.3. Results

The viable fungus incubated for72 hours was found to be optimal since greater recovery of gold(III) from solution and subsequently utilised for the optimisation studies.

5.3.1. Batch optimization studies

5.3.1.1. Effect of biomass concentration

The percentage removal of gold(III) at biomass concentrations of 1 to 7 mg/mL revealed that the optimal biomass concentrations were 3 and 7 mg/mL with 80% removal. For subsequent experiments the lower concentration of biomass, 3 mg/mL, was utilized (Figure 5.1).

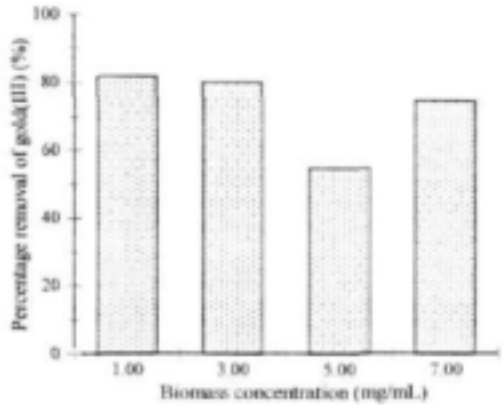


Figure 5.1: The effect biomass concentration on the removal of gold(III) from solution. The following parameters were applied: initial gold(III) concentration of 1 mg/mL, 25°C, pH 2, and agitation speed: 200 rpm.

5.3.1.2. Effect of initial gold(III) concentration on binding

Figure 5.2 indicates that 1 mg/mL gold(III) was found to be optimal with 80% removal occurring. This is significant since this is a typical concentration of gold that is found in mine effluents.

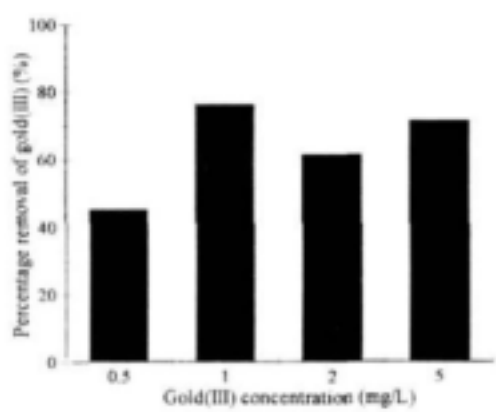


Figure 5.2: The effect of initial gold(III) concentration on the removal of gold(III) from solution. The following parameters were applied: biomass concentration of 3 mg/mL, 25°C, pH 2, and agitation speed: 200 rpm.

5.3.1.3. Effect of pH on binding

Figure 5.3 demonstrates a substantial change in the removal of gold(III) over the pH range investigated, with pH 5 being the most effective. The adsorption of gold is therefore appears to be ionic rather than covalent as shown by the dependency of the removal with pH. The removal may be due to the protonation of the functional groups on the biomass as well as a change in the metal chemistry.

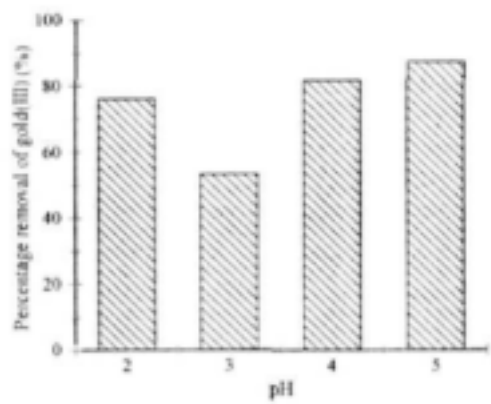


Figure 5.3: The effect of pH on the removal of gold(III) from solution. The following parameters were applied: gold(III) and biomass concentration of 1 mg/mL and 3 mg/mL, pH 2, and agitation speed: 200 rpm.

5.3.1.4. Effect of temperature on binding

Previous biomass studies (Kuyucak and Volesky, 1988) have shown that variation of temperature results in a change in the removal capacity of the biomass for gold. A temperature of 25°C was found to be optimal with 90% removal occurring (Figure 5.4). The dependency on temperature for gold(III) removal from solution indicates that the process is energy-dependent.

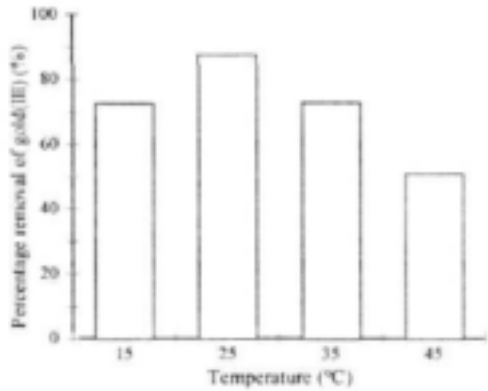
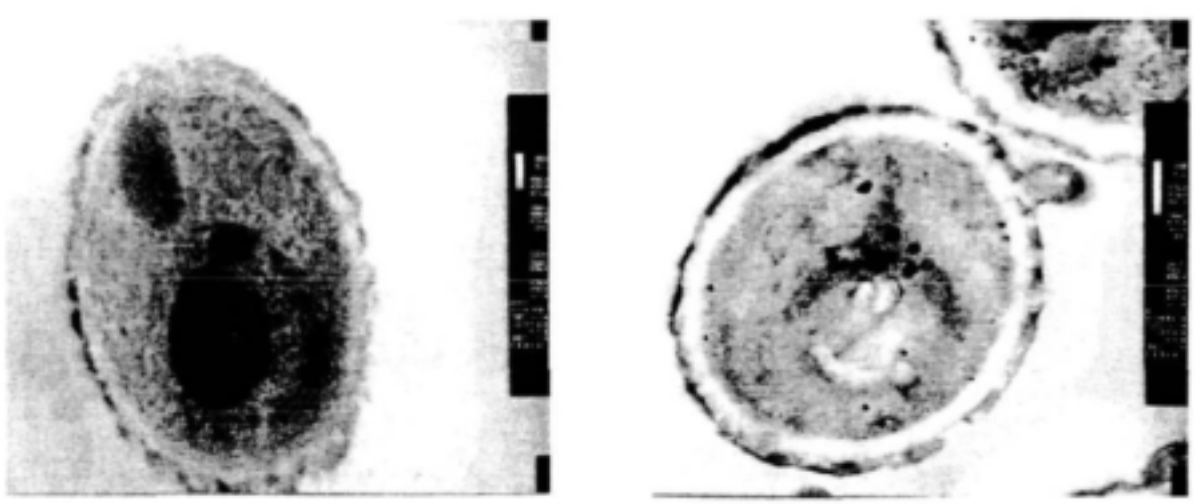


Figure 5.4: The effect of temperature on the removal of gold(III) from solution. The following parameters were applied: gold(III) and biomass concentration of 1 mg/mL and 3 mg/mL respectively, pH 2, and agitation speed: 200 rpm

5.3.2. Electron Microscopy Studies and Energy Dispersive X-ray analysis

The removal of gold(III) is bioaccumulative and adsorptive. Figures 5.5a and 5.5b show random electron dense areas on the cell and the cell surface to be gold precipitates. This is confirmed by energy dispersive X-ray analysis with gold shown by the distinct gold peak (Au) (the copper (Cu) peak is from the grid on which the biomass was placed) (Figure 5.6).



(a) **(b)**

Figure 5.5: Transmission electron micrographs of a cross-section of a *Phoma sp.* cell after exposure to: 10 mg/mL **(a)**, 80 mg/mL **(b)** gold(III) solution for a period of 24 hours at pH 2.

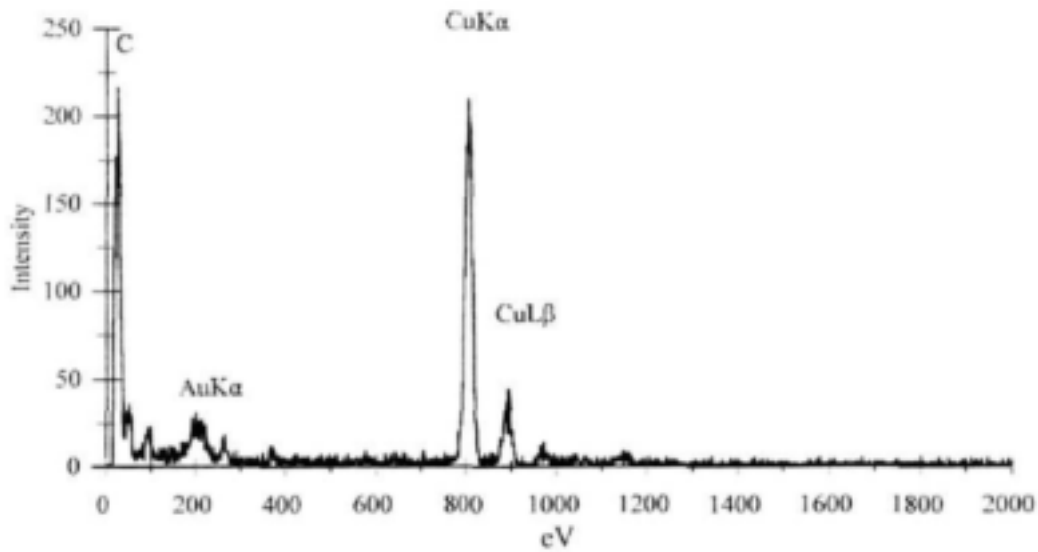


Figure 5.6: Energy Dispersive X-ray analysis of *Phoma sp.*. Abbreviations are as follows: **Au**: gold, **C**: carbon and **Cu**: copper.

5.4. SUMMARY

The fungal species, *Phoma sp.*, is able to remove gold(III) from dilute solutions, typical of the concentrations of gold found in mine effluent. Batch studies indicate that under the following optimal conditions: biomass and gold(III) concentration of 3 mg/mL and 1 mg/mL respectively, pH 5 and temperature of 25°C, the fungus is able to remove 90% of gold(III) from solution. Other studies have also shown that the biomass is capable of removing 60% of gold from effluent wastewater (results not shown). Gold removal is both by bioaccumulation and adsorption, this data demonstrates the feasibility of utilizing the microbial biomass in wastewater bioremediation.

CHAPTER 6

REMOVAL OF PLATINUM(IV) FROM AQUEOUS SOLUTIONS BY *Azolla filiculoides*

6.1. INTRODUCTION

The biosorption process of accumulating metals via active or non-active processes has been utilised for the removal and/or recovery of precious metals such as gold from aqueous solutions. Darnall *et al.* (1986), Greene *et al.* (1986a), Gee and Dudeney (1988), Kuyucak and Volesky (1988a) and Brierley and Vance (1988) have all demonstrated that gold(III) is able to be both adsorbed by various viable and non-viable biomasses from dilute simulated solutions as well as from waste water. The ability of *Azolla filiculoides* to adsorb gold(III) efficiently and rapidly has led to an explorative study to determine whether similar results would be obtained for another precious metal, platinum. As a valuable commodity with international reserves in the region of 939 million ounces for the next fifty years, platinum's importance in various industries such as medical research and catalytic converters will focus research on improving its value and recovery over the next few years (Cottington, 2000).

As shown in Chapters 2-4 the biomass is capable of rapidly removing the anionic gold(III) complex from aqueous solutions with high affinity. As a soft acid capable of pH-independent binding, its adsorption characteristics differ from other base metals. Similar characteristics are possibly expected with platinum. However, for any covalent bonding, square planar $[PtCl_4]^{2-}$ would act similarly to $[AuCl_4]^-$ but $[PtCl_6]^{2-}$ is octahedral and thus its binding to the biomass may be different. Square planar Pt(II) and Au(III) complexes can possibly bind to a donor atom (from tannins for example) to form a trigonal bipyramidal intermediate by an associative mechanism. For $[PtCl_6]^{2-}$ it would require the loss of a ligand such as a chloride before binding occurs to the biomass and thus is required to use a dissociative mechanism of adsorption. Since Pt(IV) is found to occur in the platinum recovery process, it was decided to utilise the +4 oxidation state in the present study using synthetic solutions, this is in contrast to the bioremediation study reported by

Greene *et al.* (1987) who investigated the adsorption of Pt(II).

The present study involves use of a novel method for the recovery of platinum from simulated waste solutions and ultimately from mine waste solutions by a plant material. The biomass of choice is *Azolla filiculoides* and as previously discussed, the main impetus for its utilisation is its excellent biosorption characteristics, ready availability and its present status as a "weed", which provides an incentive for the plant material to be harvested (Hill, 1998). Greene *et al.* (1987) previously demonstrated that the binding of tetrachloroplatinate(II), $[PtCl_4]^{2-}$, to *Chlorella vulgaris* was pH-dependent, with maximum adsorption at pH 2.

Batch and column systems were investigated in this study. Optimal conditions involved in the adsorption of chloroplatinic acid(IV) from solution in batch reactors were determined. The parameters investigated were: biomass concentration, initial platinum(IV) concentration, pH and temperature. Once optimal conditions were established, the utilisation of a packed column for recovery was explored, with parameters such as flow-rates and initial platinum(IV) concentrations being investigated. Once the optimal conditions were established, the plant material's viability in the treatment of platinum refinery effluent was investigated in batch and column systems.

6.2. Batch Optimisation Studies

6.2.1. Materials and Method

6.2.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 2.2.1.1. Chloroplatinic acid(IV) was obtained from Saarchem, South Africa. Platinum atomic absorption standards were prepared from a platinum atomic absorption solution (1000 mg/L) (Wirsam, South Africa) and diluted with deionised water until the desired concentration was achieved.

6.2.1.2. Method

All experiments were conducted in duplicate. Biomass (1, 3, 5 and 7 g/L) and platinum(IV) (5, 10, 15 and 20 mg/L) concentrations were adjusted according to the respective experiment. A volume of 100 mL of a specific platinum(IV) and biomass concentration were placed in an Erlenmeyer flask (300 mL) and constantly agitated at 200 rpm at room temperature. Aliquots (3 mL) were withdrawn at regular intervals (every five minutes for the first hour, every ten minutes for the second hour and every twenty minutes for the final third hour) and filtered using cellulose-acetate filters (25 mm diameter, 0.45 μM pore size). The filtrate was then analysed for platinum using AA spectrophotometry. In the pH study, the pH was adjusted every half-hour with NaOH or HCl. In the temperature study, the flasks were shaken in a thermostatically-controlled incubator (Labcon, South Africa). Control experiments were conducted using platinum(IV) solutions in the absence of biomass to exclude the possibility of platinum precipitation. The results were expressed as percentage removal of platinum(IV) from solution.

6.2.2. Results and Discussion

Greene *et al.* (1987) found that maximum removal of Pt(II) occurred at pH 2, most likely due to Pt(II) existing as an anionic complex. For this reason the following studies were initiated at this pH.

6.2.2.1. Effect of biomass concentration

The results in Figure 6.1 demonstrate that the percentage removal of platinum(IV) is dependent on the biomass concentration over the range investigated. Maximum metal removal occurred within 5 minutes with 77.9%, 69.0%, 59.1%, and 49.5% removal for 1, 3, 5 and 7 g/L respectively after the three hour incubation period. Consequently, a biomass concentration of 1 g/L was utilised for all subsequent experiments. The data suggests that there may be a limited number of binding sites for platinum(IV) at pH 2.

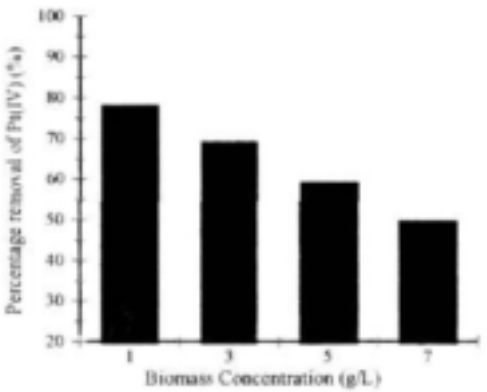


Figure 6.1. Effect of biomass concentrations on the adsorption of platinum(IV) by *Azolla filiculoides*. The following parameters were utilised: room temperature, agitation speed of 200 rpm, platinum(IV) concentration of 15 mg/L and pH 2.

6.2.2.2. Effect of initial concentration of chloroplatinic acid(IV)

Concentrations of platinum found in waste water are usually in the region of 1-10 mg/L. Due to inherent insensitivity of the atomic absorption spectrophotometer to platinum, concentrations in the region of 5-20 mg/L (at intervals of 5 mg/L) were utilised at pH 2. The results in Figure 6.2 show that the removal rates of platinum(IV) from solution increased, i.e., 56.4%, 75.8%, 85.2% and 89.0% as the concentration of the initial platinum(IV) increased (5, 10, 15 and 20 mg/L respectively). At an initial concentration of 5 mg/L of Pt(IV) the rate of removal gradually decreased as the experiment reached its conclusion which may suggest that Pt(IV) may be reduced to Pt(II) by the various functional groups present on the biomass surface. Since 20 mg/L was found to have a removal of 89%, this concentration was utilised in the subsequent experiments.

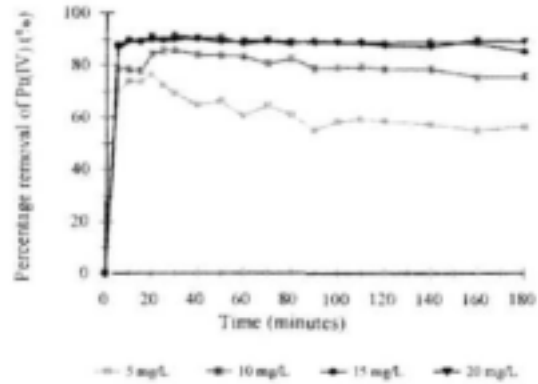


Figure 6.2: Effect of initial platinum(IV) concentration on the adsorption of platinum(IV) by *Azolla filiculoides*. The following parameters were utilised: room temperature, agitation speed of 200 rpm, biomass concentration of 1 g/L and pH 2.

6.2.2.3. Effect of pH

Platinum(IV) is "class-b" or "soft" in character and is capable of being reduced to Pt(II) with various ligands such as *P* or *As*-donors. This suggests that the binding of the metal is likely to be covalent and pH-independent and invariably not influenced by the presence of ions such as H^+ in the surrounding aqueous environment (Greenwood and Earnshaw, 1989). But Pt(IV), as a soft acid, although slightly harder than Pt(II), may have a tendency to bind to hard bases such as oxygen-donor ligands, for example OH^-. The addition of alkali to the solution therefore resulted in up to a 50% platinum precipitation at pH's 3, 4 and 5. The percent removal of platinum from the pH-adjusted solution was therefore calculated after determining the platinum concentration on pH-adjustment and again after adsorption. Figure 6.3 demonstrates that as the pH increases, i.e., at 2, 3, 4 and 5, the removal of platinum from solution decreased from 89.0%, to 66.2%, 57.1%, and 41.3% respectively. It seems that the addition of alkali forms a range of hydroxide complexes containing the halide according to the following formula: $[PtX_n(OH)_{6-n}]^{2-}$ where X= Cl^- or Br^- and thus facilitates conditions for precipitation (Greenwood and Earnshaw, 1989; Hartley, 1973). Comparing the rate of removal curves of platinum(IV) and gold(III), there is a similar low removal at pH 5 suggesting lower protonation of *Azolla* sites and therefore a loss of

ionic bonding of the anionic complex $[PtCl_6]^{2-}$ (or $[PtCl_4]^{2-}$). The more rapid equilibrium (not as slow as seen for gold(III) at pH's 5 and 6, Chapter 2, Figure 2.3) suggests the rapid formation of insoluble hydroxy species with platinum at pH 3, 4 and 5 (Greenwood and Earnshaw, 1989). Note that at pH 2, the rate of binding of platinum(IV) to *Azolla* (Figure 6.3) is faster than gold(III) but is not as efficient. A pH of 2 was utilised for further experiments.

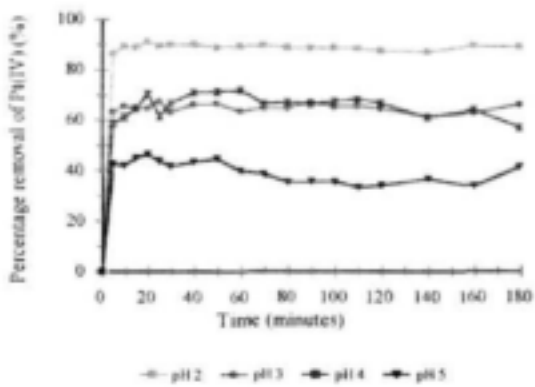


Figure 6.3: Effect of pH on the adsorption of platinum(IV) by *Azolla filiculoides*. The following parameters were utilised: room temperature, agitation speed of 200 rpm, platinum(IV) concentration of 20 mg/L and biomass concentration of 1 g/L.

6.2.2.4. Effect of temperature

Previous studies using non-viable *Azolla filiculoides* with lead(II) and gold(III) have shown the metal removal is not temperature-dependent (Antunes *et al.*, 2001; Sanyahumbi *et al.*, 1998). In studies on the removal of Pt(IV) at temperatures of 10°, 20°, 30° and 40°C a removal of 86.1%, 91.0%, 83.1% and 90.0% occurred respectively (Figure 6.4). The removal remained fairly constant over the temperature range investigated suggesting that the adsorption process is energy-independent.

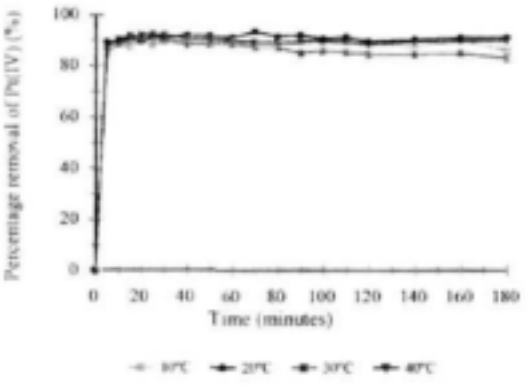


Figure 6.4: Effect of temperature on the adsorption of platinum(IV) by *Azolla filiculoides*. The following parameters were utilised: agitation speed of 200 rpm, platinum(IV) concentration of 20 mg/L, biomass concentration of 1 g/L and pH 2.

6.3. Column Optimisation Studies

6.3.1. Materials and Method

6.3.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 3.2.1.1. Chloroplatinic acid(IV) was obtained from Saarchem, South Africa. Platinum atomic absorption standards were prepared from a platinum atomic absorption solution (1000 mg/L) (Wirsam, South Africa). The standards were diluted with deionised water until the final concentration was achieved.

6.3.1.2. Method

All experimental work was conducted in duplicate. Platinum(IV) solutions at concentrations of 10, 20, 40 and 60 mg/L were utilised. A solution (1 litre) was then pumped through a packed column in an up-flow mode containing 1 g of whole *Azolla filiculoides* in a bed volume of 10 mL. The solution was pumped at the desired flow-rate (2, 5, 10 and 20 mL/min). The pH of the

solution was adjusted prior to adsorption to 2. Samples (5 mL) were then collected at regular intervals (every 20 mL for the first 100 mL, thereafter every 100 mL) using a fraction collector and analysed for platinum using an AA spectrophotometer. The results were expressed as percentage removal (effluent concentration relative to initial platinum concentration) of Pt(IV) from solution.

6.3.2. Results and Discussion

6.3.2.1. Effect of flow-rate on the adsorption of chloroplatinic acid(IV)

Batch studies demonstrated that pH 2 was optimal for platinum(IV) adsorption (Figure 6.3). For this reason it was decided to utilise this pH for the following studies. The effect of flow-rate on the adsorption of platinum(IV) from solution is seen in Figure 6.5. Maximum removal occurred within 100 mL and a removal of 50.3%, 56.3%, 58.7% and 57.0% at the end of the study was obtained for the 2, 5, 10 and 20 mL/min flow-rates respectively. The percentage removal of the metal gradually decreases for all flow-rates investigated at the end of the experimental period. This may be due to a speciation change via the reduction of Pt(IV) to Pt(II) which may be followed by desorption from the oxygen donors due to loss of hardness (Pt(IV) to Pt(II)), or else, due to a change in electrostatic attraction. Since maximum adsorption occurred at a 10 mL/min flow-rate, this flow-rate was utilised for subsequent studies.

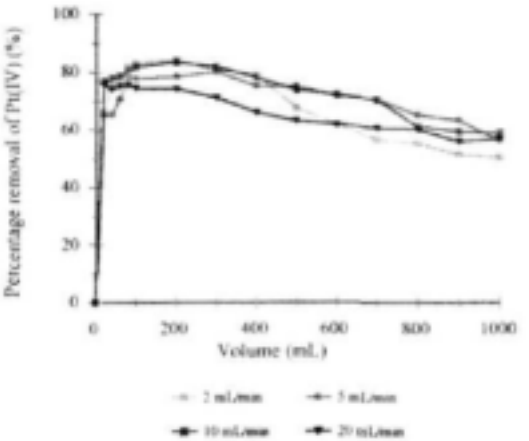


Figure 6.5: Effect of flow-rate on the removal of platinum(IV) from solution by *Azolla filiculoides*. The following parameters were utilised: biomass concentration: 1 g/L, platinum(IV) concentration of 20 mg/L, pH 2 and room temperature.

