REMOVAL AND RECOVERY OF GOLD AND PLATINUM FROM AQUEOUS SOLUTIONS UTILISING THE NON-VIABLE BIOMASS *Azolla filiculoides.*

THESIS

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ABSTRACT

Waste water from the mining industry is generally extremely complex and contains numerous species which influence the adsorption of the metals to any biomass. A variety of factors need to be addressed before treatment is considered viable. It is also beneficial to establish 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. The adsorption of gold(III) is principally pH-dependent with optimal removal 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, 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. 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, 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. 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).

For selective metal recovery of lead and copper, pre-washing the plant material with water, utilising an acid (0.3 M nitric acid), pumping in an up-flow mode, and recycling the desorbent six times was found to be optimal. Cost analysis of utilising elutant versus incinerating the biomass for gold recovery indicated the latter as the most economical. Over a 5 cycle adsorption and desorption series, acid desorption before each adsorption cycle was found to result in greater than 92% desorption for lead and 96% for copper. Gold recovery was 97% with incineration. A preliminary study with gold effluent (Mine C) indicated that nickel and sulphate was removed in batch and column studies. Gold removal was found to be 100% and 4% in batch and column studies respectively. Adsorption of gold in the effluent study was accompanied by the release of H^+.

Modifying the plant material with various reagents failed to identify the primary binding sites and the role of polysaccharides, proteins and lipids in gold(III) uptake. The mode of gold binding is suggested as being initially ionic, this is very rapid, with the interaction of the anionic complex, $[AuCl_4]^-$, with the cationic biomass (pH 2). This eventually leads to the displacement of the chloride ligand(s) initiating covalent binding. Spectral studies of the chemical interaction between gold and the representative tannins indicated the protonated hydroxy groups to be responsible. All evidence suggests that the binding mechanisms of gold are not simple.

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. Again adsorption was accompanied by H^+ release.

Azolla filiculoides demonstrated its feasibility in the removal of gold and platinum from simulated as well as waste water solutions. Its potential viability as a biosorbent was demonstrated by the high recovery from synthetic solutions of greater than 99% for gold (2-10 mg/L), and greater than 89% for platinum (20 mg/L).

List of Abbreviations

β	Overall formation constant
μM	Micromolar
π	Pi
AA	Atomic absorption
ATP-ase	Adenosine triphosphatase
$Au^{+/3+}$	Gold ion
$AuCN_2$	Gold cyanide
BSA	Bovine serum albumin
$C_4H_4O_3$	Succinic anhydride
CAT	Catechol
CIP	Carbon-in-pulp
cm^{-3}	Cubic centimetre
CN^-	Cyanide ion
CO	Carbon monoxide
CSTR	Conventional stirred tank reactors
DNA	Deoxyribose nucleic acid
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
DWAF	Department of water affairs
EDTA	Ethylenediaminetetraacetic acid
FT-IR	Fourier-Transform infrared
g/L	Gram per litre
g	Gram
GA	Gallic acid
h	Height
H_2PtCl_6	Chloroplatinic acid
$HAuCl_4$	Hydrogen tetrachloroaurate
HSAB	Hard and soft acid base
ICP-MS	Inductive coupled plasma-mass spectrometry
k	Stepwise formation constant
kg	Kilogram
L	Litre
M^{2+}	Divalent metal ion
MFS	Mimosa FS
mg/L	Milligram per litre
mL	Millilitre
mM	Millimolar
MME	Mimosa ME
MRA	Metal recovery agent
N_2	Nitrogen
Na_2SO_3	Sodium sulphite
Na_2SO_4	Sodium sulphate

NaBr	Sodium bromide
PBR	Packed-bed reactors
PGM	Platinum group metals
ppm	Parts per million
$Pt(NH_3)_2(Cl)_2$	Cisplatin
$Pt^{2+/4+}$	Platinum ion
$PtCl_4$	Tetrachloroplatinate(II)
QB	Quebracho
r^2	Radius squared
rpm	Revolutions per minute
SCN^-	Thiocyanate ion
SO_3^{2-}	Sulphite ion
SO_4^{2-}	Sulphate ion
TA	Tannic acid
TT	Trupotan MT
V_T	Total volume

δ	Bends:	vibrations involving atoms moving perpendicular to the bond.
ν	Stretches:	vibrations involving atoms moving along a bond.
ω	Wag:	vibrations involving three atoms with two atoms moving in the plane in phase
δ_s	Scissors:	vibrations involving three atoms with two atoms moving in the plane out of phase
ρ	Rock:	vibrations involving three atoms with two atoms moving out of the plane out of phase.

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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. To do so, we need to consider the following problems: first, the average rainfall in South Africa is about 900mm/annum, but is variable nationwide; second, South Africa has limited groundwater and, third, the evaporation rate exceeds

the precipitation rate. 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).

It is also necessary to manage and regulate water resources to achieve long term protection of the water quality. 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).