6.3.2.2. Effect of initial platinum(IV) concentration at pH 2

Maximum removal occurred within the first 20 mL fraction for all initial metal concentrations except for 10 mg/L where equilibration between the biomass and the metal was slightly inhibited and a maximum removal of 85.0% (500 mL fraction) occurred before gradually decreasing to 76.2% (Figure 6.6). A maximum removal of 91.5% at 20 mg/L was found with a gradual decline to 82%. At concentrations of 40 and 60 mg/L a maximum removal of 81.5% and 85.4% occurred respectively, followed by an immediate decrease in removal to 50% and 41% respectively. This may be as a result of the gradual saturation of the column. At these two concentrations a total of 40 and 60 mg respectively had passed through the column. The maximum uptake capacity of *Azolla* for platinum(IV) at room temperature was found to be 25 mg/g biomass (data not presented).

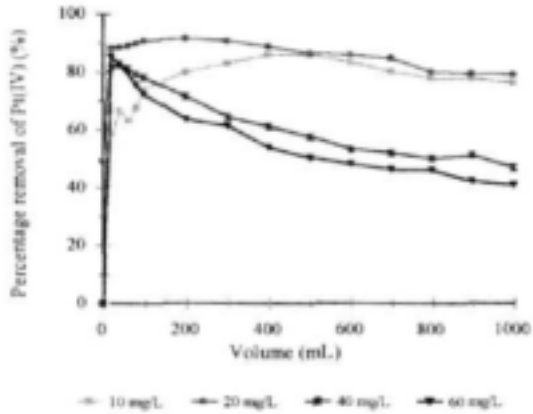


Figure 6.6: Effect of initial platinum(IV) concentration on the removal of Pt(IV) from solution by *Azolla filiculoides*. The following parameters were utilised: biomass concentration: 1 g/L, pH 2, flow-rate of 10 mL/min and room temperature.

6.4. Platinum Effluent Studies

Sections 6.2 and 6.3 have shown that platinum was able to be adsorbed with high affinity from solution. The aim of the following studies were to determine whether *Azolla filiculoides* would be able to remove various metals, such as platinum, from a waste water solution obtained from Mine C.

6.4.1 Batch Studies

6.4.1.1. Materials and Method

6.4.1.1.1. Materials

The plant material was obtained and prepared as described in Section 2.2.1.1. The platinum effluent was obtained from a platinum refinery, Mine C, and utilised with no modification. The five metals, other than platinum presented in Table 6.1. depict the highest concentrations found in the effluent.

6.4.1.1.2. Method

The study was carried out in duplicate. A volume of 100 mL of effluent was placed in contact with *Azolla* (1 g/L) in an Erlenmeyer flask and constantly agitated at 200 rpm for a period of 3 hours at room temperature. Aliquots (3 mL) were removed at regular intervals and filtered using nylon filters (25 mm diameter, 0.45 μM pore size). The concentrations of the non-precious metals were analysed utilising an AA spectrophotometer. Due to the presence of a low concentration of platinum in the effluent, ICP-MS (Perkin-Elmer) was utilised for this metal. Sulphate and chloride levels were analysed with the Spectraquant® sulphate and chloride test kits (Appendix II and III respectively) (Merck, Germany). The results were expressed as percentage removal from solution for each of the metals studied.

6.4.1.2. Results and Discussion

Due to the low pH of the effluent (approximately 0.10), the removal of metals such as iron, zinc and copper were minimal. The presence of large amounts of Cl^- ions may also play an important role in the non-removal of these hard acids. Lead, as a borderline acid, had a 25% removal, while nickel removal is impeded in contrast to the gold study, possibly due to the high concentrations of these anions present. A removal of 22.5% of platinum occurred. The pH decreased by 0.03 units.

Table 6.1. Percentage removal of metal from the waste water of Mine C in batch studies

Metal	*Pre-Treatment (mg/L)*	*Post -Treatment (mg/L)*	*Percentage Removal (%)*	**Max. Specifications (mg/L)*
Iron (Fe)	598.87	598.13	0.12	9.00
Zinc (Zn)	0.74	0.74	0	0.70
Lead (Pb)	0.28	0.21	25.00	0.10
Copper (Cu)	4.31	4.24	1.62	0.10
Nickel (Ni)	0.62	0.62	0	1.14
Platinum (Pt)	0.0267	0.0207	22.47	-
Choride (Cl^-)	60 040.00	52 820.00	12.03	NA
Sulphate (SO_4^{2-})	NS	NS	-	NA
pH	0.12	0.09	-	9.00

* Maximum specifications for Aquatic Ecosystems (DWAF, 1998), **NS:** not significant, **NA**: not available.

6.4.2. Column Studies

6.4.2.1. Materials and Method

6.4.2.1.1. Materials

The plant material was obtained and prepared as described in Section 3.2.1.1. The platinum effluent was obtained from a platinum refinery, Mine C, and utilised without modification. The metals chosen for this study was described in Section 6.4.1.1.1 and is represented in Table 6.2.

6.4.2.1.2. Method

The study was carried out in duplicate. A volume of 1 litre of platinum effluent was pumped in an up-flow mode through a packed column containing whole *Azolla* (1 g/L). A flow-rate of 10 mL/min was utilised (optimal). Samples (5 mL) were collected at regular intervals utilising a

fraction collector. The concentration of the non-precious metals present were analysed utilising an AA spectrophotometer. Platinum, due to the low concentration in the effluent, was analysed with ICP-MS (Perkin-Elmer). The results were expressed as percentage removal (effluent concentration relative to influent platinum concentration) from solution for each of the metals studied.

6.4.2.2. Results and Discussion

The low pH seems to have a large influence on the removal of the metals studied as no significant adsorption of iron, lead, copper and nickel (Table 6.2) occurred. Zinc removal was found to be 8% from solution. Platinum corresponded closely with the batch studies in that 21% removal occurred, thus suggesting that residence time does not play a role, as with gold in the gold effluent, in the adsorption process and thus a column process could be employed. A decrease of 0.07 pH units occurred.

Table 6.2. Percentage removal of metal from the waste water of Mine C in column studies

Metal	*Pre-Treatment (mg/L)*	*Post-Treatment (mg/L)*	*Percentage Removal (%)*	**Max. Specifications (mg/L)*
Iron (Fe)	594.82	594.52	0	9.00
Zinc (Zn)	0.76	0.70	7.89	0.70
Lead (Pb)	0.19	0.19	0	0.10
Copper (Cu)	3.75	3.69	1.60	0.10
Nickel (Ni)	0.66	0.66	0	1.14
Platinum (Pt)	0.0233	0.0184	21.03	-
Choride (Cl^-)	26 030.00	22 023.00	15.39	NA
Sulphate (SO_4^{2-})	NS	NS	-	NA
pH	*0.11*	*0.04*	-	*9.00*

* Maximum specifications for Aquatic Ecosystems (DWAF, 1998). **NS:** not significant, **NA**: not available.

6.5. SUMMARY

The demand for platinum in the world, for jewellery, catalysis and medicinal purposes is ever increasing, and with its limited reserves the demand for recovery from waste water has been expanding. Preliminary batch and column studies on the ability of *Azolla filiculoides* to adsorb platinum(IV) were investigated.

In batch studies various parameters were investigated. A range of biomass concentrations (1, 3, 5 and 7 g/L) demonstrated that platinum removal decreased as the biomass concentration increased suggesting a limited number of sites. At initial platinum(IV) concentrations of 5- 20 mg/L removal increased as the metal concentration increased, from 56% to 89% respectively. pH had a marked effect on the adsorptive capacity of *Azolla filiculoides* decreasing from 89% to 41% removal between pH 2 and 5. Temperature had no significant effect on adsorption.

For the column studies a pH of 2 was utilised for optimal removal. The maximum removal at various flow-rates (2-20 mL/min) remained constant. A 10 mL/min flow-rate was utilised for further studies. Initial metal concentration studies showed a maximum removal at 20 mL for all concentrations except for the initial concentration of 10 mg/L where equilibration was slightly retarded. At initial 40 and 60 mg/L, removal decreased after maximum which suggests that the saturation of the biomass had been achieved.

For the platinum effluent batch studies, no removal of iron, zinc and nickel was observed, whilst lead and copper showed a 25% and 1.6% removal respectively. Platinum removal was 22.5%. In the effluent column studies no adsorption of iron, lead and nickel was found. Copper, zinc and platinum removal was found to be 1.6%, 7% and 21% respectively. As observed with the gold effluent batch and column adsorption studies, the platinum effluent showed that adsorption of various metals is accompanied by H^+ release.

The utilisation of *Azolla filiculoides* in the recovery of platinum from synthetic solutions at pH 2 has demonstrated a potential viability for the removal and recovery of the metal from aqueous

solutions containing less than 25 mg/L platinum, although further characterisation studies need to be undertaken to determine its feasibility for recovery in waste water when concentrations of the metal are dilute (1 mg/L). The biomass appears to be able to selectively adsorb the metal of interest, platinum, from the effluent. Ultimately a pilot scale study would be required to fully validate the use of *Azolla filiculoides* for the biosorption of platinum.

CHAPTER 7

LEAD REMOVAL FROM AQUEOUS SOLUTION IN BATCH SYSTEMS BY *Azolla filiculoides*

7.1 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.

7.2 Materials and Method

7.2.1 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.

7.2.2. 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.

7.2.3 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.

7.2.4 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.

7.2.5 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 7.1 below.

Table 7.1: 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)

7.3 Results and Discussion

7.3.1 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 7.1). 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 7.1 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.

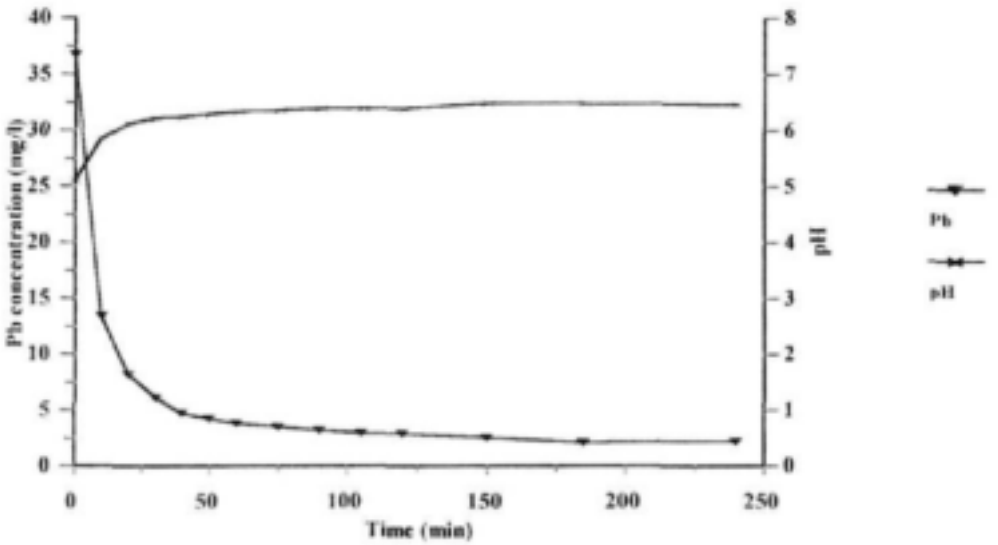


Figure 7.1: Rate of lead removal from aqueous solution and pH profile. Initial Pb concentration, 37 mg/l; biomass concentration, 4 g *Azolla* / l solution; initial pH, 5.1; temperature, 25 °C; shaking rate, 170 rpm.

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).

7.3.2 pH profile for lead nitrate precipitation

A lead nitrate ($PbNO_3$) precipitation profile with respect to pH was generated (figure 7.2), 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.

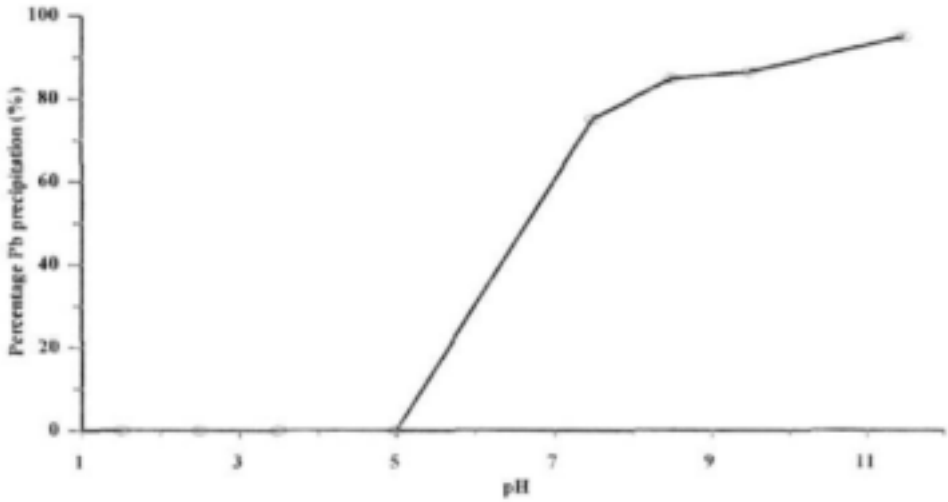


Figure 7.2: Percentage lead precipitation with varying pH values for $Pb(NO_3)_2$. Initial Pb concentration 83 mg/l; temperature, room temperature (~20 °C); NaOH & HNO_3 to adjust pH.

7.3.3 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 7.3 and 7.4). 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 7.3) 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 7.4). 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.

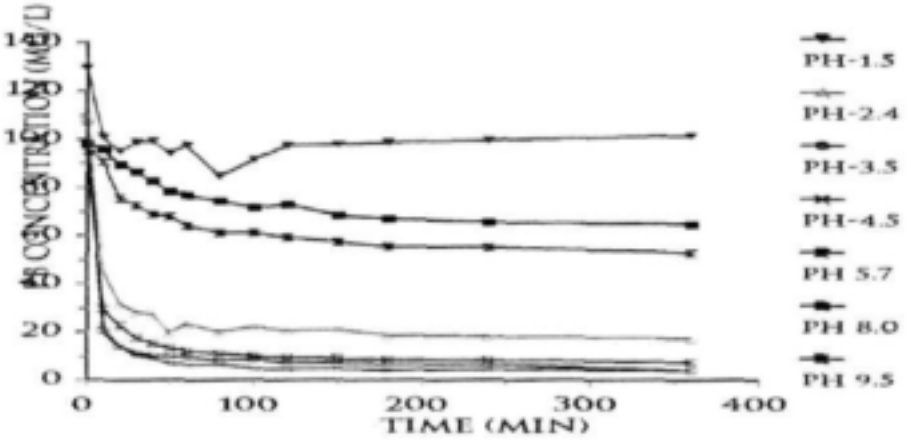


Figure 7.3: The rate of lead removal from aqueous solution with varying pH values. Initial Pb concentration 110 (± 10) mg/l; biomass concentration, 5 g *Azolla* / l solution; temperature, 25 °C; shaking rate, 170rpm.

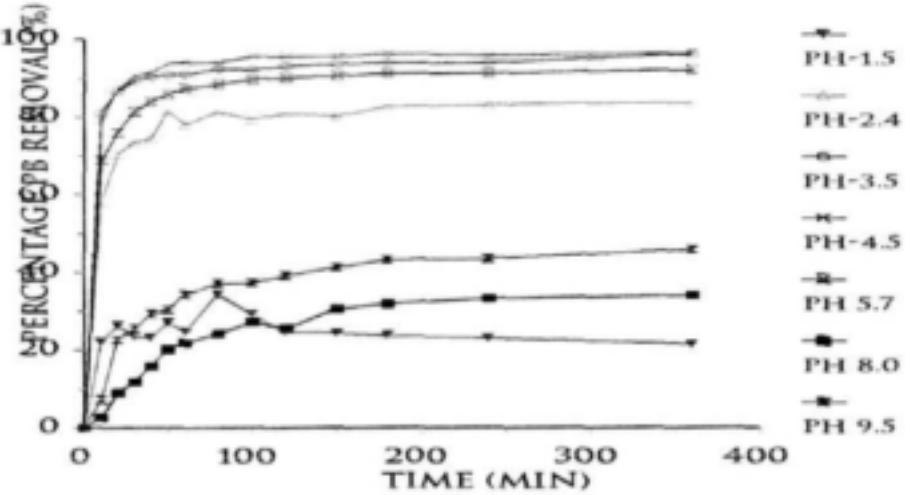


Figure 7.4: Percentage lead removal with time, from aqueous solution with varying pH values. Initial Pb concentration, 110 (± 10) mg/l; biomass concentration, 5 g *Azolla* / l solution; temperature, 25 °C; shaking rate, 170 rpm.

Figure 7.5 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.

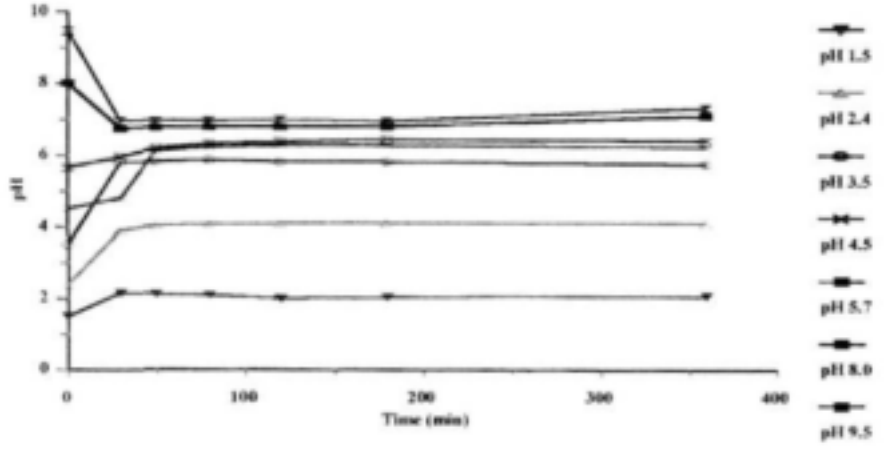


Figure 7.5: pH profiles for lead removal from aqueous solution with varying initial pH values. Initial Pb concentration, 110 (±10) mg/l; biomass concentration, 5 g *Azolla* / l solution; temperature, 25 °C; shaking rate, 170rpm.

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 (1988b) 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 7.5) 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.

7.3.4. Effect of biomass concentration on lead removal

Figure 7.6 shows the concentration of lead remaining in solution with varying biomass concentrations. Figure 7.7 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 g/l, for the system, and a biomass concentration of 5 g/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.

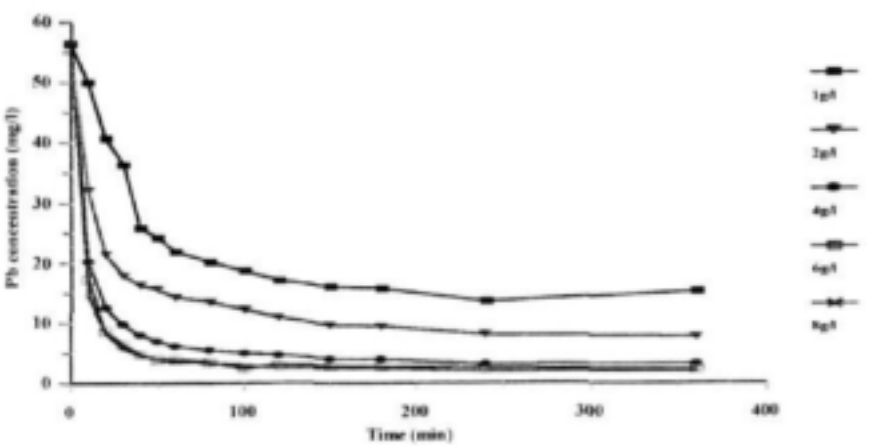


Figure 7.6: Rate of lead removal from aqueous solution with varying concentrations of *Azolla* biomass. pH, 5.7; initial Pb concentration, 56.5 mg/l; temperature, 25 °C; shaking rate, 170 rpm.

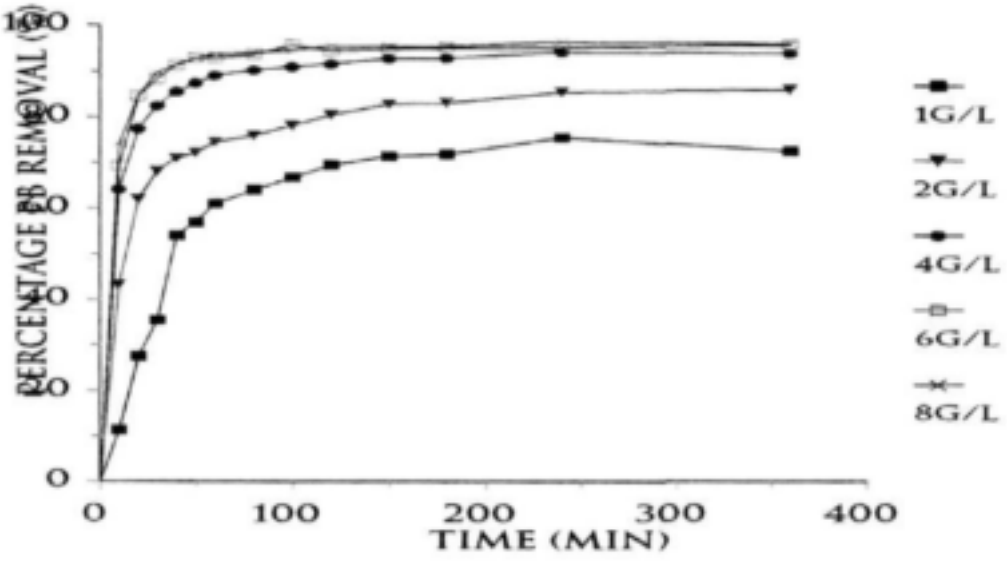


Figure 7.7: Percentage lead removal with time, from aqueous solution with varying concentrations of *Azolla* biomass. pH, 5.7; initial Pb concentration, 56.5 mg/l; temperature, 25 °C; shaking rate, 170 rpm.

7.3.5 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 7.8 and 7.9). Figure 7.8 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 7.9, 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.

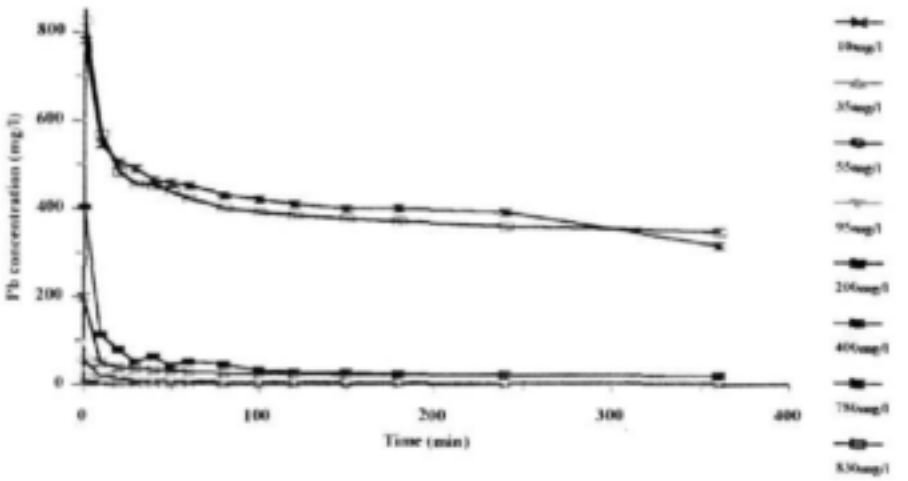


Figure 7.8: Rate of lead removal from aqueous solution with varying initial lead concentrations. pH, 5.7; biomass concentration, 5 g *Azolla* / l solution, temperature, 25 °C; shaking rate, 170 rpm.

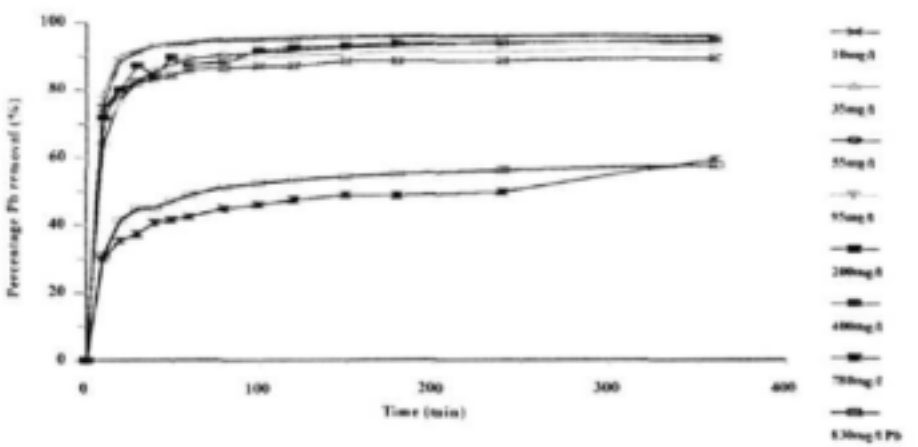


Figure 7.9: Percentage lead removal with time, from aqueous solution with varying initial lead concentrations. pH, 5.7; biomass concentration, 5 g *Azolla* / l solution; temperature, 25 °C; shaking rate, 170 rpm.

7.3.6 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 7.10) nor on the percentage lead removal from aqueous solution (figure 7.11). 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.

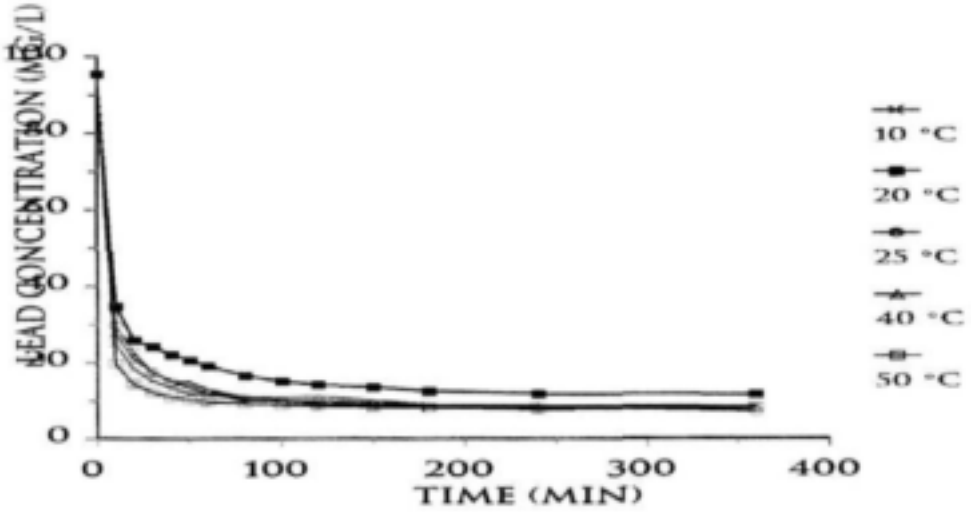


Figure 7.10: Rate of lead removal from aqueous solution with varying temperatures. Initial Pb concentration, 95 mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 4.9; shaking rate, 170 rpm.

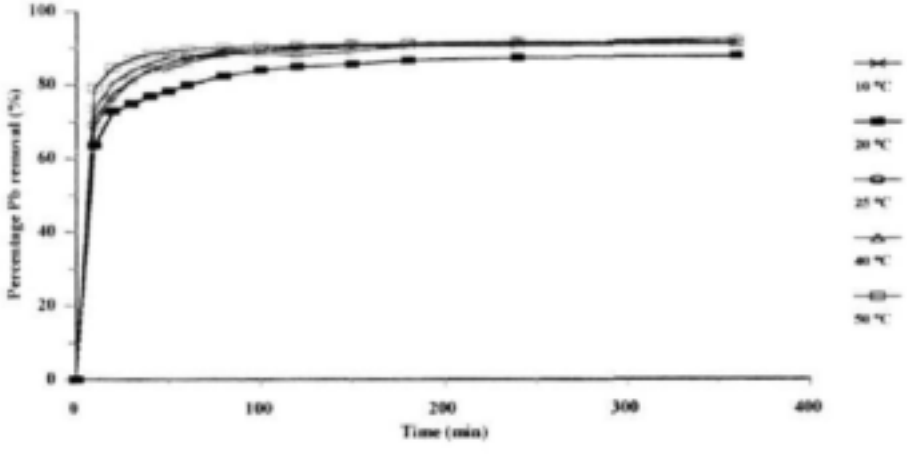


Figure 7.11: Percentage lead removal with time, from aqueous solution with varying temperatures. Initial Pb concentration, 95 mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 4.9; shaking rate, 170 rpm.

7.3.7 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 7.12. 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 7.2 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.

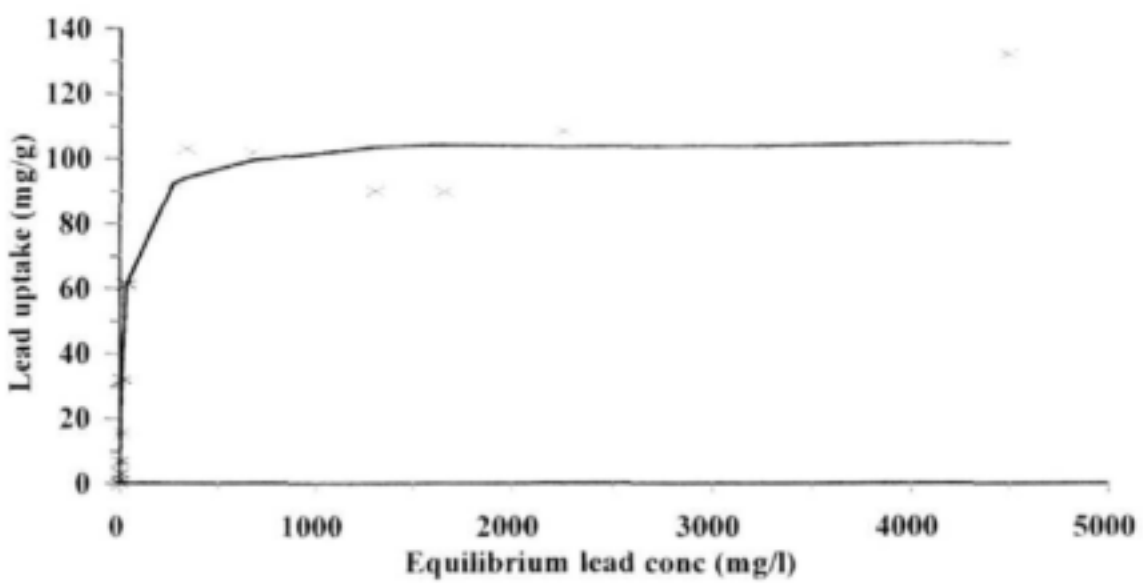


Figure 7.12: Equilibrium sorption isotherm for lead removal from aqueous solution by *Azolla* biomass. pH, 5.2; temperature, 25 °C; shaking rate, 170 rpm.

table 7.2: 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)

7.3.8 Effect of different lead salts on lead removal

Figures 7.13 and 7.14 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 7.14).

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.

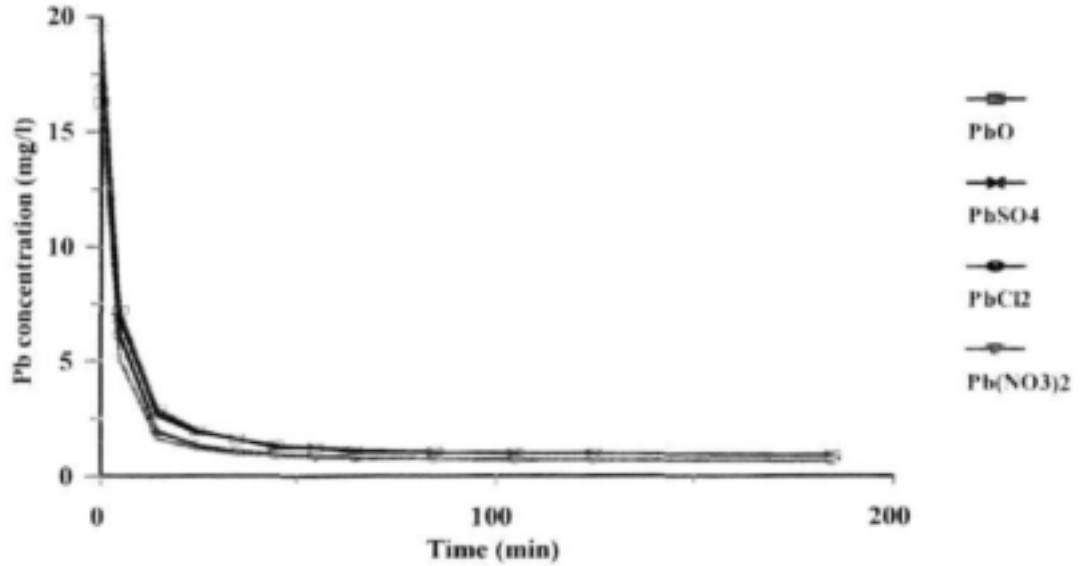


Figure 7.13: Rate of lead removal from aqueous solution with different lead salts. Initial Pb concentration, 17 (±2) mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, 25 °C; shaking rate, 170 rpm.

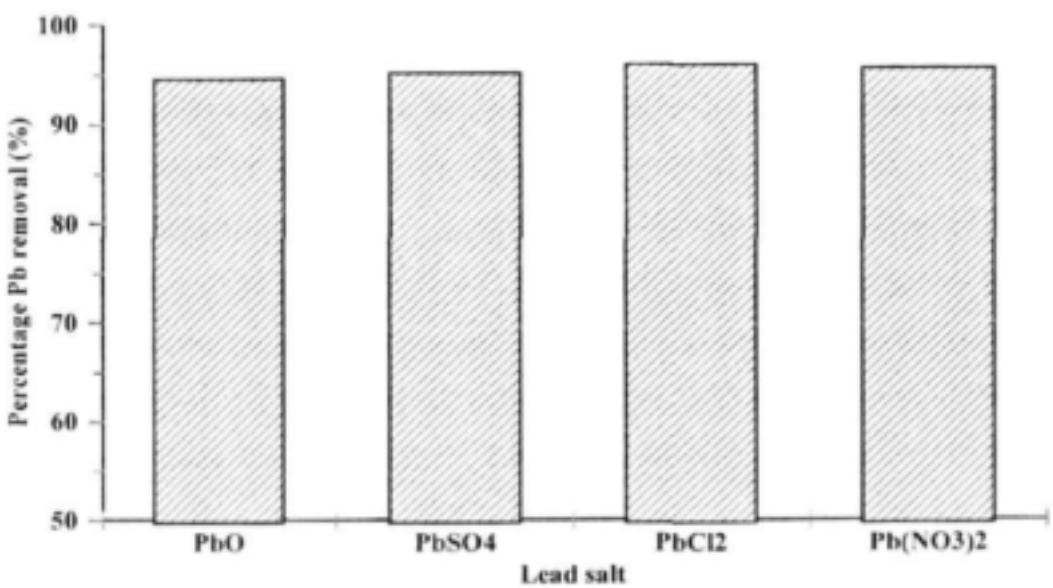


Figure 7.14: Percentage lead removal from aqueous solution after three hours with different lead salts. Initial Pb concentration, 17 (±2) mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, 25 °C; shaking rate, 170 rpm.

7.3.9 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.

7.3.9.1 Precipitation studies

Depending on the lead, copper and iron salt used in the competition studies, some precipitation was observed. Figures 7.15 and 7.16 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 7.15), and approximately 45 to 58 % when added to ferric chloride (figure 7.16). 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.

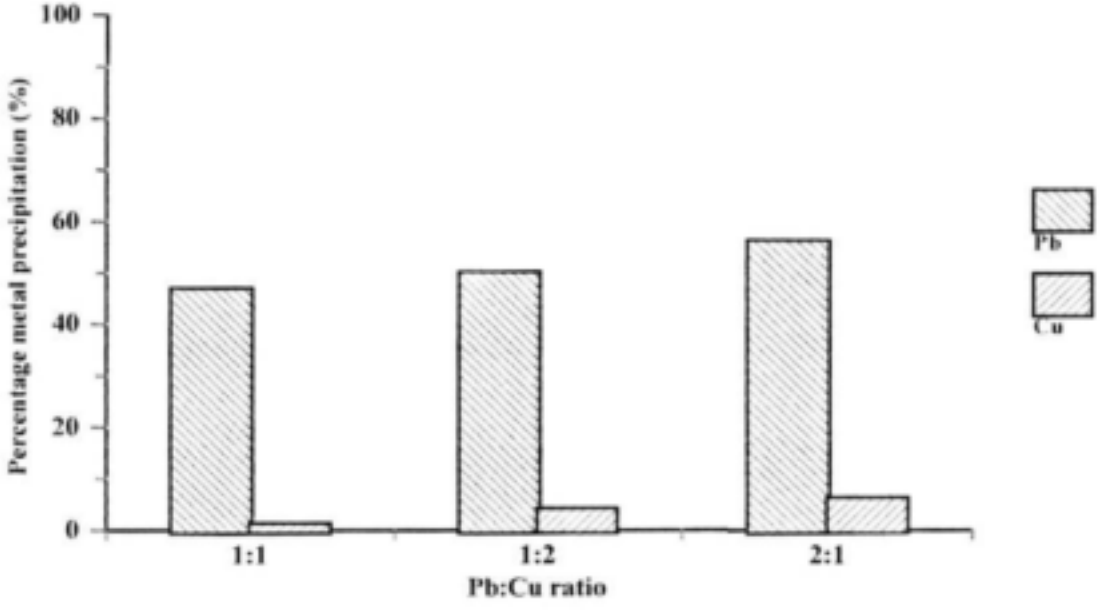


Figure 7.15: Percentage lead and copper precipitation from aqueous solution after 2½ hours. 1 part = 40 mg/l; pH, 5.4; temperature 25 °C, shaking rate, 170 rpm.

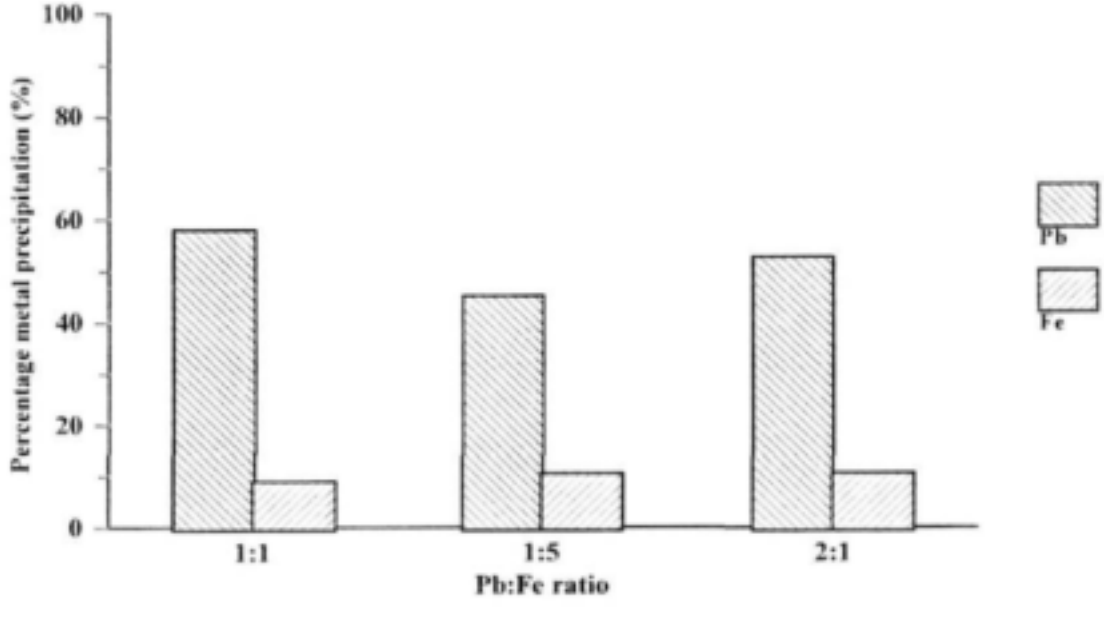


Figure 7.16: Percentage lead and iron precipitation from aqueous solution after 2½ hours. 1 part = 40 mg/l (except for the Pb:Fe=1:5 sample where 1 part = 20 mg/l); pH, 4.2; temperature, 25 °C, shaking rate, 170 rpm.

7.3.9.2 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 7.17 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 7.17).

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.

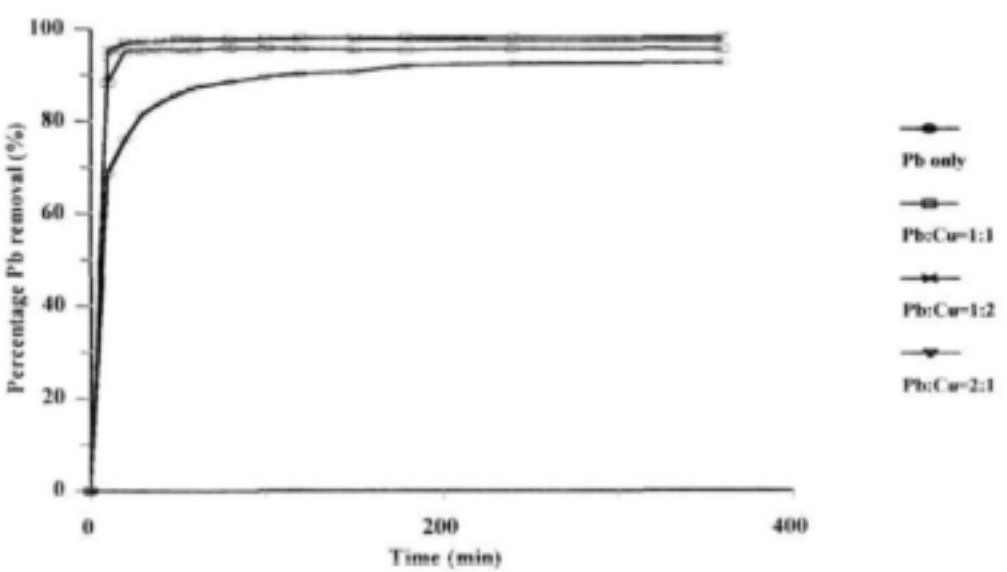


Figure 7.17: Percentage lead removal with time, in the presence of varying initial copper concentrations in aqueous solution. 1 part Pb or Cu = 40 mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 5.1; temperature, 25 °C; shaking rate, 170 rpm.

The effect of lead on percentage copper removal is shown in figure 7.18, 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.

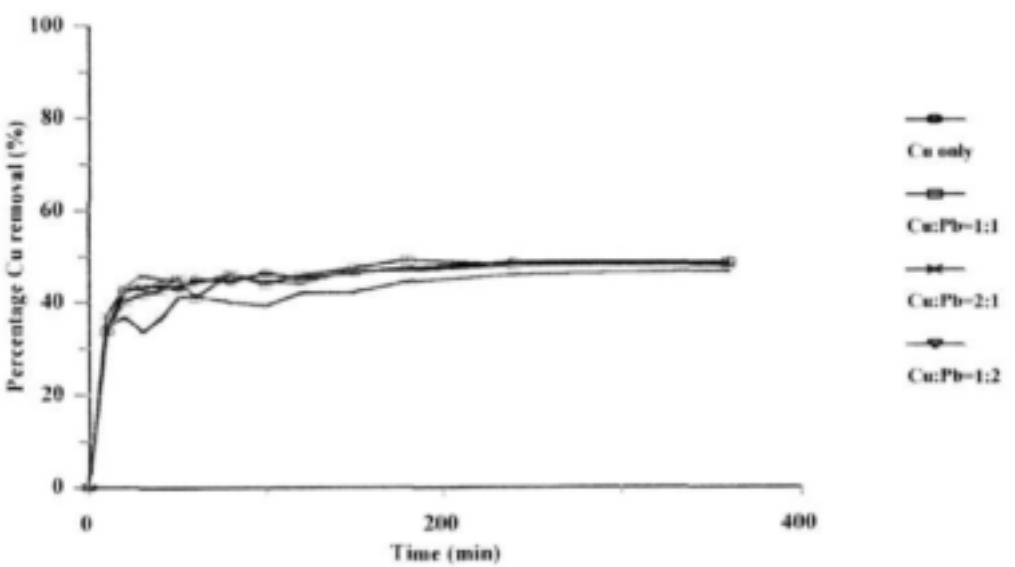


Figure 7.18: Percentage copper removal with time, in the presence of varying initial lead concentrations in aqueous solution. 1 part Cu or Pb = 40 mg/l; biomass concentration, 5 g *Azolla* / l solution; pH, 5.1; temperature, 25 °C; shaking rate, 170 rpm.

7.3.9.3 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 7.19). Figure 7.20 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.

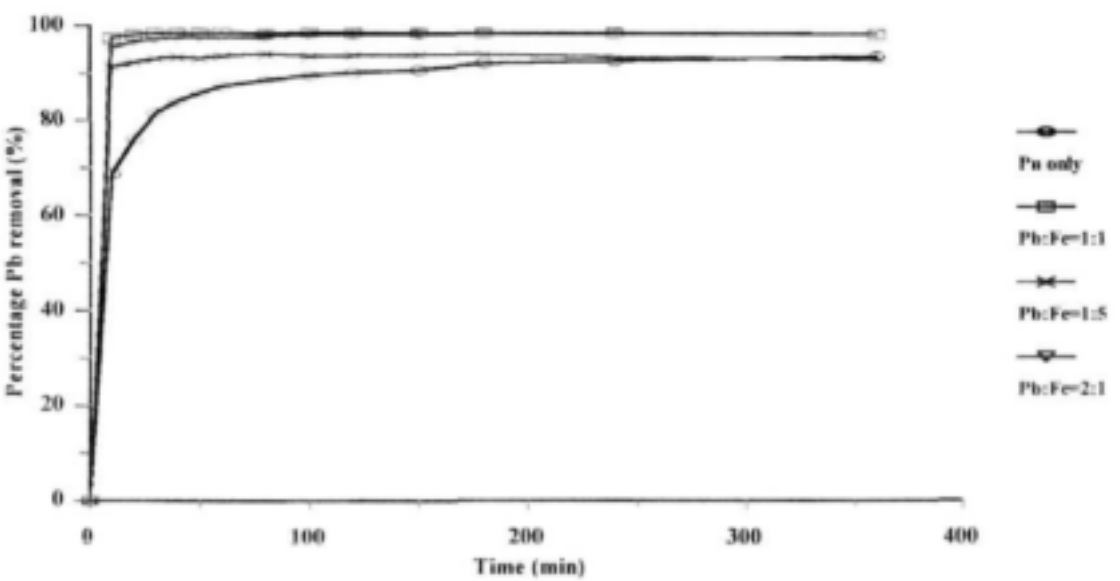


Figure 7.19: Percentage lead removal with time, in the presence of varying initial iron concentrations in aqueous solution. 1 part Pb or Fe = 40 mg/l (except for the Pb:Fe = 1:5 sample where 1 part = 20 mg/l); biomass concentration, 5 g *Azolla* / l solution; pH, 4.7; temperature, 25 °C; shaking rate, 170 rpm.

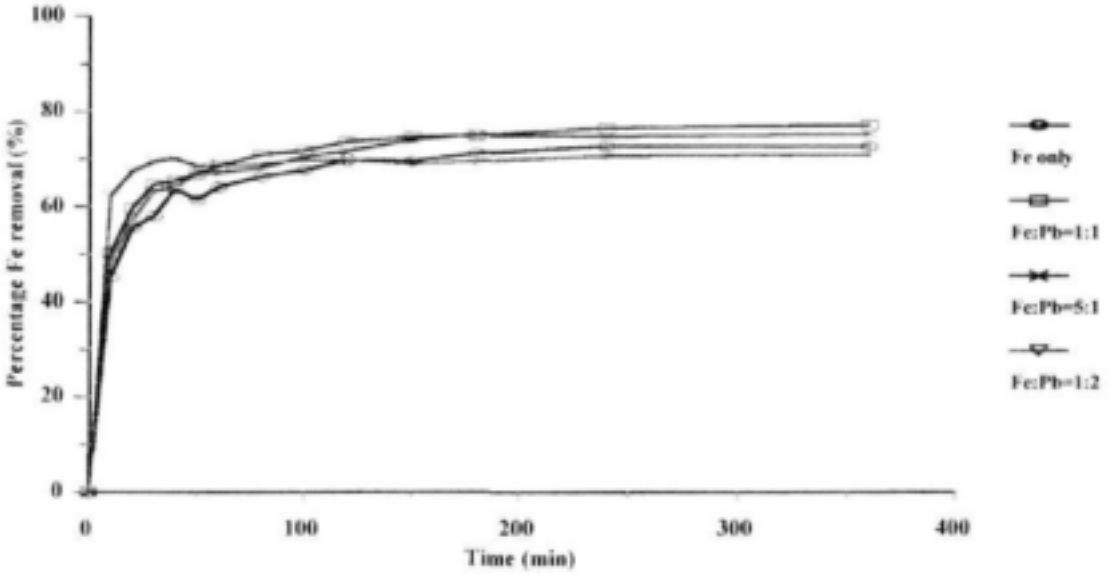


Figure 7.20: Percentage iron removal with time, in the presence of varying initial lead concentrations in aqueous solution. 1 part Fe or Pb = 40 mg/l (except for the Fe:Pb = 5:1 sample, where 1 part = 20 mg/l); biomass concentration, 5 g *Azolla* / l solution; pH, 4.7; temperature, 25 °C, shaking rate, 170 rpm.