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.

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

1.2. 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).

1.3. 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).

1.3.1. Metal Removal and Recovery from Waste Waters

Heavy metals must be removed from waste waters in a specially designed "pre-treatment step" which has to feature low costs before it is regarded as feasible (Volesky, 1999). Application of conventional treatment methods, either chemical (precipitation, neutralisation) or physical (ion-exchange, activated carbon adsorption) have limitations. Table 1.2 outlines some of the technologies available for the removal of metals.

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)

Conventional methods are generally costly in that they use materials that may not be recovered for consecutive treatment cycles. This results in a low volume, high metal concentrated sludge that is difficult to dispose of. For example in the gold mining industry, the metal-cyanide complexes cannot be precipitated. Rather the cyanide needs to be converted to a cyanate ion through oxidation or be completely oxidised to nitrogen and carbon dioxide. In addition, ion-exchange resins used in the water and waste treatment plants are prone to oxidation by chemicals, sensitive to the presence of calcium or magnesium ions, and fouling by organics (Atkinson *et al.*, 1998). A further difficulty, in metal-finishing plants for example, is the production of variable effluent metal loads which complicates the choice of treatment process. Some alternative physical treatment methods remove metals using membranes that are not resistant to pH changes and thus have a limited tolerance for metal removal.

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.

1.4. 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.

1.4.1. 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.

1.4.2. 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).

1.4.3. 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 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).

1.4.4. 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).

1.4.4.1. 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).

1.4.5. 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.

1.5. 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 1.1). 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.

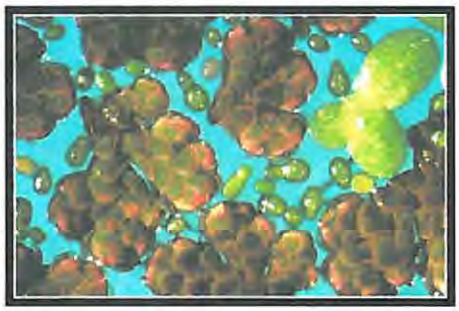


Figure 1.1: Several water fern plants floating on the water surface. *Azolla filiculoides* (dark brown overlapping leaves) is represented with duckweed (oval green fronds).

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 1.2) 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).

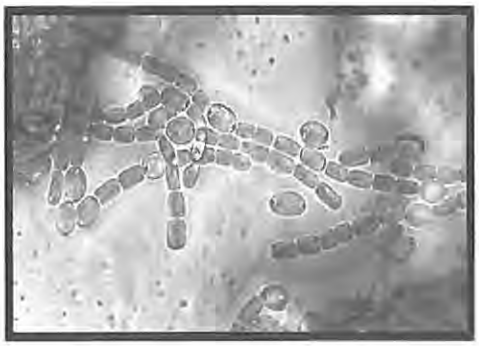


Figure 1.2: Filimentous cyanobacterium *(Anabeana azollae)* from cavities within the leaves of *Azolla filiculoides*. The heterocysts (large oval cells) are responsible for nitrogen fixation ($N_2 \rightarrow NH_3$).

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 1.3).

(A) (B)

Figure1.3: 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.

1.6. 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).

1.6.1. 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 1.4 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 1.4: 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):

$$4\ Au + 8CN^- + O_2 + 2H_2O \rightarrow 4[Au(CN)_2]^- + 4OH^- \qquad (1)$$

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 1). It has since been concluded that most of the gold dissolves according to the following reaction (Equation 2) (Förstner and Wittmann, 1976):

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

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.

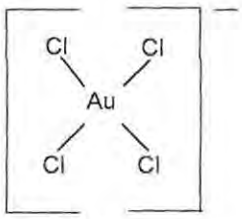
The biggest challenge to the industry arises from the intense exploitation of gold deposits in the past and the near exhaustion of many ores close to the surface, namely the development of better recovery techniques. Gold-bearing metal scrap is being returned to refiners for recovery (West, 1975). A 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*.

1.6.2. 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 1.5).