7.3.9.4 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 7.21. 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.

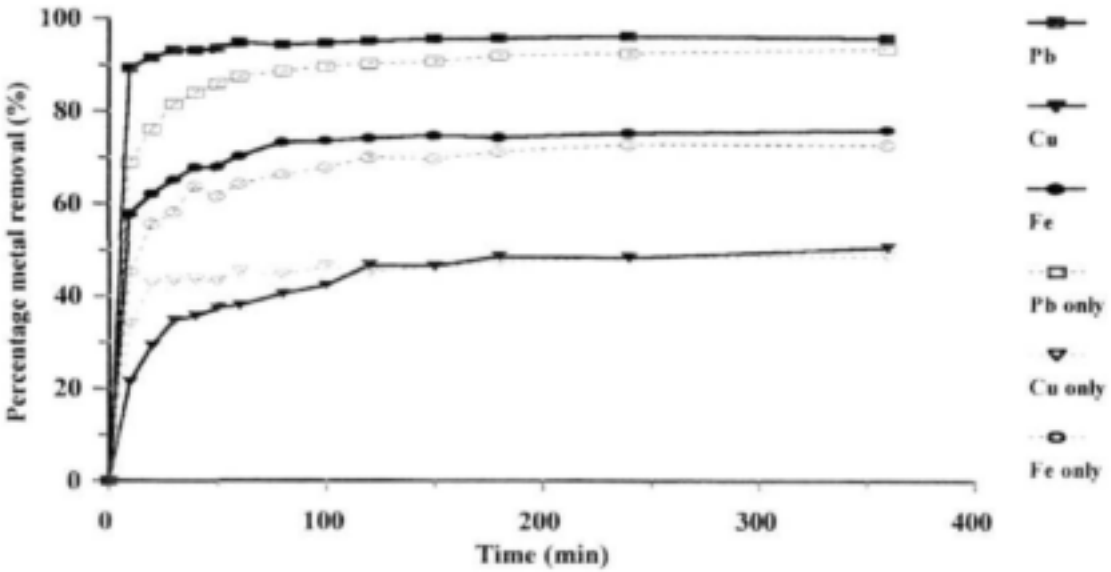


Figure 7.21: Percentage metal removal from a multiple-ions aqueous solution. Pb:Cu:Fe = 1:1:1, one part = 250 μM; single metal concentration, 250 μM; biomass concentration, 5 g *Azolla* / l metal solution; pH, 4.8; temperature, 25 °C; shaking rate, 170 rpm.

7.4 SUMMARY

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 7.2). 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 8

LEAD REMOVAL FROM AQUEOUS SOLUTION IN COLUMN SYSTEMS BY *Azolla filiculoides*

8.1 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.

8.2 Materials and Method

8.2.1. Biomass

Azolla filiculoides biomass was obtained locally and prepared as outlined in chapter 7, with the

exception that dried whole *Azolla* biomass was used instead of ground biomass in subsequent column studies.

8.2.2 Solutions

All solutions were prepared as detailed in chapter 7.

8.2.3 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.

8.2.4 Metal analysis

Analysis of metal in solution was as described in chapter 7.

8.3 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.

8.3.1 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 8.1). 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 8.1 below contains a summary of the data observed in and calculated from figure 8.1.

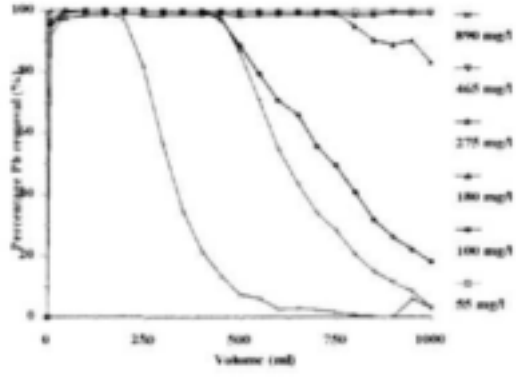


Figure 8.1: Break-through curves for lead removal from aqueous solution, with varying initial lead concentrations. Flow rate, 2 ml/min; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, RT ~ 20 °C, bed volume, 49 ml.

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 *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 8.1: 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 8.1 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.

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

Figure 8.2 shows the break through curves for the same initial lead concentrations as those given in figure 8.1, 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.

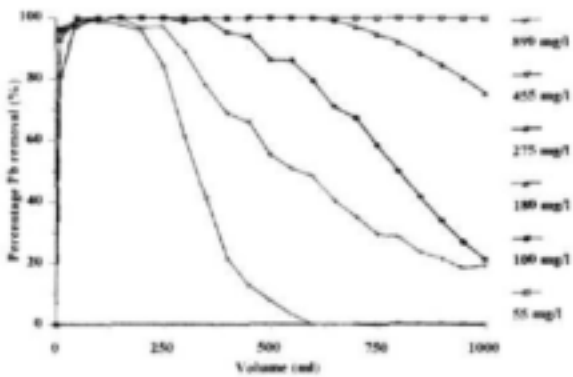


Figure 8.2: Break-through curves for lead removal from aqueous solution, with varying initial lead concentrations. Flow rate, 5 ml/min; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, RT ~ 20 °C; bed volume, 49 ml.

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 8.1). Table 8.2 below gives the data observed and calculated from figure 8.2.

Table 8.2: 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 8.3 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 8.1 at a flow rate of 2 ml/min. This was probably due to the same reasons as explained for figure 8.1, 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%.

8.3.3 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 8.3 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.

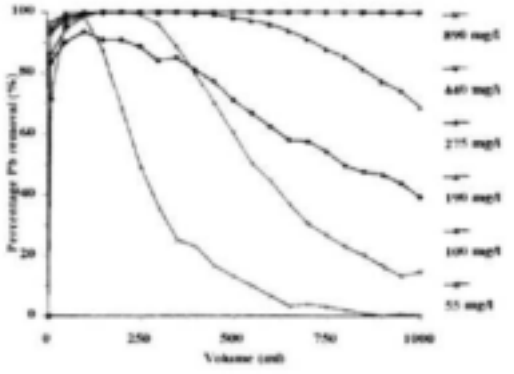


Figure 8.3: Break-through curves for lead removal from aqueous solution with varying initial lead concentrations. Flow rate, 10 ml/min; biomass concentration, 5 g *Azolla* / l solution; pH, 5.2; temperature, RT ~ 20 °C; bed volume, 49 ml.

The data observed and calculated from figure 8.3 is given in table 8.3 below. The break-through points (V_b) given in table 8.3 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 8.3: 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 8.1 to 8.3, 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 8.1-8.3). 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 8.4, 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 8.4: 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

8.3.4 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 8.4 and figure 8.5. 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 8.5) which was mirrored by an increase in the amount of copper in solution (figure 8.4). The percentage lead removal equilibrium values were approximately 100, 95 and 70 % for lead, copper and iron respectively.

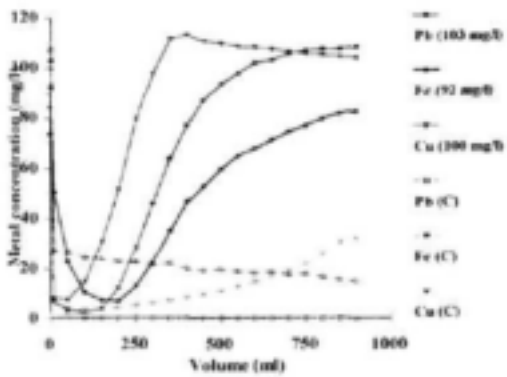


Figure 8.4: Break-through curves for lead, iron and copper removal from multiple-metal aqueous solution at an initial metal concentration of approximately 100 mg/l. Pb(C), Fe(C) and Cu(C) are single-metal control studies. Biomass concentration, 5 g *Azolla* / l solution; pH, 3.0; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

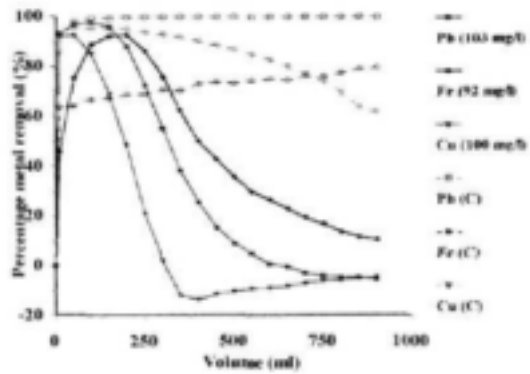


Figure 8.5: Percentage lead, iron and copper removal from multiple-metal aqueous solution at an initial metal concentration of approximately 100 mg/l. Pb(C), Fe(C) and Cu(C) are single-metal control studies. Biomass concentration, 5 g *Azolla* / l solution; pH, 3.0; flow rate, 10 ml/min; temperature, 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 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 8.4 and 8.5).

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

Figures 8.6 and 8.7 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 8.4 and 8.5, where they were 100 mg/l. The trends observed in all three curves were very similar to those observed in figures 8.4 and 8.5 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.

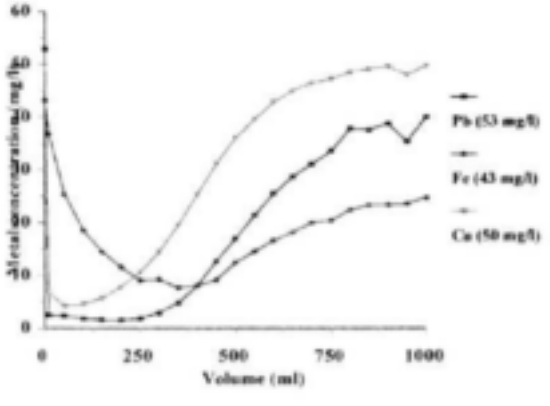


Figure 8.6: Break-through curves for lead, iron and copper removal from multiple-metal aqueous solution, at an initial metal concentration of approximately 50 mg/l. Biomass concentration, 5 g *Azolla* / l solution; pH, 3.0; flow rate, 10 ml/min, temperature, RT – 20 °C; bed volume, 49 ml.

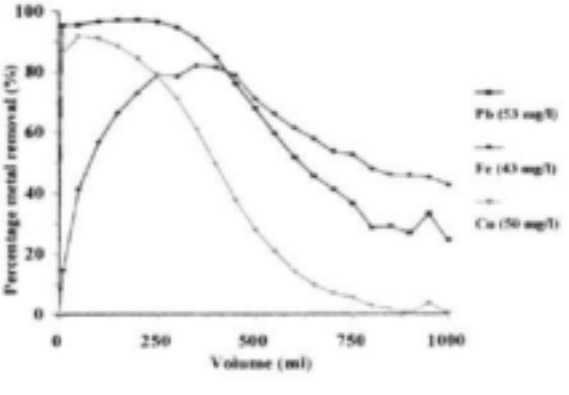


Figure 8.7: Percentage lead, iron and copper removal from multiple-metal aqueous solution at an initial metal concentration of approximately 50 mg/l. Biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; pH, 3.0; temperature, RT – 20 °C; bed volume, 49 ml.

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 8.5: 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.

8.3.6 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 8.8. 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 %.

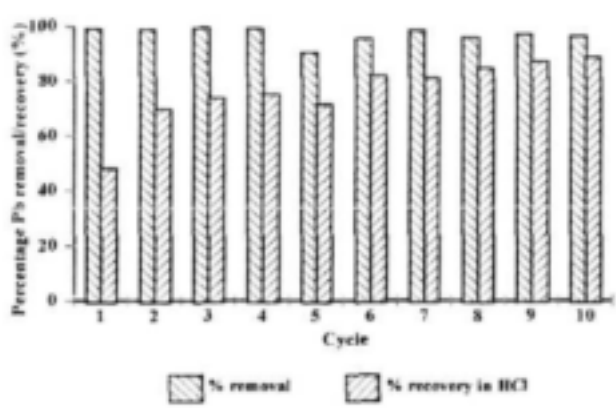


Figure 8.8: Percentage lead removal and recovery for10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50ml 0.1 M HCl as the desorbent. Initial Pb concentration, 100 mg/l; eluant pH, 5.4; biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; reconditioning base, 50 ml 0.05M NaOH, temperature, RT ~ 20 °C, bed volume, 49 ml.

Figure 8.9 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.

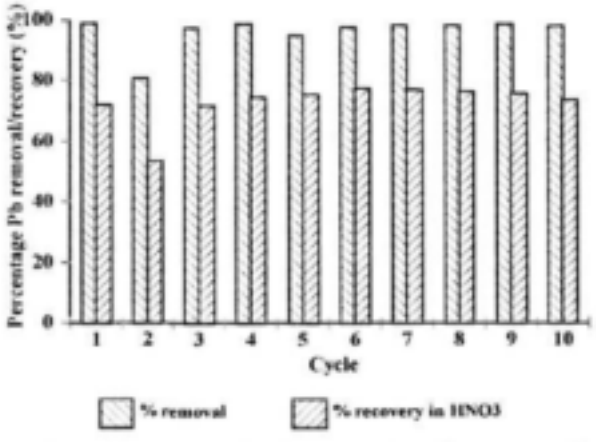


Figure 8.9: Percentage lead removal and recovery for10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml 0.1 M HNO_3 as the desorbent. Initial Pb concentration, 100 mg/l; eluant pH, 5.4; biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; reconditioning base, 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 8.10 and 8.11. 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.

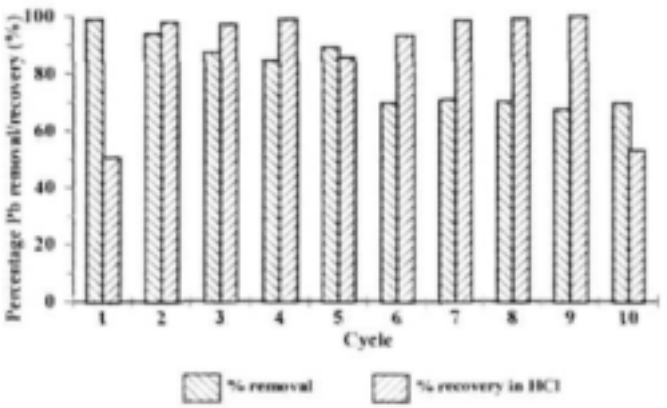


Figure 8.10: Percentage lead removal and recovery for 10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml 0.1 M HCl as the desorbent. Initial Pb concentration, 100 mg/l; eluant pH, 5.4; biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; reconditioning base, 50 ml 0.05M $NaHCO_3$, temperature, RT ~ 20 °C, bed volume, 49 ml.

The adsorption and desorption results using HNO_3 are shown in figure 8.11. 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.

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.

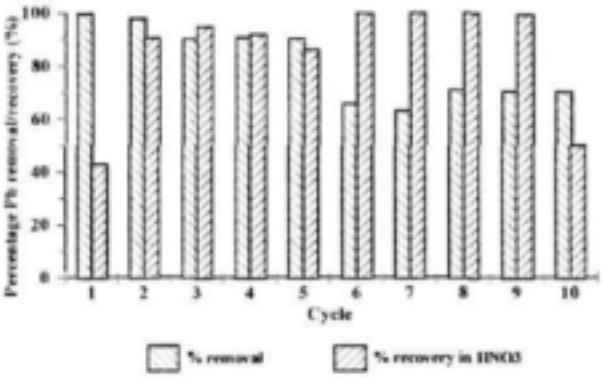


Figure 8.11: Percentage lead removal and recovery for 10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml 0.1 M HNO_3 as the desorbent. Initial Pb concentration, 100 mg/l; eluant pH, 5.4; biomass concentration, 5 g *Azolla* / l solution; flow rate, 10 ml/min; reconditioning 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 ex-change 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.

8.4 SUMMARY

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 9

LEAD REMOVAL FROM EFFLUENT BY *Azolla filiculoides*

9.1 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.

9.2 . Materials and Method

9.2.1 Biomass

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

9.2.2 Solutions

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

9.2.3 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.

9.2.4 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).

9.2.5 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.

9.2.6 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).

9.2.7 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

9.2.8 Metal analysis

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

9.3. Results and Discussion

9.3.1 Metal composition of the lead-acid battery effluent

Table 9.1 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 9.1: 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)

9.3.2 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.

9.3.2.1 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.

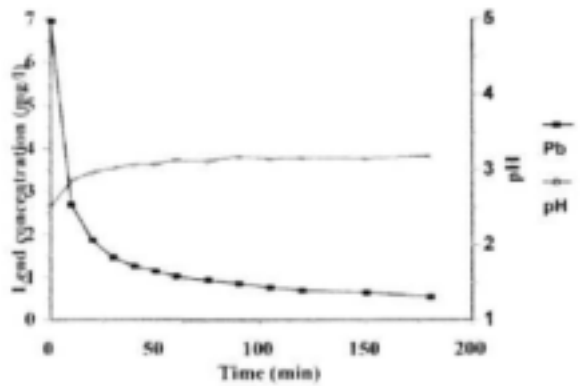


Figure 9.1: Rate of lead removal from effluent A1, and pH profile. Initial Pb concentration, 7.0 mg/l; initial pH, 2.5; biomass concentration, 5 g *Azolla* / 1 effluent; temperature, 25 °C, shaking rate, 170 rpm.

In figure 9.1, 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 9.2 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.

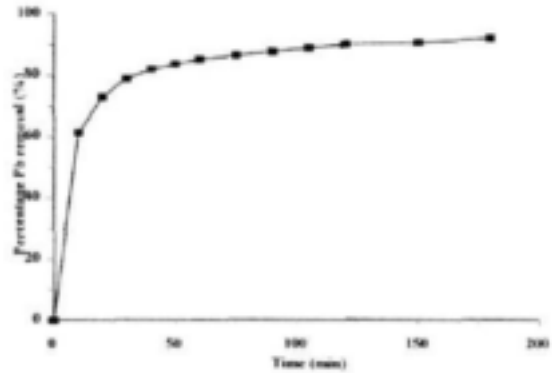


Figure 9.2: Percentage lead removal from effluent A1. Initial Pb concentration, 7.0 mg/l; initial pH, 2.5; biomass concentration, 5 g *Azolla* / 1 effluent; temperature, 25 °C, shaking rate, 170 rpm.

Effluent B1 had a lower lead content (4 mg/l) and a higher initial pH value of 6.3 compared to effluent A1. Figure 9.3 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 7), 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 9.4). 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.

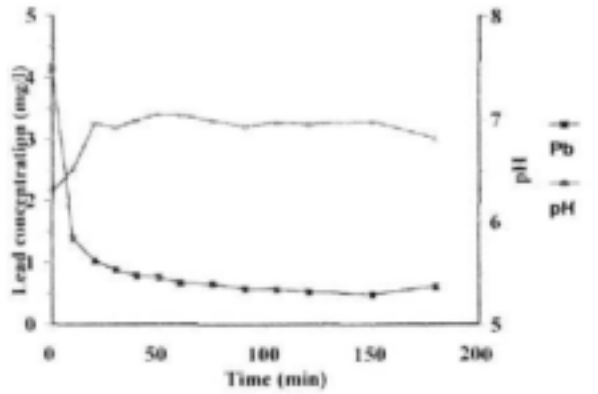


Figure 9.3: Rate of lead removal from effluent B1, and pH profile. Initial Pb concentration, 4.1 mg/l; initial pH,6.3; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

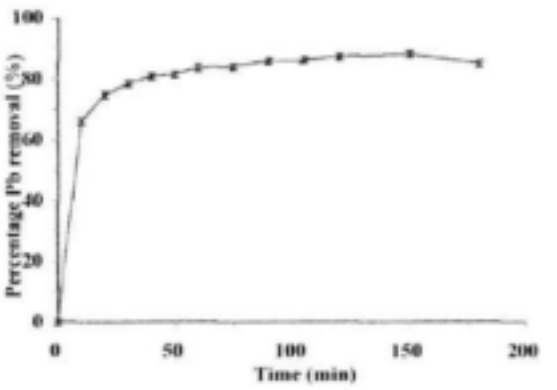


Figure 9.4: Percentage lead removal from effluent B1. Initial Pb concentration, 4.1 mg/l; initial pH, 6.3; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

9.3.2.2 Lead removal from effluent samples A2 and B2

Figures 9.5 and 9.6 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.

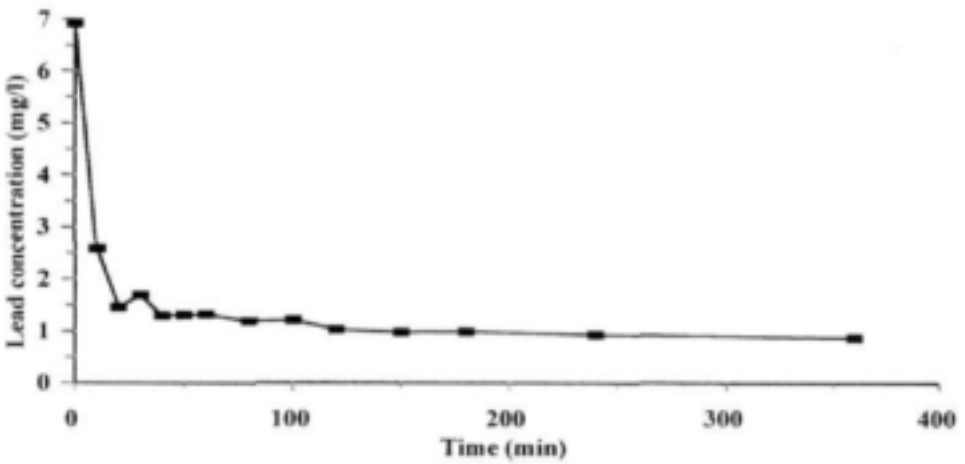


Figure 9.5: Rate of lead removal from effluent A2. Initial Pb concentration, 6.9 mg/l; initial pH, 2.6; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

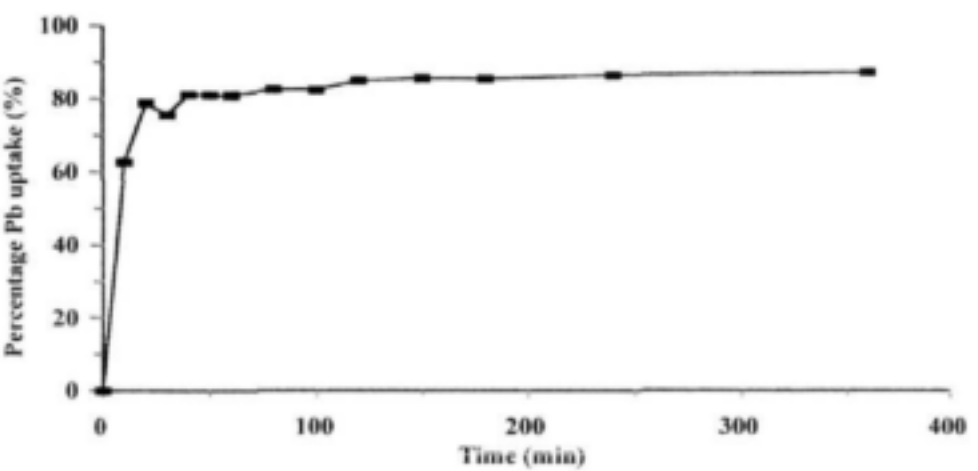


Figure 9.6: Percentage lead removal from effluent A2. Initial Pb concentration, 6.9 mg/l; initial pH, 2.6; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C, shaking rate, 170 rpm.

In figures 9.7 and 9.8 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 9.7). The percentage lead removal equilibrium of approximately 50 % is achieved for effluent B2 (figure 9.8). 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.

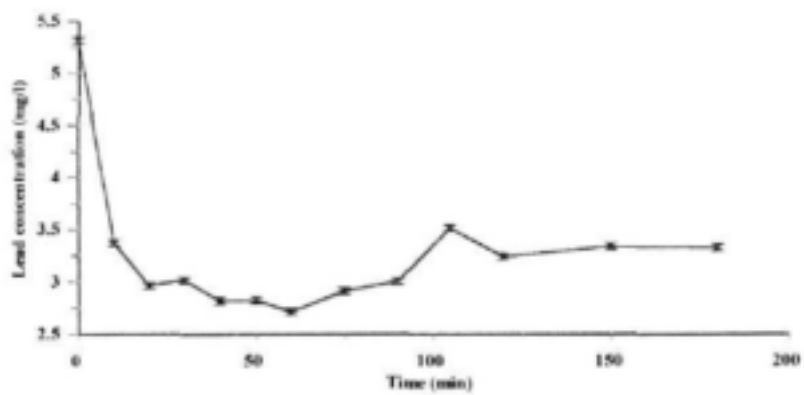


Figure 9.7: Rate of lead removal from effluent B2 by *Azolla* biomass. Initial Pb concentration, 5.3 mg/l; initial pH, 1.4; biomass concentration, 5 g *Azolla* / l effluent; temperature, 25 °C; shaking rate, 170 rpm.

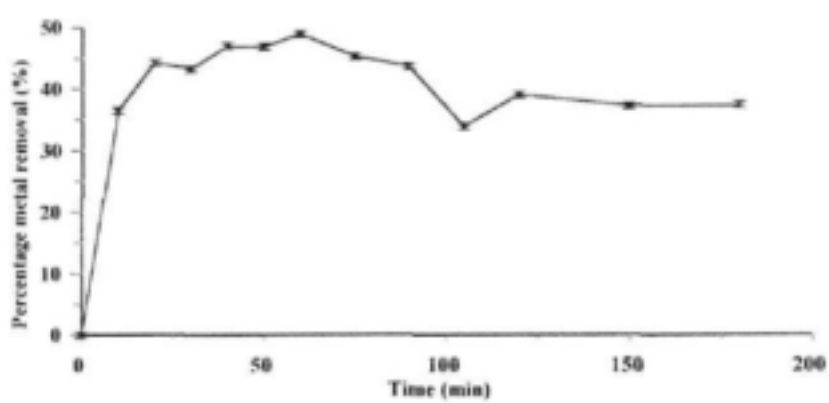


Figure 9.8: Percentage lead removal with time, from effluent B2 by *Azolla* biomass. Initial Pb concentration, 5.3 mg/l; initial pH, 1.4; biomass concentration; 5 g *Azolla* / l effluent; temperature, 25 °C; shaking rate, 170 rpm.

9.3.3 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.

9.3.3.1 Lead removal from effluent in a column system

Figure 9.9 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.

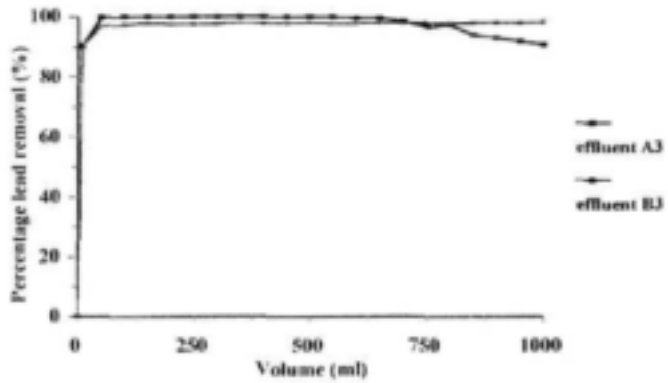


Figure 9.9: Percentage lead removal from effluents A3 and B3, by *Azolla* in column systems. Biomass concentration, 5 g *Azolla* / l effluent; flow rate, 2 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml; initial Pb concentration, 7.5 mg/l (A), 16.6 mg/l (B); initial pH, 2.6 (A), 4.8 (B).

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 9.1).

9.3.3.2 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 9.10 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.

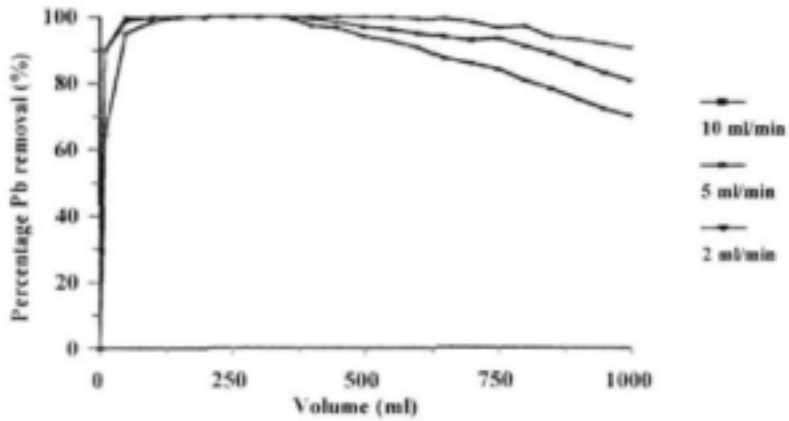


Figure 9.10: Percentage lead removal from effluent A3, at varying flow rates. Initial Pb concentration, 7.5 mg/l, initial pH, 2.6; biomass concentration, 5 g *Azolla* / l effluent; temperature, RT ~ 20 °C; bed volume, 49 ml.

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 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 9.11), 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.

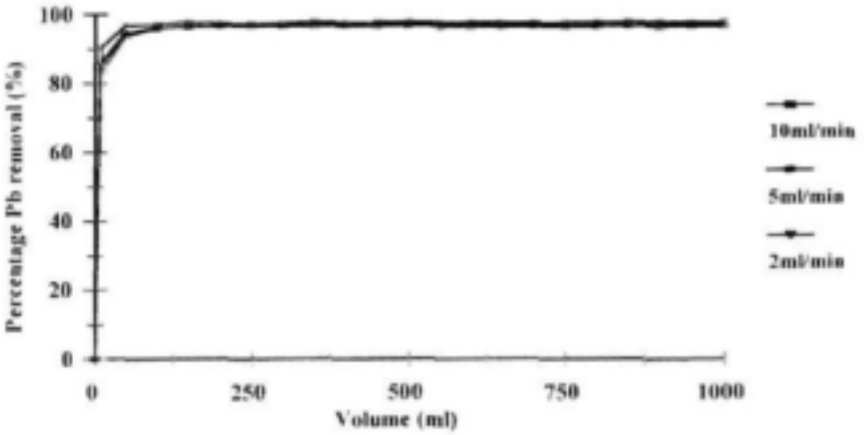


Figure 9.11: Percentage lead removal from effluent B3, at varying flow rates. Initial Pb concentration, 16.6 mg/l, initial pH, 4.8; biomass concentration, 5 g *Azolla* / l effluent; temperature, *RT* ~ 20 °C; bed volume, 49 ml.

9.3.3.3 Effect of flow rate on lead uptake capacity

Figures 9.12 and 9.13 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 9.12 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 9.13). 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.

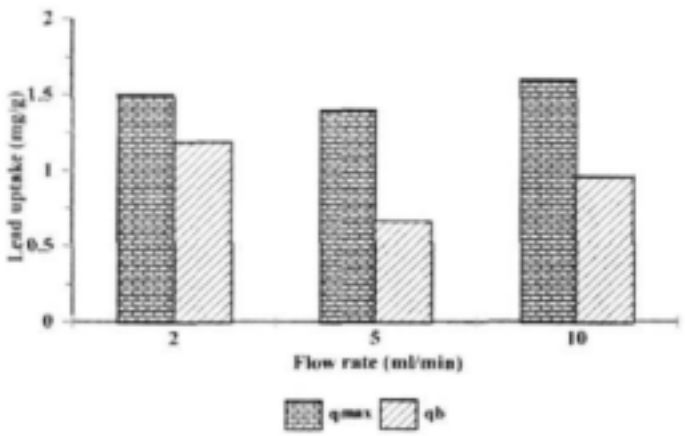


Figure 9.12: Lead uptake from effluent A3, at varying flow rates. Initial Pb concentration, 7.5 mg/l, initial pH, 2.6; biomass concentration, 5 g *Azolla* / l effluent; temperature, RT ~ 20 °C; bed volume, 49 ml; qmax = maximum lead uptake possible; qb = uptake at break-through point or equilibrium.

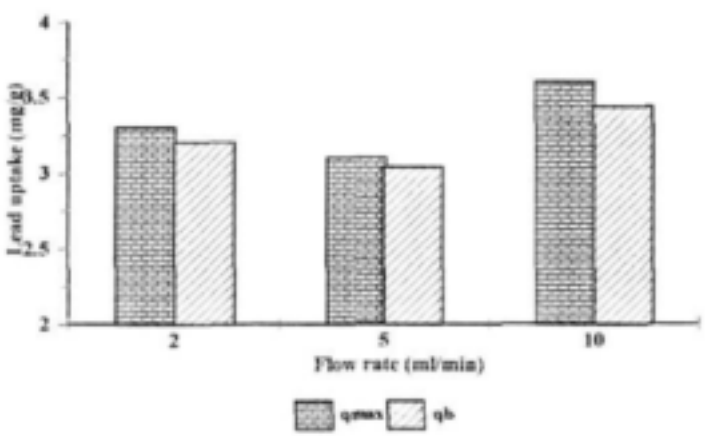


Figure 9.13: Lead uptake from effluent B3, at varying flow rates. Initial Pb concentration, 16.6 mg/l, initial pH, 4.8; biomass concentration, 5 g *Azolla* / l effluent; temperature, RT ~ 20 °C; bed volume, 49 ml; qmax = maximum lead uptake possible; qb = uptake at break-through point or equilibrium.

9.3.3.4 Removal of lead, copper and iron from effluent

Figures 9.14 and 9.15 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 9.15. 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.

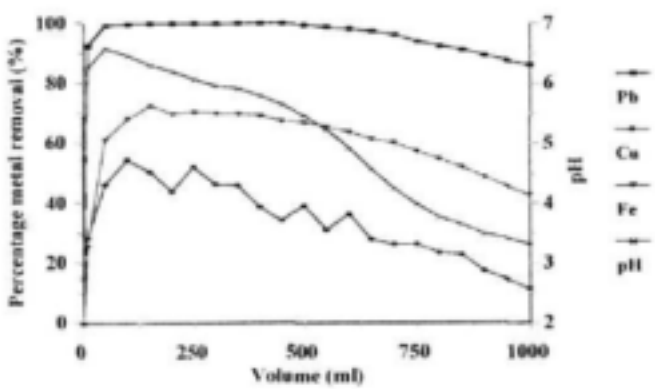


Figure 9.14: Percentage lead, copper and iron removal from effluent A4, Initial metal concentration, 17.2 mg/l - Pb, 0.3 mg/l - Cu, 6.1 mg/l Fe; initial pH, 2.8; biomass concentration, 5 g *Azolla* / l effluent; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

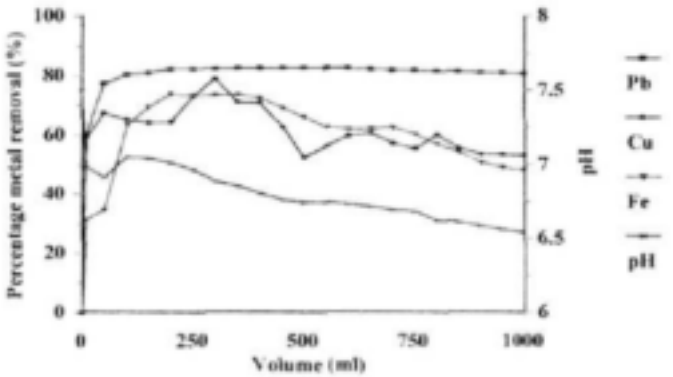


Figure 9.15: Percentage lead, copper and iron removal from effluent B4. Initial metal concentration, 79 mg/l - Pb, 0.5 mg/l - Cu, 0.9 mg/l Fe; initial pH, 7.5; biomass concentration, 5 g *Azolla* / l effluent; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

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).

9.3.3.5 Lead recovery from the biomass

Table 9.2: 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 9.2 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 8) may be due interference from other ions present in the effluent system. HNO_3 at 0.5 M concentration was used in subsequent experiments.

9.3.3.6 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 9.16 and 9.17. 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 %.

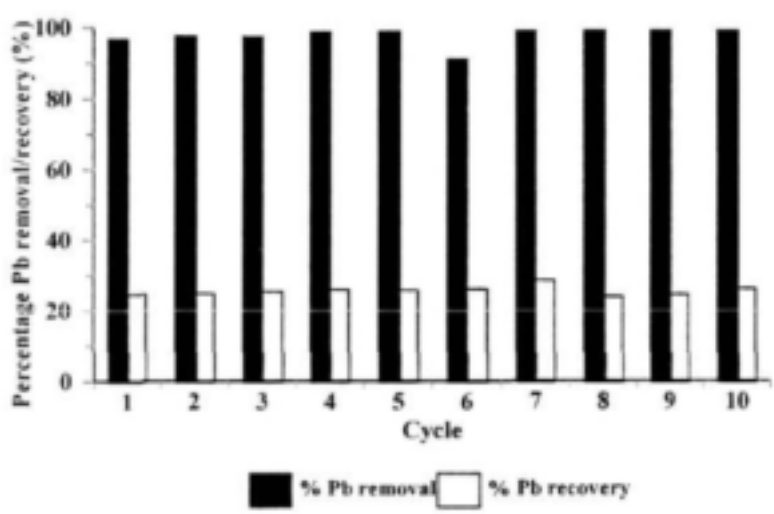


Figure 9.16: Percentage lead removal and recovery from effluent A5, for 10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml, 0.5 M HNO_3 as the desorbent and 50 ml, 0.05 M NaOH to regenerate the biomass. Initial Pb concentration, 14.7 mg/l; initial pH, 2.7; biomass concentration, 5 g *Azolla* / l effluent; flow rate, 10 ml/min; temperature, RT ~ 20 °C; bed volume, 49 ml.

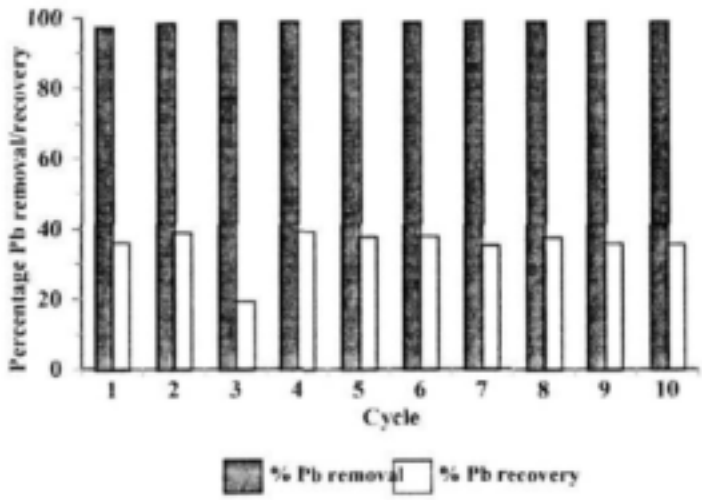


Figure 9.17: Percentage lead removal and recovery from effluent B5, for 10 repeated adsorption and desorption cycles by *Azolla* biomass, using 50 ml, 0.5 M HNO_3 as the desorbent and 50 ml, 0.05 M NaOH to regenerate the biomass. Initial Pb concentration, 43.7 mg/l; initial pH, 7.5; biomass concentration, 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 9.2), 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 9.16 and 9.17).

9.4 SUMMARY

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 8), 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 10

Spirulina Sp. AS A BIOREMEDIATION AGENT

10.1. Spirulina sp. as a bioremediation agent and it's interaction with metals

South Africa is a country rich in natural resources, with the critical exception of fresh water. This fresh water is essential to life, social development and economic progress. The country's average annual rainfall of 497 mm is well below the world average of 860 mm. Only a comparatively narrow region along the eastern and southern coastlines experience rainy conditions, while 65 % of the country receives less than 500 mm of rain annually. This is usually regarded as the minimum for successful dry-land farming. Twenty-one percent receives less than 200 mm. In addition, high evaporation rates result in the runoff-to-rainfall ratio being amongst the lowest for any populated region on earth (Department of Water Affairs, 1986; Kidd, 1997).

Coupled to the natural scarcity of water, is a simultaneous increase in human demand for water resources. This has arisen from the growth of the population and economy, as well as rising standards of living. In addition, the scarce, unutilised supplies are geographically mismatched in relation to demand. Water quality is also rapidly deteriorating due to increasing development. It has been estimated, from present growth rates of population and industry, that by the year 2020 the demand for potable water in South Africa will exceed supply. (Department of Water Affairs, 1986; Kidd, 1997).

Heavy metal ions are a major source of water contamination, and increasing levels are being released into local water supplies via effluents from industrial, military and mining sites. Metallic species released into the environment tend to persist indefinitely, eventually accumulating throughout the food chain, thus posing a serious threat to the environment, animals and humans. Not only are these effluents highly toxic but, in the case of the mining industry, valuable metals are being lost.

Removal of these metal ions and recycling of the water is consequently vital in order to avoid significant contamination of watercourses and loss of potential resources.

Traditional technologies for the removal of heavy metals from water, such as ion exchange or lime precipitation have proved ineffective and/or very expensive, especially when the metals are in the lower concentration ranges. New technologies are required that can reduce heavy metal concentrations to environmentally acceptable levels at affordable costs. Biotechnology based processes have the potential to contribute significantly to the achievement of this goal.

10.2. Algal Accumulation of Heavy Metals

Microalgae bioremoval technologies are still being developed and much more work is required. Some practical applications have been achieved, and the fundamentals look promising. Microalgae have the potential to remove metal ions to very low concentrations, to grow on light energy, and to accumulate large amounts of specific toxic elements. They appear to function well even in the presence of other ions, in particular Ca^{2+} and Mg^{2+}, and organics. Only future research and the discipline of the market place will determine their role in the clean up of the environment.

10.2.1. Spirulina sp. as a Bioremediation Agent

Spirulina is currently being investigated as a potential bioremediation agent for the removal of heavy metals from mining and other industrial effluents. An initial aim of this research was to determine the threshold levels of *Spirulina* for toxic metals, as it was an attractive possibility that *Spirulina* would be capable of binding heavy metal ions and could be used for biosorption of heavy metals form industrial waste-water.

10.2.1.1.The Effects Of Toxic Metals on *Spirulina*

Spirulina is one of the few algal species that has been investigated for its potential as a bioremediation agent, although little data is available to support its use for bioremoval of heavy metals. An attractive possibility is that *Spirulina* may be capable of binding heavy metal ions as

well as removing nutrients from the surrounding medium, thereby decreasing the eutrophication potential of waters which receive treated fluids. The advantage of using *Spirulina* is that it can be grown in ponds with little nutritional input or maintenance. Laboratory or small-scale experiments have also been performed on the growth of *Spirulina* on city waste-waters, cow manure and swine wastes. In addition *Spirulina* is non-pathogenic, which gives it an advantage over other forms of microbial biomass (Brady *et al.* 1994; Ciferri, 1983; Hulse, 1982; Oswald, 1988; Shelef *et al*, 1980). The indications that *Spirulina* has potential as a bioremediation agent and the availability of the algae warranted this investigation into the use of *Spirulina* as a biosorbent.

10.2.1.2. *Spirulina* As A Biosorption Agent

A potential problem associated with the use of a living biomass as a biosorbent is the potential toxic effects of the heavy metals in solution (Wilde & Bennemann, 1993). Therefore, in order to determine the feasibility of Spirulina as a biosorption agent it is necessary to determine the effects of various metals n the growth of *Spirulina.*

The aim of the research dealt with in this chapter was to determine the threshold levels of *Spirulina* for the toxic metals copper, zinc and lead. Electron microscopy was also used in order to determine the effects of metal accumulation on the morphology and internal organelles of the organism.

10.2.1.2.1. Materials And Methods

10.2.1.2.1.1. Determination Of Algal Concentration

Due to the filamentous nature of *Spirulina*, cell counts or photometric methods cannot be used to determine the strength of the culture. A novel method of determining culture strength is therefore required. And chlorophyll determination is one of the most rapid methods for estimating quantities of living plant material (Richmond, 1986).

10.2.1.2.1.2 .Batch Experiments

The following experiments were carried out using copper, zinc and lead and all experiments were performed in duplicate.

Healthy *Spirulina* from the existing culture was filtered through a nylon mesh and washed with distilled water to remove any traces of EDTA. The algal slurry was then resuspended in 50 % Zarrouk's mixture (Zarrouk's medium made up with no EDTA or $FeSO_4$ and diluted 50 % with water). Twelve 500ml flasks were inoculated with 30 ml concentrated algal slurry and 270 ml 50 % Zarrouk's mixture. A chlorophyll extraction, an acid digestion and a pH reading was performed on each culture. The cultures were then allowed to grow overnight, at 120-130 rpm on an orbital shaker, under constant environment conditions.

On the following day appropriate volumes of metal stock solution were added to each flask to make the final concentrations up to 5, 10, 20, 30 and 50 µM. Chlorophyll extractions, acid digestions and pH readings were performed again, and every second day thereafter.

10.2.1.2.2. Results and Discussion

a) Copper

The results for algal concentration are shown Figure 10.1. From these results it can be seen that for the first three days all six groups exhibited essentially similar behaviour. Both the algal concentration and the pH (data not shown) increased steadily, after which the 20, 30 and 50 µM groups showed a decline in both pH and algal concentration. The 50 µM cultures were the first to start declining and by day eleven all of the 20, 30 and 50 µM cultures were dead. The controls and the 5 and 10 µM flasks were all still healthy, although the growth in the 10 µM cultures was starting to decline.

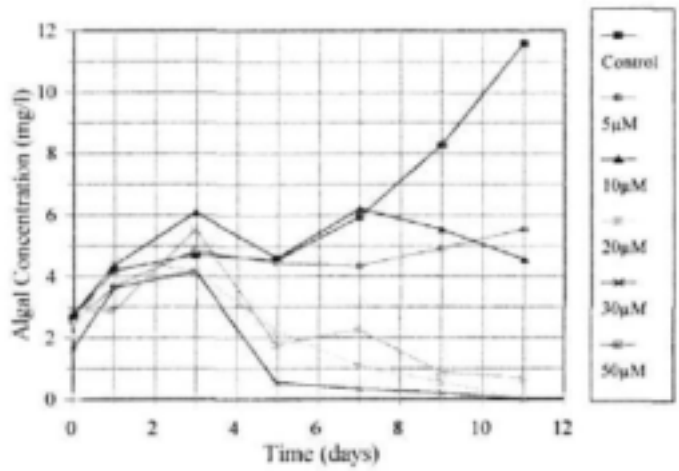


Figure 10.1: Growth curves of *Spirulina* cultures containing $CuSO_4$

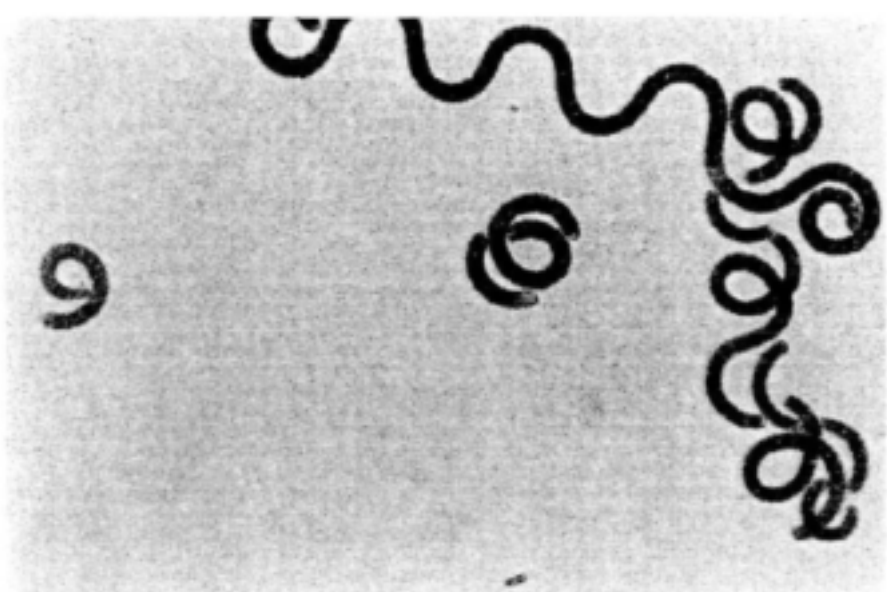


Figure 10.2: Light microscope preparation of *Spirulina sp.* after 2 days in 50 µM $CuSO_4$.

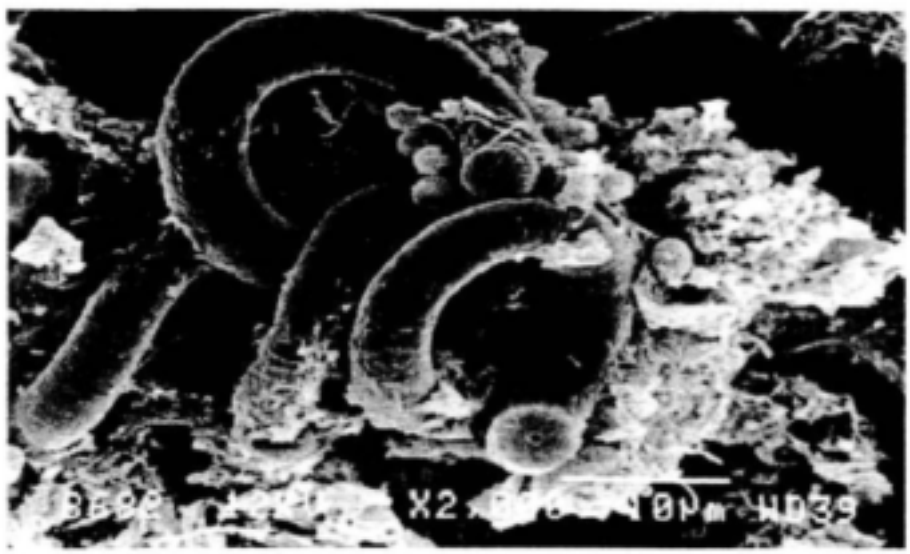


Figure 10.3: SEM preparation of *Spirulina sp.* after 2 days of 50 µM of $CuSO_4$.

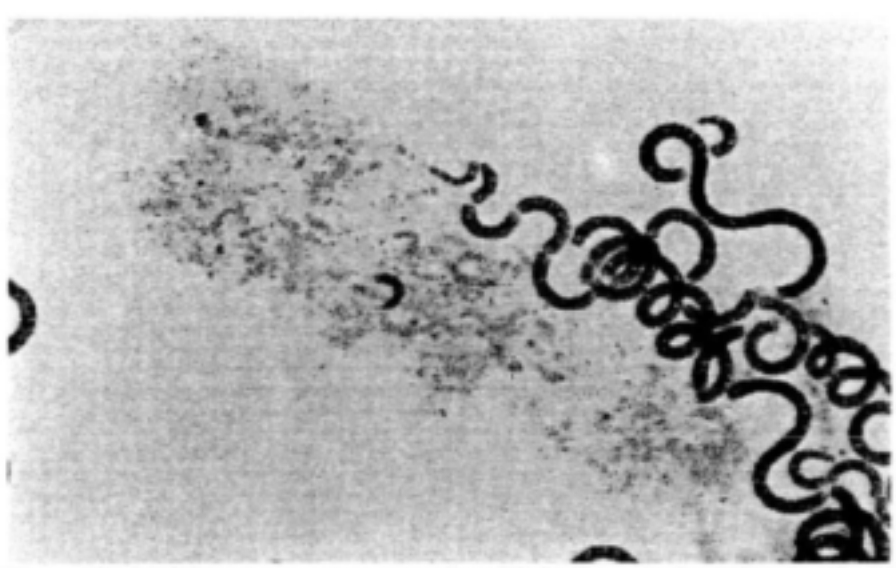


Figure 10.4: Light microscope preparation of *Spirulina sp.* after 4 days in 50 µM $CuSO_4$.

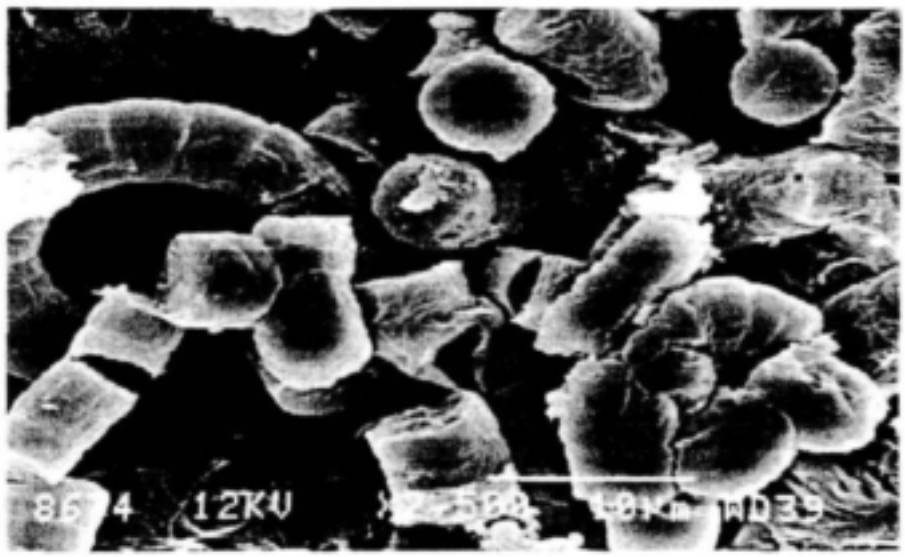


Figure 10.5: SEM preparation of *Spirulina sp.* after 4 days in *50* μM in Cu SO_4.

b) Zinc

The results obtained for zinc showed essentially the same trends as those for copper. If the growth curve for zinc (Figure 10.6) is compared to that of copper (Figure 10.1) it can be seen that the copper appeared to take slightly longer before taking effect. Even in the 20, 30, and 50 μM groups, a slight increase in algal concentration was observed before decline began (Figure 10.1). In the case of zinc, the same groups started to decline immediately (Figure 10.6), with the exception of the 20 μM group. At first glance it would appear that, once the cells started deteriorating, death occurred more rapidly in the case of copper (Figure 10.1). However, as the starting concentration of *Spirulina* in the zinc experiment was far higher than in the copper experiment, the cultures took longer to deteriorate to the state of copper cultures.

Results suggest that the tolerance level of *Spirulina* for zinc is approximately 5 μmoles/g, because as soon as the Zn accumulated exceeded 5 μmoles/g the cells started to die. In the case of copper this occurred at approximately 7 μmoles/g.

The deterioration of the cells can be seen in the photographs of *Spirulina* grown in 50 μM $ZnSO_4$ over a period of two days (photographs not shown in this report). The *Spirulina* in these

photographs looked much healthier than those at the same stage of the copper experiments (Figures 10.2 & 10.3). Very little damage had occurred to the filaments at this stage, they were just starting to unwind. This was probably due to the difference in the starting concentrations of the cultures. By day two in the zinc experiments, the cultures were already in a state of decline, but the algal concentration was still relatively high and therefore the culture would not yet have reached the state of the copper cultures of the same stage.

Figures 10.7 and 10.8 show the *Spirulina* after four days in $ZnSO_4$. Although the cells appeared to be very broken up at this stage, they were still a lot greener (Figure 10.7) and they looked a lot healthier (Figure 10.8) than those of the copper experiment (Figures 10.4 & 10.5). The cells in Figure 10.8 have retained their shape completely, and don't appear to have lost as much of their cell contents as those in Figure 10.5. This could be because the culture had not reached the same state of the copper cultures due to the increased starting concentration. However, it is known that copper has a tendency to interfere with the photosynthetic electron transport, especially in photosystem II. In addition, copper toxicity interferes with pigment and lipid biosynthesis and consequently chloroplast ultrastructure, thus it negatively affects photosynthetic efficiency (Barón *et al.* 1995). Zinc has also been shown to inhibit photosynthesis, but it takes higher concentrations of zinc to have an equivalent effect on photosynthesis as a lower concentration of copper (Davies, 1983).

McBrien and Hassel (cited in Overnell, 1975) found that copper also increased the permeability of algal cells, whereas Overnell (1975) showed that zinc did not exhibit this effect. This could explain the loss of shape and slightly wrinkled effect of the cells that had been exposed to copper for four days (Figure 10.5). The *Spirulina* from the zinc experiments (Figure 10.8) did not exhibit this effect at all.

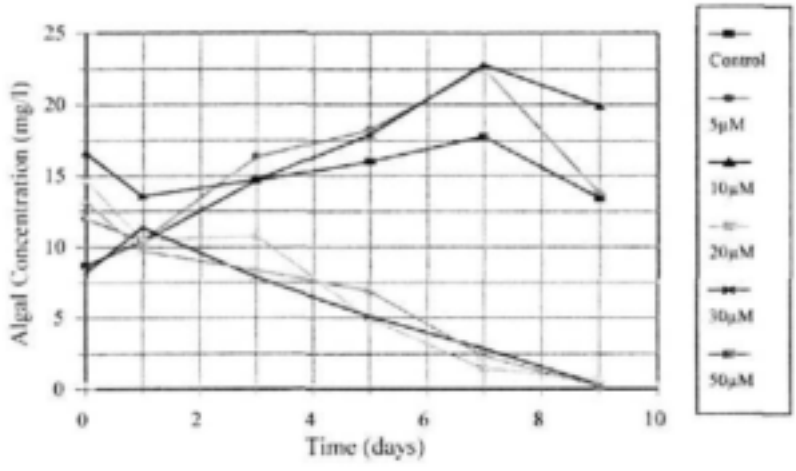


Figure 10.6: Growth curves of *Spirulina* cultures containing 50 μM $ZnSO_4$.

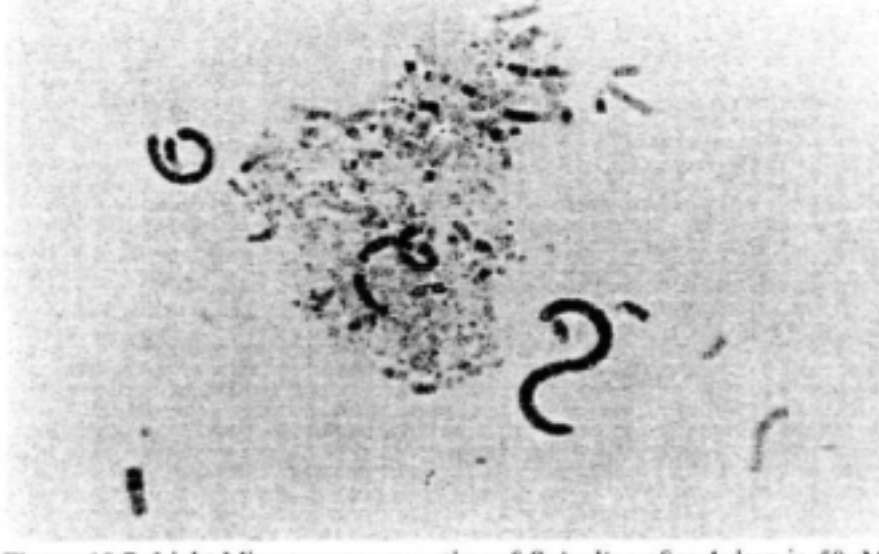


Figure 10.7: Light Microscope preparation of *Spirulina* after 4 days in 50μM $ZnSO_4$.

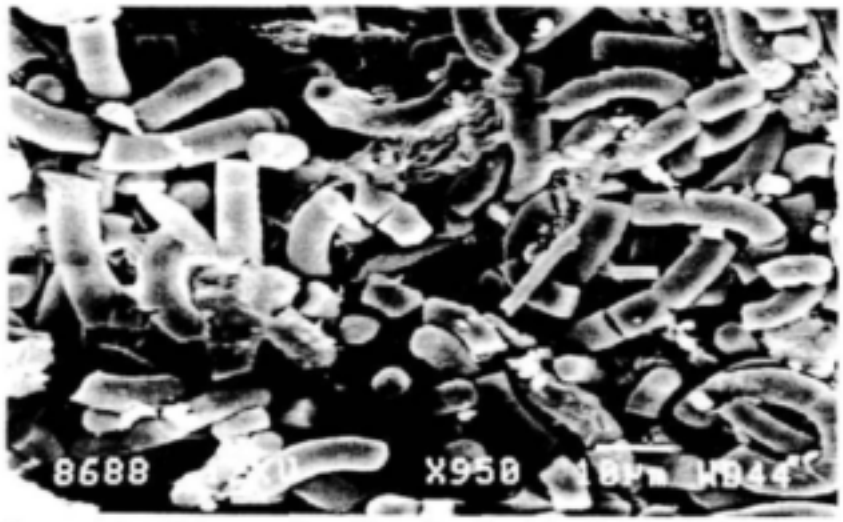


Figure 10.8: SEM preparation of *Spirulina* after 4 days in 50μM $ZnSO_4$.

The results thus far suggest that *Spirulina* has approximately the same threshold levels for both zinc and copper, but the specific toxic effects vary between the two metals.

c) Lead

The growth curves obtained from the lead experiments suggest that lead is not as toxic to *Spirulina* as copper or zinc. The first sign of any significant decline in algal concentration (Figure 10.9) was on day seven, at which stage growth in all groups, including the control started to decline. This suggests that the decline could be due to a depletion of nutrients in addition to lead toxicity. There was definitely some form of toxic effect seen in the 50:M cultures (Figure 10.9), as there was no increase in algal concentration beyond day three. These results indicate that there was no active growth in the 50:M culture after day three. All the other cultures appear to have grown unaffected until day seven, when they all started to decline.

Although the lead did not appear to have a serious effect on the growth of *Spirulina*, it did cause drastic changes to the cell morphology (Figures 10.10 to 10.13). By day four, a lot of debris is seen under the light microscope (Figure 10.12). The SEM photographs (Figures 10.11 & 10.13)

suggest that the cell debris seen in Figure 10.12 was cell contents alone, as the filaments were still more-or-less intact, although grossly distorted. Whole filaments could still be seen under the light microscope on day four (Figure 10.12).

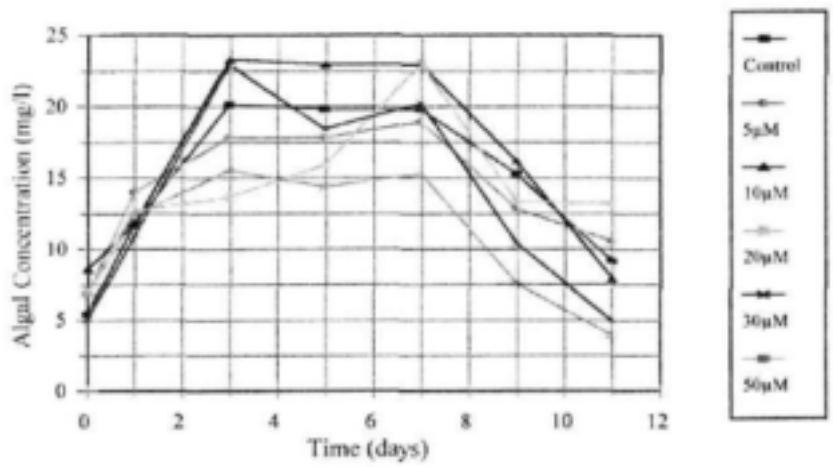


Figure 10.9: Growth curve of *Spirulina* cultures containing 50 µM $Pb(NO_3)_2$.

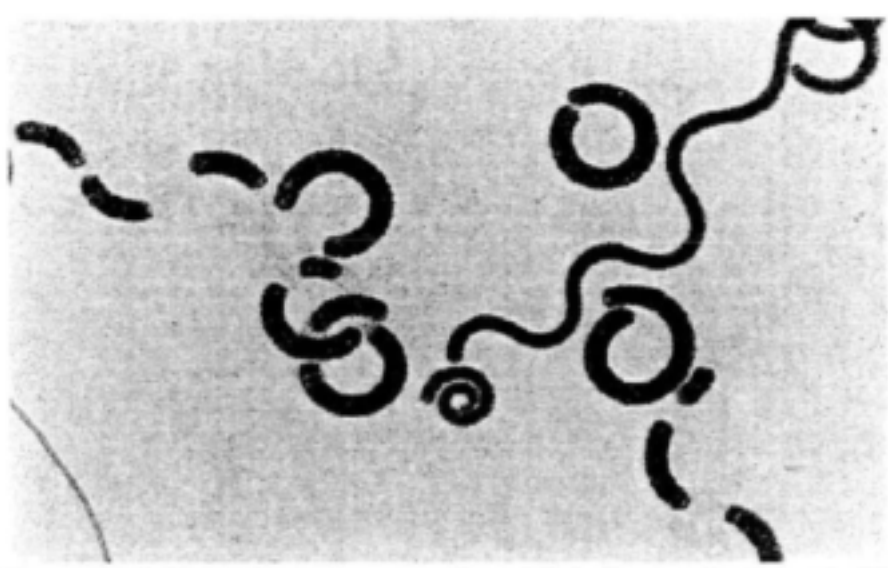


Figure 10.10: Light microscope preparation of *Spirulina sp.* after 2 days in 50 µM $Pb(NO_3)_2$.

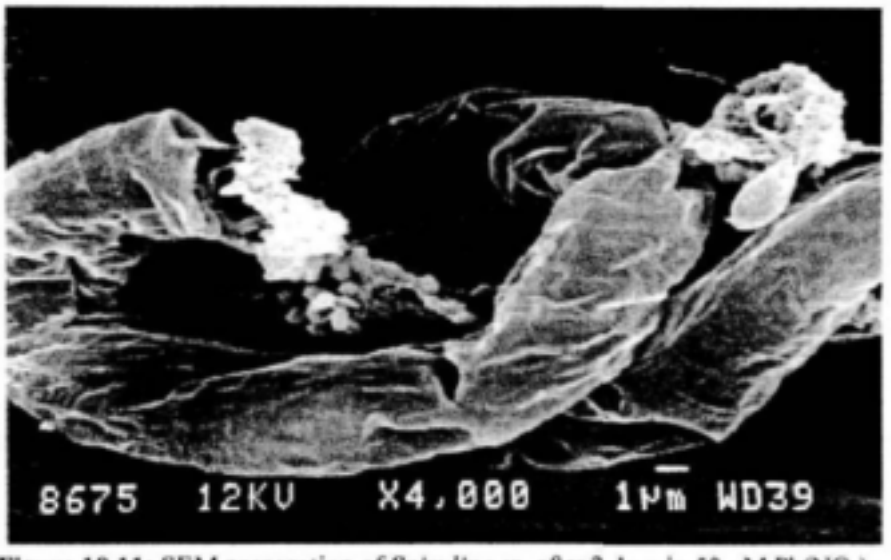


Figure 10.11: SEM preparation of *Spirulina sp.* after 2 days in 50 µM $Pb(NO_3)_2$.

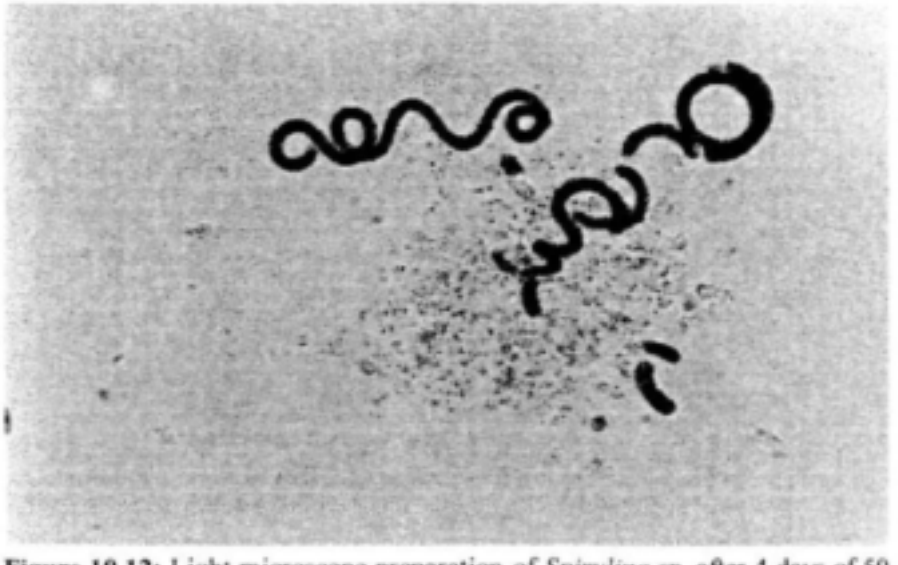


Figure 10.12: Light microscope preparation of *Spirulina sp.* after 4 days of 50 µM $Pb(NO_3)_2$.

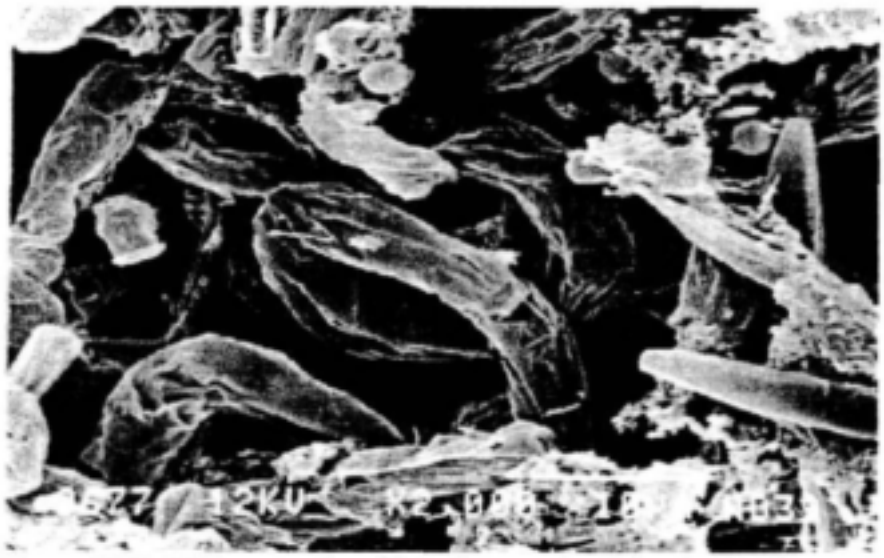


Figure 10.13: SEM preparation of Spirulina sp. after 4 days in 50 µM $Pb(NO_3)$.

10.3 The mechanism of inorganic carbon uptake by Spirulina and the possible use of the bicarbonate/carbonate equilibrium for heavy metal precipitation

Pollution of the environment by toxic metals arises as a result of many activities, largely industrial and include processes such as mining, electroplating, just to name a few. Many of these pollutants are discharged into the environment and may reach extremely high concentrations. Some of the main processes that remove, immobilize or detoxify heavy metals in the natural environment result from microbial activities. Biotechnological approaches to the abatement of toxic metal pollution consist of selectively using and enhancing these natural processes to treat wastes. Microorganisms interact with toxic metals in a variety of ways, however, the three main processes used are biosorption, extracellular precipitation and uptake by metal-binding proteins and other specialist molecules derived from microbial cells. These processes are not exclusive and several physico-chemical and biological processes may be involved. Biosorption and bioprecipitation are two of the most successfully utilized biotechnological processes to date.

The conventional processes for the treatment of acidic, metal laden effluents from industry, involves neutralization by the addition of alkaline chemicals such as limestone, lime, sodium hydroxide or sodium carbonate which increases the pH and causes precipitation of the metals from

solution. However, these methods are relatively expensive when used on a large scale and as a result there has been a trend toward the implementation of passive treatment schemes with minimal operational and maintenance requirements.

Research conducted thus far, on the use of algae for the removal of metal ions in solution, has predominantly concentrated on their ability to adsorb or accumulate the metal. Little research has focused on the possibility of using the alkalinity generated by the algae, for the precipitation of metals.

A limitation of using an algal system, in which the cells are in direct contact with the metal-containing solution, is that the algal culture is only effective over a short period of time because of the effects of heavy metal toxicity. To overcome this problem, it would prove to be more beneficial, to keep the culture separate from the metal-containing solution or effluent. Therefore, it would be more feasible to use a system whereby metal precipitation occurs when the alkaline water from an algal growth vessel is transported to a reaction vessel containing the effluent.

Extensive research has been conducted on the effects of heavy metal ions on the physical properties of the algal biomass and on photosystem activities. It is known that small amounts of certain metals are required for maintenance of some components or metabolic functions in the cell, but if a heavy metal is present in too high a concentration, the effect could prove to be detrimental or even fatal to the organism.

Algae differ from the other groups of small or microscopic organisms in that they possess an internal green pigment called chlorophyll, sometimes hidden or partially masked by other pigments, which enables them in the presence of sunlight to combine water and carbon dioxide to form starch or related substances, and to release oxygen into the water. In algae and other green plants, the rate of photosynthesis is normally faster than that of respiration, therefore more oxygen is released than used and they absorb more carbon dioxide than they release.

An important chemical effect of algae is the continuous removal of CO_2 from the water during the daylight hours as a result of photosynthesis. This process brings about an alteration in the

relative amounts of soluble carbonic acid, partially soluble bicarbonates, and the nearly insoluble monocarbonates, often causing some of the latter to precipitate. These changes in CO_2 also tend to change the pH of the water. The pH rises as the algae increase their photosynthetic activity during daylight hours, and the pH then decreases at night, when the algae are not carrying out photosynthesis but are releasing CO_2 during respiration.

It is clear that both cyanobacteria and microalgae possess an environmental adaptation for survival at low CO_2 concentrations, this is known as a CO_2 concentrating mechanism which elevates CO_2 around the active site of the primary photosynthetic carboxylating enzyme Rubisco. This process actively transports and accumulates inorganic carbon within the cell and then uses this pool to provide elevated CO_2 concentrations (through the enzymatic function of carbonic anhydrase), which in turn allow them to perform efficient photosynthesis in their aquatic environments.

We have found that under CO_2- limiting conditions the algae begin to utilise the bicarbonate in the medium, CO_2 (which is taken up by the cell) and OH^- ions are released into solution. More than half of the OH^- ions, react with HCO_3^- ions in solution to form carbonate, which then complex with the metal ions, causing them to precipitate as carbonates. Some of the remaining OH^- ions react with free H^+ ions in solution and this removal of H^+ ions from solution results in an increase in pH.

10.3.1. The effect of algae on the chemical composition of the surrounding medium

An extensive amount of research has been conducted, investigating the effect of CO_2 on the carbon concentrating mechanism (CCM) present in algae. When algae experience conditions of limited carbon dioxide, they are able to utilise inorganic carbon, in the form of bicarbonate, through the use of this mechanism, for photosynthesis. Carbonic anhydrase (CA) is the enzyme responsible for the dehydration of HCO_3^- to CO_2 and OH^-. This results in a decrease in acidity, as the resulting OH^- ions in solution are responsible for the alkalization of the culture medium and a shift in the bicarbonate/carbonate equilibrium results in more carbonate in the medium.

The major focus of this section is to determine the effect the algae have on the chemical

composition of their surrounding medium by quantifying bicarbonate utilization and carbonate production.

10.3.1.1. Batch flask cultures

Batch flask cultures were prepared using varying concentrations of $NaHCO_3$ to quantify bicarbonate utilization and to investigate the effect of the algae on carbonate speciation of the medium over a period of about two weeks. The culture flasks were stoppered and sparged with nitrogen to ensure removal of any CO_2 and O_2 in solution, resulting in low-C_i cells. The algal CCM was induced by the limitation of CO_2 and as a result utilisation of bicarbonate was made possible by the enzyme, CA, which is known to be responsible for the dehydration of HCO_3^- to CO_2 and OH^-.

10.3.1.2. Chlorophyll extractions

Chlorophyll is one of the most important chelates in nature and is capable of channeling the energy of sunlight into chemical energy through the process of photosynthesis. In photosynthesis, the energy absorbed by chlorophyll transforms carbon dioxide and water into carbohydrates and oxygen (http://www.herbuniverse.com). The chemical energy stored by carbohydrates drives biochemical reactions in nearly all living organisms. As a result, the chlorophyll *a* concentrations observed were used as an indication of the health of the cultures.

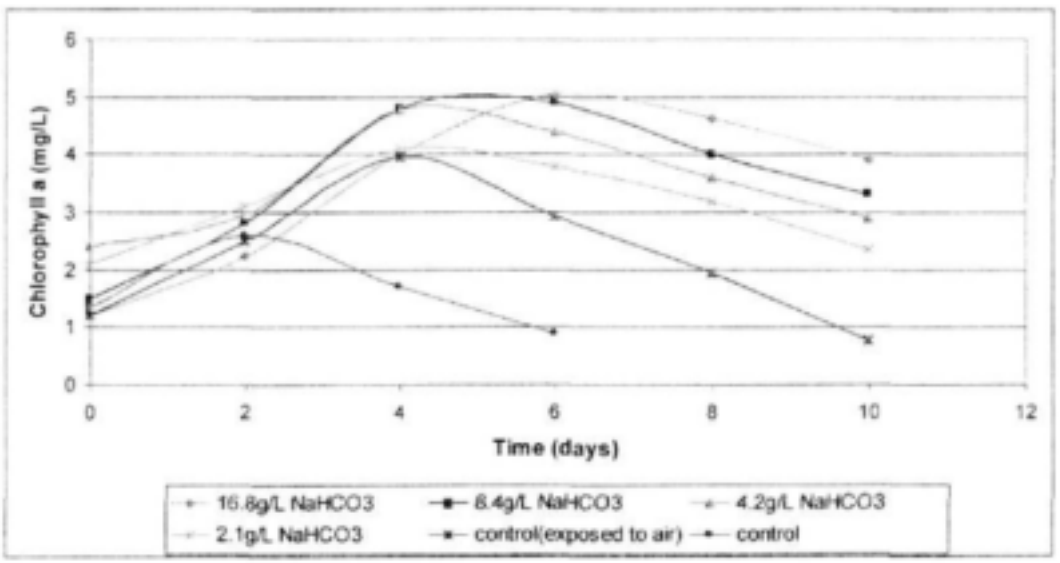


Figure 10.14: Chlorophyll *a* concentrations at varying concentrations of $NaHCO_3$

Referring to Figure 10.14, it can be seen that the cultures supplied with the highest concentration of $NaHCO_3$, had the highest chlorophyll concentrations. Chlorophyll *a* is considered to be at the center of the photosynthetic reaction and is therefore responsible for supplying the algae with the necessary energy required for growth.

The one set of control flasks, which contained no bicarbonate, was discarded within the first few days, as they were not supplied with an inorganic carbon source for growth. The chlorophyll *a* concentrations in these flasks were seen to decrease quite rapidly as the biomass perished, indicating that the method of chlorophyll extraction is a reasonably suitable method for determining the health of the cultures.

The other set of control flasks, which were also not supplied with an inorganic carbon source, were not stoppered (as above), but exposed to air. These flasks showed a higher increase in chlorophyll concentration over a period of a few days, as the algae were able to utilise the small amount of CO_2 which entered the unstoppered flasks. Because the amount of CO_2 going into solution was not sufficient to maintain the photosynthetic demand of the algae, they too began to perish at a rapid rate.

10.3.2. Determination of the carbonate species in solution and measurement of pH

Titrations were performed on 10ml of filtered algal solution from each flask using 0.02N H_2SO_4, and the molarities of the carbonate species in solution were determined.

The pH of the first set of batch flasks investigated was adjusted to 8.3 at the start of the experiment, to ensure that there was only bicarbonate in solution. From the carbon dioxide equilibria equations it has been determined that below values of pH 8.3, CO_2 appears to serve as the main carbon source, whereas above pH 8.3, HCO_3^- appears to be the predominant species. The alkalinities (Figure 10.16) were determined and the pH (Figure 10.15) of the cultures were recorded over a two week period.

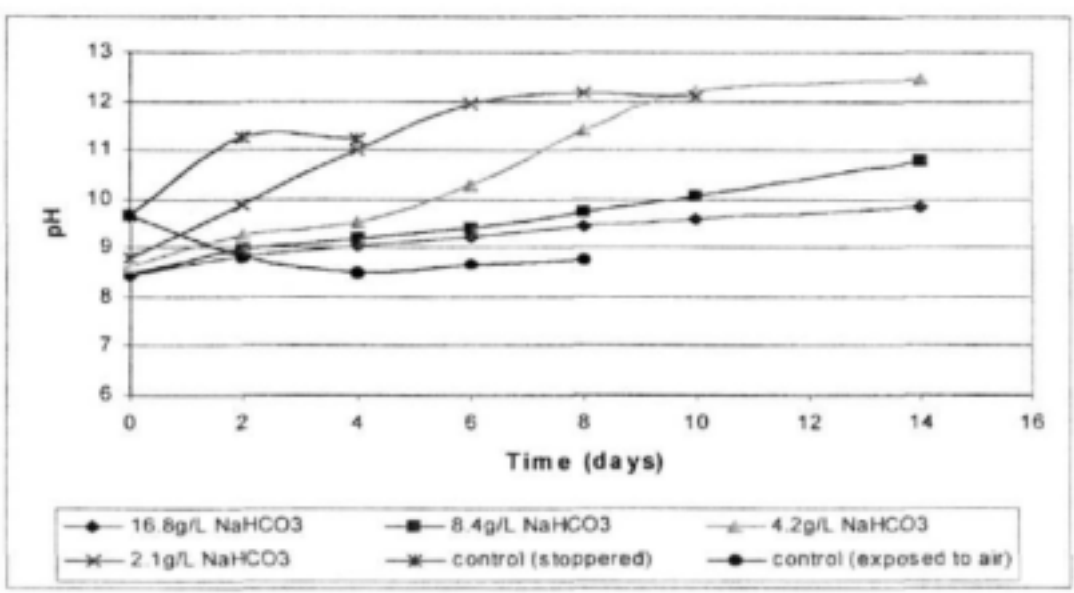


Figure 10.15: The pH of the batch flasks containing varying concentrations of $NaHCO_3$

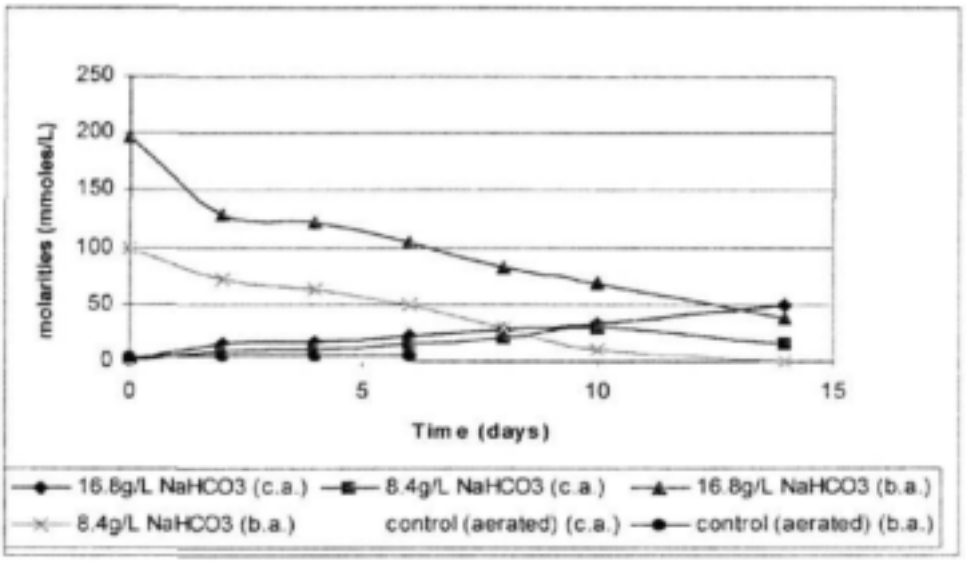


Figure 10.16: Carbonate speciation in solution (pH of media initially adjusted to 8.3)

Figure 10.15 and Figure 10.16 will be discussed in conjunction with one another. Referring to Figure 10.15, it can be seen that there was an initial rapid increase in the pH of the stoppered control flasks, but after 2 days the pH began to decrease. The increase in pH was most likely as a result of the utilisation of traces of DIC in the water and carry over from the cell suspension. Because the control flasks contained no bicarbonate to buffer the pH, the increase in pH was more pronounced. As the DIC in solution was used up, the photosynthetic rate begins to exceed the respiration rate. As photosynthesis is inhibited, more CO_2 is released than is taken up and the pH decreases.

In the control flasks, which were not stoppered, but exposed to air, a decrease and then a slight increase in the pH was observed. These cultures survived about 4 days longer than the stoppered control, as they were able to utilise the small amounts of CO_2 entering the flasks. The decrease in pH occurs when the photosynthetic rate exceeds the respiration rate. Photosynthesis is inhibited as a result of this and more CO_2 is released into the medium, forming carbonic acid.

The flasks which were supplied with varying concentrations of bicarbonate demonstrated that alkalization of the medium was dependent on the concentration of the external bicarbonate. An initial quick increase in the pH was observed and then the pH of the flasks containing the higher

concentrations of bicarbonate leveled off due to the bicarbonate/carbonate buffering capacity of the medium. This observation is clearly demonstrated in Figure 10.15, where it can be seen that the flasks containing 16.8g/L $NaHCO_3$ had a less rapid rate of pH increase as compared to flasks containing 2.1g/L $NaHCO_3$, because of the effective buffering capacity of the medium. Once all the DIC has been utilised, the pH of the medium begins to level off and may even decrease when the respiration rate exceeds the photosynthetic rate.

10.3.3. Predictive modelling

To confirm the results already observed, the pH and alkalinity data from an experiment containing 16.8g/L $NaHCO_3$ were used for carbonate speciation modeling, using the chemical speciation software, MINTEQA2. MINTEQ is unable to predict photosynthesis, so the titration data for HCO_3^- and CO_3^{2-} was used as input data. The modeled data obtained confirmed that there was a rapid decrease in bicarbonate concentration as the algae photosynthesised, while at the same time there was a corresponding increase in the carbonate concentration in the medium, though not as rapid (Figure 10.17).

The pH (Figure 10.17) was also predicted to increase, due to alkalisation of the medium as the result of OH^- ion release caused by dehydration of the bicarbonate supplied in the external medium.

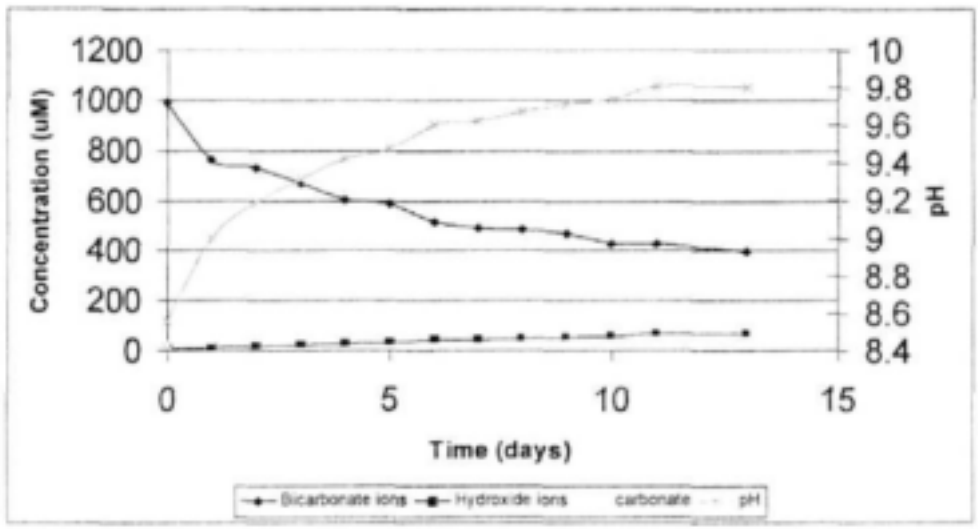


Figure 10.17: Predicted carbonate species in solution and pH readings (MINTEQA2).

10.4. Alkalization of the medium and the effect of carbonic anhydrase inhibitors on inorganic carbon accumulation

Due to its biological importance and the fact that it has one of the highest turnover rates of any known enzyme, carbonic anhydrase has been studied for many decades and is the subject of a large body of literature (Payne, 1997).

The aim of these experiments was to assay CA activity of *Spirulina* under normal conditions and inhibitors were used to assess the actual contribution of CA to the generation and maintenance of alkalinity in the medium.

10.4.1 Alkalization dependency on external bicarbonate

Shiraiwa *et al* (1993) found that alkalization of the medium did not occur when the external dissolved inorganic carbon (DIC) was predominantly CO_2, this is when the pH is below 5, or when the internal DIC was used for photosynthesis. However, at pH 6.6 equal amounts of both CO_2 and HCO_3^- have been found to be present, and at pH 8.3 DIC is mainly HCO_3^-. Therefore, to investigate bicarbonate utilization and the dependency of alkalization on external bicarbonate, different bicarbonate concentrations (pH adjusted to 8.3) were used and the pH of the medium was monitored on an hourly basis for 5hrs and then again after 30hrs (Figure 10.18).

Measurements of the pH increase of the medium by *Spirulina* showed that the initial rate of alkalization was dependent on the concentration of the added external DIC, as can be seen in Figure 10.18. Initial quick increase in the pH was observed because of the addition of $NaHCO_3$,thereafter there was a decreasing rate of pH increase due to the bicarbonate/carbonate buffering capacity of the medium (this is shown by cultures supplemented with 10mM, 5mM and 1mM $NaHCO_3$).

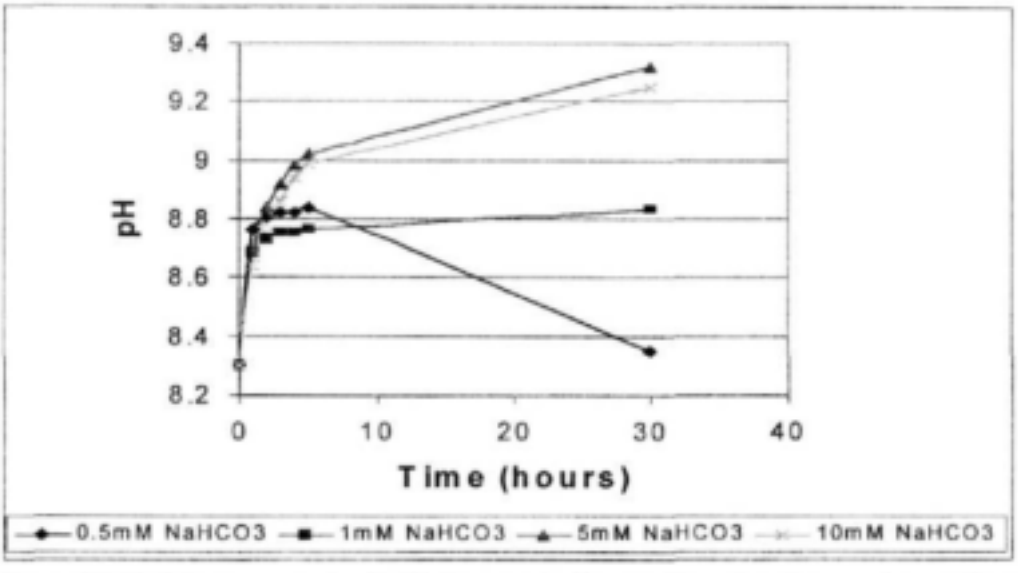


Figure 10.18: Measurement of pH changes in the medium by algae using various concentrations of bicarbonate

The pH of the flasks containing 5mM $NaHCO_3$ were found to be slightly higher than the 10mM $NaHCO_3$ flasks, because the flasks containing the higher concentration of $NaHCO_3$ result in a better pH buffering system.

Initially, a higher pH was recorded in the flasks containing 0.5mM $NaHCO_3$ as compared with the 1mM $NaHCO_3$ containing flasks, as there was very little bicarbonate present to buffer the pH, so the increase in pH was more pronounced. The relatively rapid increase in pH may also have been due to the utilization of traces of DIC in the water and carry over from the cell suspension. But, as all the DIC present in the surrounding medium was exhausted, the photosynthetic rate begins to exceed the respiration rate and more CO_2 is released than is taken up, resulting in more CO_2 in solution, and a decrease in the pH as carbonic acid is formed (this can be observed in the 0.5mM $NaHCO_3$ containing cultures in Figure 10.18.

10.4.2. The effect of pH on alkalization of the medium

The equilibration between the various inorganic carbon species in solution (CO_2, bicarbonate and carbonate) will be affected by pH. CA activity is suppressed by growth at acid pH. Williams and Colman (1996) found that external CA was suppressed by growth at pHs below 8.3, with total repression at pH 5.0. This is due to the fact that below pH 8.3, CO_2 serves as the primary carbon source for photosynthesis, so there is no need for the CCM and the enzyme CA. Where as above pH 8.3, the dehydration of HCO_3^- becomes limiting and the algae must take up HCO_3^-. Therefore, increasing pH will lead to a greater bicarbonate to CO_2 ratio, and if air equilibrium is maintained, to increased total inorganic carbon concentrations.

As shown in Figure 10.19, the pH of the medium containing low-CO_2 grown cells of *Spirulina* during photosynthesis increased, upon the addition of 50mls of a 50mM $NaHCO_3$ and 17.11mM NaCl solution, from pH 8.3 to pH 8.87. This is because the HCO_3^- is converted to CO_2, by the enzyme carbonic anhydrase, which is internalised and incorporated into the photosynthetic pathway, and OH^- ions, which remain in solution. The result is a net increase of hydroxyl ions in the medium, which leads to an increase in pH. When the pH was lowered once again to 8.3 by the addition of 0.02N H_2SO_4, the algae, photosynthetically re-alkalized the medium at the initial rate until the pH had again reached about 8.8.

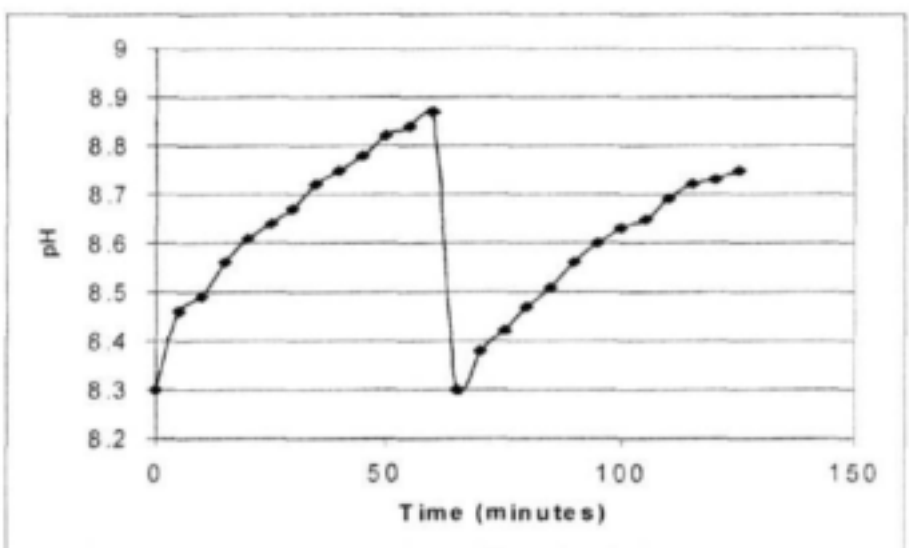


Figure 10.19: Changes in the pH of the medium containing *Spirulina* after the pH was readjusted with 0.02N H_2SO_4.

10.4.3. The effect of carbonic anhydrase inhibitors

In some green algae, the use of HCO_3^- has been correlated with the presence of external CA activity. In these cases external CA is thought to facilitate the use of HCO_3^- by maintaining equilibrium between HCO_3^- and CO_2 and thereby maintaining the supply of CO_2 to a CO_2 transporter (Williams & Colman, 1995). Direct HCO_3^- transport however has also been demonstrated in cells that have external CA activity. In some green algae it has been shown that external CA increases the overall DIC supply at alkaline pH if CO_2 is the preferred species of DIC (Sültemeyer *et al*, 1989).

It can therefore be said, that external CA functions in the algal DIC pump for rapid conversion of HCO_3^- present in the external medium to CO_2 and OH^-. Acetazolamide (AZ), an impermeable inhibitor of external carbonic anhydrase was investigated to assess the role of extracellular carbonic anhydrase on the inorganic carbon concentrating system in *Spirulina*.

Firstly, the effect of AZ on extracellular carbonic anhydrase in *Spirulina* was investigated using varying concentrations of the inhibitor to assess the importance of the enzyme on the inorganic carbon concentrating mechanism (Figure 10.20).

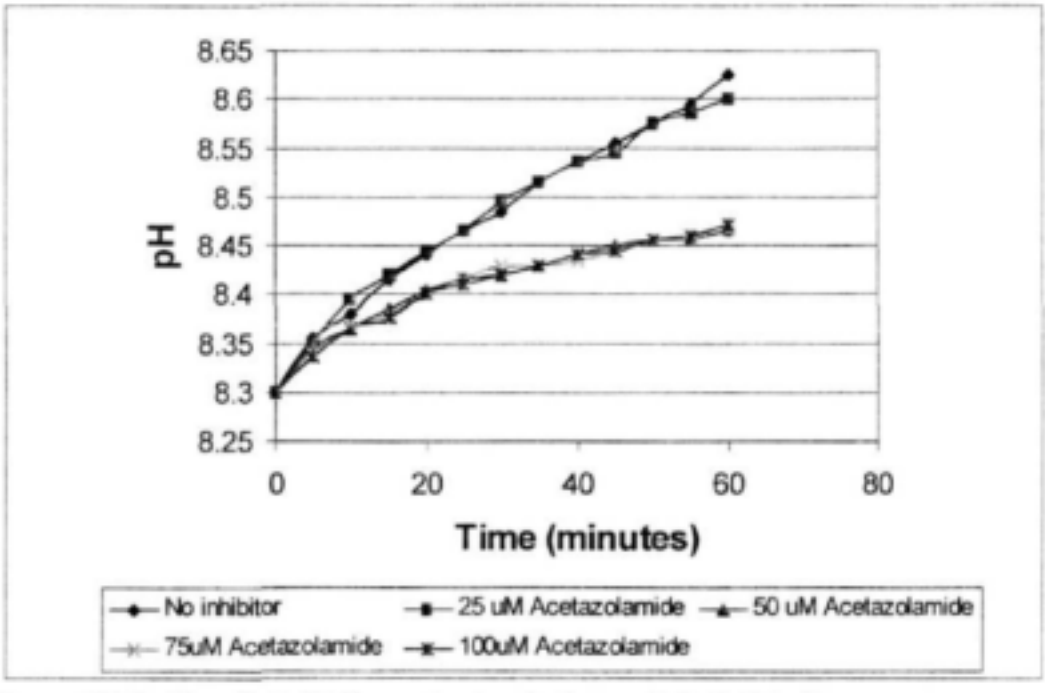


Figure 10.20: The effect of AZ on carbonic anhydrase activity in *Spirulina*.

When referring to Figure 10.20, it can be seen that AZ inhibited the alkalization of the medium at a concentration of 50μM as compared to the control experiment. When 25μM AZ was used, there was very little if any inhibition of the inorganic carbon concentrating mechanism, as the algae continued to alkalize the medium and an almost identical rate of pH increase was observed when compared with the control experiment. An interesting point to observe, is that even when higher concentrations of AZ were utilized, i.e. 75 and 100μM, the inhibition was almost identical to those obtained for 50μM AZ.

10.5. The use of the alkalinity generated by Spirulina for the precipitation of metals

Considering the toxic effects that high concentrations of heavy metals have had on the photosystems and the general health of the algal culture, it is evident that alternative methods of metal removal need to be investigated.

A considerable amount of work has been conducted on algae as a bioremediation agent, but this research has focussed on the ability of the algae to adsorb or accumulate metal ions from solution (van Hille *et al*, 1999). These methods are usually limited by the toxicity caused to algal systems by the presence of even relatively low concentrations of metals in solution. It has been documented that algae are able to alter the alkaline species of the surrounding medium through the use of their inorganic carbon accumulating mechanism (this has also been demonstrated in Sections 2 and 3). Very little research however has been conducted on the use of the alkalinity generated for the precipitation of metals. This section will focus on the feasibility of using this system for metal precipitation.

A limitation of using an algal system, in which the cells are in direct contact with the metal-containing solution, is that the algal culture is only effective over a short period of time because of the effects of heavy metal toxicity. To overcome this problem, it would prove to be more beneficial, to keep the culture separate from the metal-containing solution or effluent. Therefore, it would be more feasible to use a system whereby metal precipitation occurs when the alkaline water from an algal growth vessel is transported to a reaction vessel containing the effluent.

10.5.1. Comparison of predicted MINTEQ modeling results with experimental results

10.5.1.1. Metal solutions

Metal solutions for precipitation experiments were made by dissolving an appropriate amount of a metal salt in Milli-Q water to give a concentration of 500μM. The following metal salts were used:

- Nickel sulphate ($NiSO_4$)
- Zinc sulphate ($ZnSO_4$)
- Ferrous sulphate ($FeSO_4.7H_2O$)
- Magnesium chloride ($MgCl_2$)
- Ferric nitrate ($Fe(NO_3)_3.9H_2O$)
- Lead nitrate ($Pb(NO_{32})$)
- Copper sulphate ($CuSO_4$)
- Calcium chloride ($CaCl_2$)

10.5.1.2. Experimental procedure

Titrations were carried out on both concentrations of media, before inoculating with algae, to determine the bicarbonate alkalinity in the media. It was decided that a concentration of 30mmoles was required for the precipitation experiment, which meant that 333ml of the filtered media would be added to 100ml conical flasks containing 60mls of each of the metal solutions. The flasks were left to react for 30 minutes, after which the pH was recorded. A 5ml sample was then removed with a syringe and filtered through 0.22μm OSMONICS nylon membrane filter. Another 5ml sample was removed from each flask and pipetted into tubes, which were then centrifuged at 5000g for 15 minutes. The two procedures were used to determine which was the most effective way of removing the precipitate from the solution, before analysing the samples on the Atomic Absorption Spectrophotometer (AA spec).

The alkalinity and pH values recorded were used to model what we would expect to find after a

metal precipitation reaction occurred, and to determine the effect of the metal on the chemical species in solution. The predicted results from the chemical speciation software, MINTEQ A2, coincide with those obtained from the experimental flasks.

From the results, it was possible to categorise the precipitation reactions into three different groups, namely, those which:

- Precipitate as hydroxides, eg. Copper, nickel and zinc
- Precipitate as carbonates, and are able to generate CO_3^{2-} from the dissociation of HCO_3^- in solution, eg. Iron and lead
- Precipitate as carbonates only if there is free CO_3^{2-} present in solution, eg. Magnesium and calcium

According to the conditions specified, the chemical speciation software program, MINTEQ, is able to predict the formation of a number of super-saturated compounds which could possibly precipitate from the reaction. Many of these predicted compounds will take days or even weeks to form, therefore only the compounds which were most likely to form in a reasonably short period of time were considered.

Table 10.1: The percentage metal precipitated compared with those predicted by the speciation software program MINTEQ A2.

Metal	% precipitation observed	% precipitation predicted
Copper	43 – 50%	45-50%
Nickel	Approx 20%	0-10%
Zinc	Approx 18%	18-30%
Iron	60 – 93%	50-60%
Lead	80 – 100%	70-93%
Magnesium	30 – 44%	0-26%

In some cases it was observed that there was a higher percentage precipitation in the experimental flasks when compared with the predicted results from the chemical speciation modelling software, MINTEQ (Table 10.1). Total organic carbon (TOC) measurements were performed to try and determine if any soluble organic carbon was present in the medium, which may have been

responsible for the irregularities observed in the precipitation experiments (refer to Figure 10.21). Results indicated that there was organic carbon present in solution. And the variation in the predicted and observed results were most likely as a result of the complexation of metals by soluble organic carbons, such as EPS, which are produced by the algae especially during times of nutrient and environmental stress.

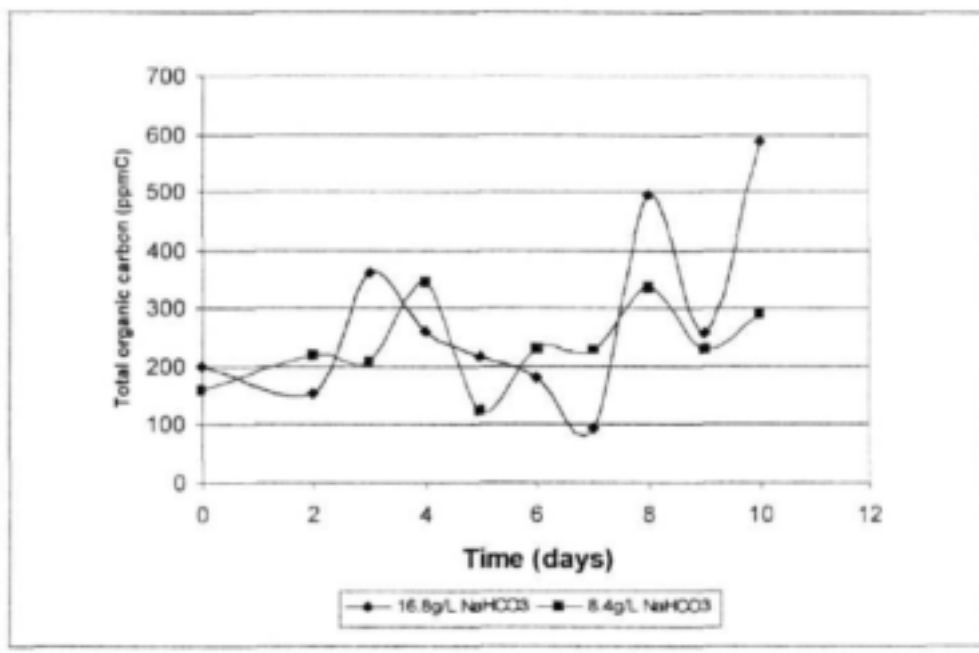


Figure 10.21: Total organic carbon readings for the flasks used in the precipitation experiments.

10.6 SUMMARY

Spirulina was found to have a threshold level of about 30 μM for copper, zinc and lead. Copper and zinc appeared to have a direct effect on the photosynthetic pathway, thereby causing a rapid decline in cell growth. Lead on the other hand seemed to affect surface properties and hence took longer to cause deterioration in growth. It can hence be concluded that *Spirulina* has little or no potential as a biosorbent.

The main objective of this project was to investigate the inorganic carbon accumulating mechanism in *Spirulina*, and to determine if the carbonate generated by the algae, under conditions of limited carbon dioxide, from bicarbonate would be a feasible method for metal precipitation.

Initial batch flask experiments were carried out to determine the change in carbonate species over the period of study. The results obtained from the experimental flasks exhibited similar trends to those predicted by the chemical speciation modelling program, MINTEQA2. A decrease in the bicarbonate concentration was observed when the algae were grown under carbon dioxide limiting conditions, as a result of the induction of their CCM. Some of the hydroxide ions formed, contribute to the alkalization of the medium, and bind to free H^+ ions in solution resulting in an increase in the pH of the surrounding medium. The remaining OH^- ions bind with some of the HCO_3^- to form carbonate, which reacts readily with metals.

The effect of the inhibitor, acetazolamide, was also investigated to try and determine the importance of the enzyme on the inorganic carbon accumulating mechanism. Results obtained were consistent with those observed in the literature, and it was found that alkalisation of the medium was almost completely inhibited by 50μM of AZ.

As it had been established in the batch flask cultures that algae are capable of affecting the carbonate species present in solution, metal precipitation experiments were performed to try and determine if the carbonate produced in the medium could be used for the precipitation of metals. The results observed in the experimental flasks were very similar to those predicted in the

modelling programme, MINTEQA2. However, a slight increase in the amount of metal precipitated was observed in the experimental flasks when compared with the modelled data, this is most likely as a result of the complexation of the metal ions with soluble organic carbons, such as extracellular polysaccharides, phytochelatins and metallothioneins.

It was possible to categorise the precipitation reactions observed into three different groups, namely those which:

- Precipitate as hydroxides (Cu, Zn and Ni)
- Precipitate as carbonates and are able to generate carbonate from the dissociation of bicarbonate (Pb and Fe)
- Precipitate as carbonates only if there is free carbonate present in solution (Mg and Ca)

It appears that this algal-based method of metal precipitation would be most suitable for the removal of Mg and Ca, as no precipitation of these metals occurs if carbonate is not present in solution. Therefore, as the algae utilize the bicarbonate supplied in the medium, when conditions of low carbon dioxide are experienced, an increase in the carbonate concentration is observed, the carbonate then complexes with the metal ions in solution resulting in the precipitation of the metals. Pb and Fe are strong metal acids capable of dissociating bicarbonate, to form carbonate, therefore all that would be required to precipitate these metals is the addition of bicarbonate.

It is clear that there are several biotechnological approaches for treating metal-containing wastes, which offer potentially efficient and cost-effective alternatives to existing treatment technologies. Whether the full potential of these biotreatments is realized, depends on further investment and exploitation by receptive industries.

GENERAL CONCLUSIONS

Escalating industrialisation and decreasing metal ore reserves, has focussed increasing wide spread attention on the recovery of these metals from low-grade ore and waste water utilising biological material which is cost-effective and selective. Plant material, such as *Azolla filiculoides,* and algae such as *Spirulina sp.*, were utilised in the removal of various metals from solution.

In batch studies, *Azolla filiculoides* is able to remove gold from dilute solutions typical of mine effluent concentrations (1-10 mg/L). The following optimal conditions were found for gold(III) uptake: a 5 g/L biomass concentration, 8 mg/L initial gold(III) concentration and pH of 2 with pH-dependent binding. The competitive effects of various metals were investigated. At equimolar concentrations, individual metal studies demonstrated a removal of 50% for lead(II), 30% for iron(III), 10% for copper(II) and 100% for gold(III). The mixed metal solution containing all four metals showed no interference in the binding of lead(II) and gold(III) while copper(II) uptake decreased slightly, with iron(III) showing a larger decrease of 30-40%. Studies at simulated effluent concentrations showed a removal of 60% for lead(II), 30% for iron(III), initially 20% for copper(II) which gradually decreased to 0%, and 90% for gold(III) in the individual metal studies. When the individual studies were compared with the single solution containing all four metals, the results suggested no interference existed between the metals. An attempt was made to relate the next study to the HSAB theory to understand gold(III) uptake behaviour in adsorption studies in the presence of various ligands. The halides, with increasing affinity for gold, i.e., $Cl^- < Br^- < I^-$, are able to affect the uptake of gold(III) with chloride having no effect, and bromide and iodide exhibiting 13% and 25% inhibition respectively. Mercaptoethanol, has no effect on the binding of gold(III) to the biomass. The presence of hard bases, such as sulphates and borderline bases, such as sulphites, are able to interfere with the uptake of gold(III). These results suggest that the presence of sulphates or sulphites at concentrations in excess of 10 mM in waste water may severely affect the binding of gold.

Column studies have demonstrated the applicability of *Azolla filiculoides* in the adsorption of gold(III) in a packed-bed column. The mechanical strength, particle size and large surface area of the plant material is particularly suited for its utilisation in a continuous-flow mode. The adsorption of dilute gold(III) concentrations (5 - 80 mg/L) at pH 2 with varying flow-rates (2 to 20 mL/min) demonstrated greater than 90% removal indicating that the biomass has a high affinity for the metal. Similarly to the batch studies, the competitive effect of metals on the uptake of gold(III) was studied. At equimolar concentrations the mixed metal study in a single solution compared well with the individual metal experiments except for iron(III) which showed an increase of 30% in removal, whilst lead(II) and copper(II) showed a slight decrease of approximately 12%. At simulated effluent concentrations for iron(III) demonstrated a marked decrease of 40% in the maximum removal, while a 25% and 10% decrease was observed for lead(II) and gold(III) respectively. The different behaviour of lead(II) and gold(III) removal at equimolar and 1:5 molar ratio concentration suggests that a borderline acid (copper(II)) can minimally affect the efficiency of gold recovery depending on their relative concentrations. A similar trend in the column studies as observed in the batch studies was found with the halides: chloride, bromide and iodide, with chloride having no effect, bromide and iodide exhibiting 15% and 35% inhibition of gold(III) uptake respectively. The column studies exhibited the same response as found with the batch studies for mercaptoethanol, whilst sulphate exhibited no effect on the adsorption of gold(III). Sulphite however affected adsorption remarkably shown by the 70% inhibition of gold(III) from solution.

A critical factor in the removal of metals is the volume of elutant to the volume of solution treated. Smaller volumes of elutant allow for a more concentrated solution, lowering disposal costs. Batch studies were conducted in an attempt to understand the binding mechanism of gold(III) to the biomass and its desorption. A ratio of adsorbent to desorbent of 1:1 was found to be optimal for desorption with the presence of an oxidant necessary to increase gold recovery from the biomass. The speciation of the metal did not alter over the 24 hour period. Gas purging with nitrogen, air and oxygen in combination with thiourea showed that oxygen and even small quantities in air aided the desorption process of gold from the biomass. A series of desorption studies, under column conditions, were investigated to determine the gold binding and desorption

characteristics for each of the elutants. The acids, HNO_3 and H_2SO_4, were inefficient as elutants. EDTA was also ineffective, whereas mercaptoethanol resulted in a low recovery. A slightly larger desorption percentage using KOH suggests that a small portion of the gold may be in the form of gold oxide. Elution of gold by KBr and ethanol suggests a small percentage of gold may be in the +3 oxidation state. Thiourea only complexes with gold(I) and removal of bound gold suggested that only a small portion of the metal exists in the +1 oxidation state. The presence of an oxidant enhances the conversion of gold(0) to gold(I) (Kuyucak and Volesky, 1989a). Examples of such oxidants utilised were: ammonium peroxodisulphate, ammonium ferric sulphate, ferric chloride, perchloric and nitric acid. Ultimately, thiourea in combination with perchloric acid and hydrochloric acid was found to be the superior desorbent with the ability to chelate most of the gold adsorbed. This suggests that most of the bound gold is in the +1 or 0 oxidation state.

An increase in concern over the pollution of natural watercourses by lead from industrial effluents has occurred. Therefore, restrictions on the discharge of effluents containing heavy metals have been intensified in the past few years (Wilson and Edyvean, 1994). This study 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).

It may be concluded that *Azolla* biomass is an efficient biosorbent for lead and has promising potential for industrial application.

Another aspect in metal removal from waste water is the utilisation of algae as a bioremediation agent for metal recovery. Current technology for the removal of heavy metals from wastewater includes precipitation, ion exchange and absorption usually using synthetic resins or activated

carbon. However, the continuous cost of reagents required for these processes makes them less financially feasible and alternative, cost-effective treatments are being implemented, which have low operational and maintenance requirements.

In determining the potential of *Spirulina* as a biosorbent the toxic effects of metals were investigated, and *Spirulina* was found to have a threshold level of about 30 μM for copper, zinc and lead. Results obtained in this study indicate that both copper and zinc have a direct effect on the photosynthetic pathway of *Spirulina*, causing cell deterioration and death. Lead, on the other hand, appears to affect structural properties, and thus takes longer to affect cell growth. Ultimately lead does lead to cell death.

Consequently, *Spirulina* does not appear to be a useful biomass for metal sorption but, although relatively low concentrations of metal may have a toxic effect on the algae, *Spirulina* may have potential as a precipitation agent. The role of *Spirulina* in the precipitation of heavy metals appears to be through its ability to maintain a high pH, possibly through the enzyme carbonic anhydrase. Hence an investigation of this enzyme was initiated.

Initial batch flask experiments were carried out to determine the change in carbonate species over the period of study. Initially, as the pH of the medium was adjusted to 8.3 using H_2SO_4, there was only bicarbonate in solution and no carbonate was present. But, as the flasks were sparged with nitrogen to remove any CO_2 and O_2 and then stoppered, the CCM in the algae was induced, to enable then to utilise the bicarbonate, thus reducing the bicarbonate alkalinity, in solution for photosynthesis. The use of bicarbonate is made possible by the enzyme, carbonic anhydrase (CA), which is responsible for the dehydration of HCO_3^- to CO_2 and OH^- ions. The CO_2 released is in turn taken up by the chloroplast for photosynthesis, and some of the OH^- ions remain in solution and are responsible for the increase in pH as they react with free H^+ ions in solution (Figure 10.14) remaining OH^- ions react with some of the HCO_3^- in solution, according to the following equation, $HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O$, to form carbonate (which reacts readily with metals), resulting in an increase in the carbonate alkalinity. When considering all the results discussed in this section, it is possible to assume that the use of algae for the precipitation of metals may be

feasible and warrants further research.

These results suggest that external CA is involved in alkalization of the medium by low-CO_2 grown cells and that AZ inhibits the enzyme responsible for the dehydration of HCO_3^- to CO_2 and OH^-. The results are also consistent with those observed in literature, which have found that alkalization of the medium was almost completely inhibited by 50μM AZ. From this it can be deduced that AZ is a potent inhibitor of external carbonic anhydrase and that the enzyme plays an important part in inorganic carbon accumulation during periods of CO_2 limitation .

These results suggest that external CA is involved in alkalization of the medium by low-CO_2 grown cells and that AZ inhibits the enzyme responsible for the dehydration of HCO_3^- to CO_2 and OH^-. The results are also consistent with those observed in literature, which have found that alkalization of the medium was almost completely inhibited by 50μM AZ. From this it can be deduced that AZ is a potent inhibitor of external carbonic anhydrase and that the enzyme plays an important part in inorganic carbon accumulation during periods of CO_2 limitation .

Studies utilising algae in batch flasks to precipitate metals indicated that metals precipitated:

- as hydroxides, eg. Copper, nickel and zinc,
- as carbonates, and are able to generate CO_3^{2-} from the dissociation of HCO_3- in solution, eg. Iron and lead,
- as carbonates only if there is free CO_3^{2-} present in solution, eg. Magnesium and calcium

According to the conditions specified, the results obtained from the batch flasks were similar to those predicted by the chemical speciation software program, MINTEQ (Table 1).

Table 1: The percentage metal precipitated compared with those predicted by the speciation software program MINTEQ A2.

Metal	% precipitation observed	% precipitation predicted
Copper	43 – 50%	45-50%
Nickel	Approx 20%	0-10%
Zinc	Approx 18%	18-30%
Iron	60 – 93%	50-60%
Lead	80 – 100%	70-93%
Magnesium	30 – 44%	0-26%

It is clear that there are several biotechnological approaches for treating metal-containing wastes, which offer potentially efficient and cost-effective alternatives to existing treatment technologies. Whether the full potential of these biotreatments is realised, depends on further investment and exploitation by receptive industries.

In terms of achieving the original objectives of the study the water fern, *Azolla,* was found to be an effective accumulator of gold, platinum, lead, iron, and copper from synthetic solutions. Lead, copper and iron could readily be recovered from the biomass while the recovery of gold and platinum was more complex but achievable. The most optimal bioreactor for continuous and on-line application was an up-flow column reactor, relatively loosely packed with biomass cultivation of the *Azolla* biomass can be achieved in simple shallow baths supplied with appropriate nutrients but variable climatic conditions can significantly effect growth rates. The most appropriate and inexpensive method of biosorbent preparation was simple air or sun drying.

While one of the initial objectives was to investigate the accumulation of metals from low volume, high metal concentration effluents this was superceded previously initiated and by on going studies in our laboratory using electroplating and battery effluents. Consequently, this study concentrated on the major metal containing effluent problem in the South Africa, *viz.* mine wastewater as well as battery manufacturing effluents.

A further objective of the project was to exploit the use of algal exopolysaccharides for metal

removal but preliminary studies showed that while these compounds were efficient metal accumulators, the difficulties and costs involved in producing and extracting these polymers did not make this a viable option for bioremediation.

Capacity building programmes in collaboration with the universities of the north and Fort Hare were not successful largely due to the lack of capacity of the staff involved and the low numbers of suitably qualified postgraduate students. A successful collaboration with staff and students at the Vaal Triangle Technikon was established and is ongoing.

While no pilot scale plants are yet in operation either in the laboratory or on-site, data related to this bioremediation process and the related technologies have successfully been transferred to a battery manufacturer in East London as well as Eskom and Anglo Platinum mining and refining operations.

Future research should focus on a detailed examination of the water chemistry related to metal containing effluents as this will largely dictate the most appropriate bioremediation technologies to utilised and the most suitable operational conditions in terms of pH, anion concentrations, etc. An integration of biological systems for effective metal removal is likely to be the most efficient process to use for metal removal. Biosorption should be investigated as a pre- or polishing step in such an integrated system. In terms of gold and platinum effluents, biosorption systems offer a great deal of potential for metal removal and recovery but the best conditions for optimum metal removal were to be established and further investigation will need to be undertaken to establish the most appropriate and cost-effective solutions to utilise for metal desorption, recovery and concentration.

APPENDICES

APPENDIX I: EFFLUENT COMPOSITION OF MINE A

Determinant	*Concentration*
Iron(Fe)	10 µM
Lead(Pb)	25 µM
Copper(Cu)	200 µM
Gold(Au)	5 µM
Sulphate(SO_4^{2-})	573 mg/L
Chemical Oxygen Demand (COD)	140 mg/L
Chlorides (Cl^-)	0
Free Cyanide (CN^-)	198 mg/L
pH	8

APPENDIX II: SULPHATE DETERMINATION
(Merck Spectraquant® Sulphate Test Kit: 1.14791)

The barium iodate reacts with sulphate ions in an organic aqueous medium to form iodate ions and barium sulphate. The tannin forms a brown-red dye with the iodate.

Method

The pH range of the sample should be within 2-10. If required, the pH should be adjusted with sodium hydroxide or hydrochloric acid. Samples are diluted if necessary, with Milli Q water (Millipore).

A sample (2.5 mL) is placed into a test tube. Two drops of SO_4-1A is added to the sample and mixed. One green microspoonful of SO_4-2A is added and vortexed until the solid substance is dissolved. The test tube is added to a water bath at 40°C for a period of 5 minutes. Thereafter 2.5 mL of SO_4-3A is added and vortexed. The solution is filtered until all the turbidity is removed. Four drops of SO_4-4A is added and left to stand in a 40°C water bath for a period of 7 minutes. The absorbance is read at 515 nm utilising a spectrophotometer. In the blank, Milli Q water replaces the sample.

APPENDIX III: CHLORIDE DETERMINATION

(Merck Spectraquant® Chloride Test Kit: 1.14897)

The chloride ions react with mercury(II) thiocyanate to form slightly dissociated mercury(II) chloride. The thiocyanate released in the process in turn reacts with iron(III) ions to form iron(III) thiocyanate that is red. The colour intensity is then measured photometrically at 468 nm.

Method

The pH range must be within the range of 1-12. If turbid, the samples need to be filtered. Samples are diluted if necessary, with Milli Q water (Millipore). In the blank, Milli Q water replaces the sample.

Measuring range: 2.5 - 25 mg/L Cl^-

Sample (5 mL) is place into a test tube. A volume of 2.5 mL of reagent Cl-1 is added to the sample and vortexed. The addition of reagent Cl-2 (0.5 mL) follows immediately and vortexed. The sample is left to stand for a period of 1 minute. The absorbance is measured utilising a spectrophotometer.

Measuring range: 10 - 250 mg/L Cl^-

Sample (1 mL) is place into a test tube. A volume of 2.5 mL of reagent Cl-1 is added to the sample and vortexed. The addition of reagent Cl-2 (0.5 mL) follows immediately and vortexed. The sample is left to stand for a period of 1 minute. The absorbance is measured utilising a spectrophotometer.

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Journal Articles

1. Sanyahumbi, D., Zhao, M. and Duncan, J.R. Removal of lead from solution by the non-variable biomass of the water fern *Azolla filiculoides*. *Biotechnol. Lett.*, **20**, 745-748, 1998.

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5. van Hille, R.P., Nightingale, L., Rose, P.D. and Duncan, J.R. Biogenic alkalinity – A viable alternative to existing treatment strategies for acid mine drainage remediation. *Biodegradation*. In Press.

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1. Duncan, J.R., Wilhelmi, B.S., Stoll, A.D., Zhao, M., van Hille, R.P. and Wallace, L. Bioaccumulation of metals from industrial and mining effluents. *Proc. 14th Intl. Congress on Biohydrometallurgy*, Sydney, 1997.

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1. Duncan, J.R., Wilhelmi, B., Stoll, A., Zhao, M., van Hille, R. and Wallace, L. Bioaccumulation of metals from industrial and mining effluents (Invited Lecture). *Proc. Biotech SA '97*, 1997.

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