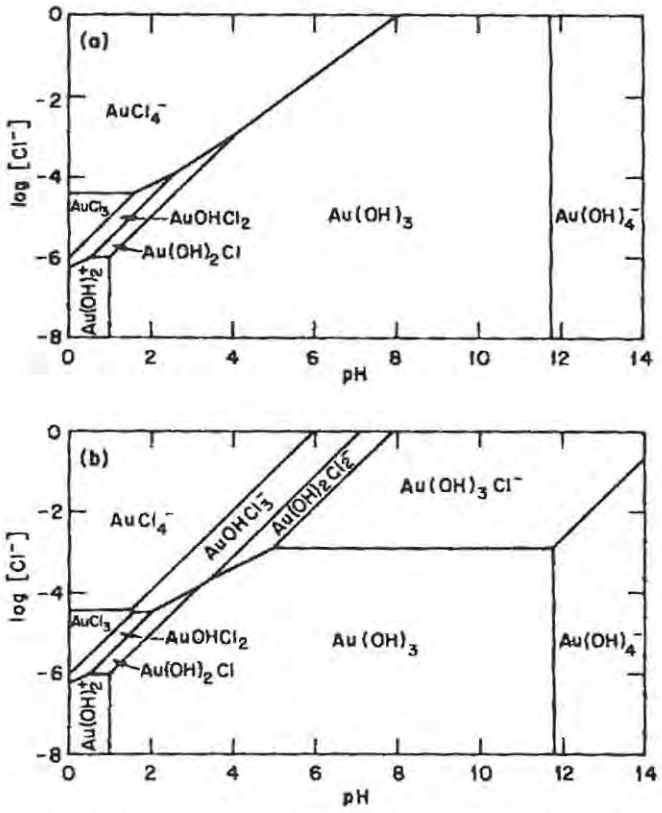
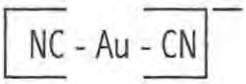


Figure 1.5: Predominance diagram of Au(III)-OH^--Cl^- species. The boundaries indicate conditions under which adjacent species are present in equal concentrations (a) based on calculated equilibrium constants and (b) based on reported equilibrium constants for mixed species (Baes Jr. and Mesmer, 1976).

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).

1.7. 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).

1.7.1. 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 1.6.

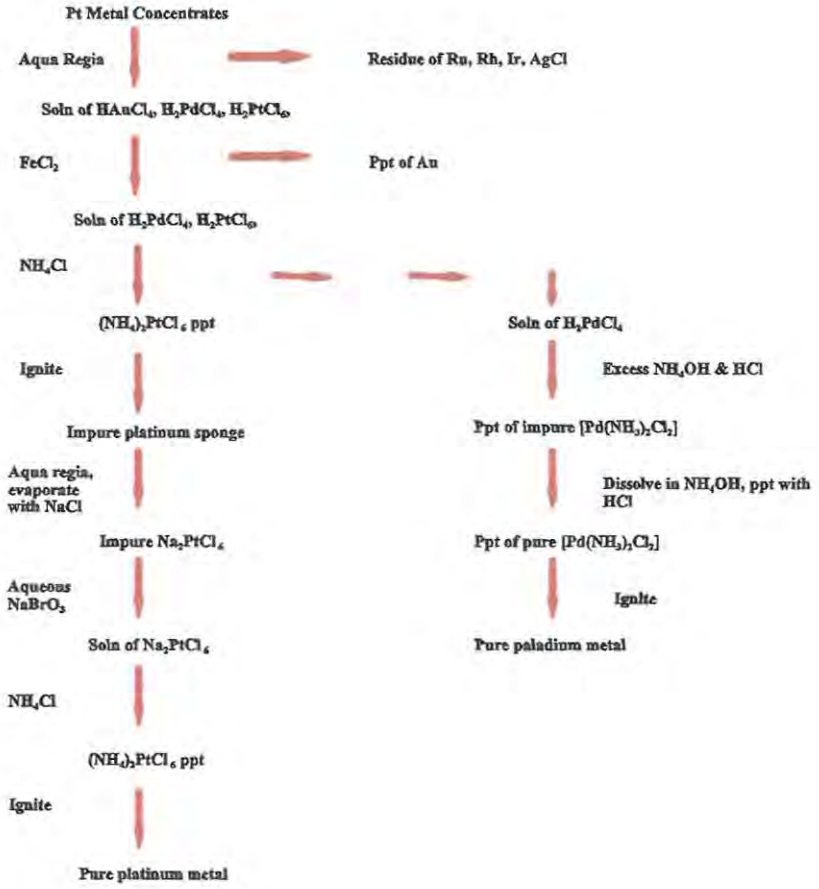


Figure 1.6: Flow-diagram of platinum and palladium extraction (Greenwood and Earnshaw, 1989).

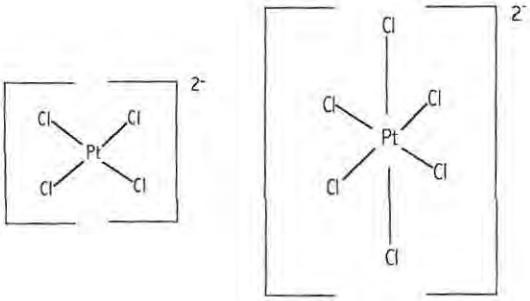
1.7.2. Chemical Properties

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 3) (Greenwood and Earnshaw, 1989; Lee, 1991):

$$Pt^0 \xrightarrow{\text{Aqua Regia}} H_2[PtCl_6] \xrightarrow{\text{Heat}} PtCl_4 + 2HCl \qquad (3)$$

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 4) (Hartley, 1973). Hydroxy compounds are most stable for the +4 oxidation state.

$$[PtCl_4]^{2-} + H_2O \rightleftharpoons Cl^- + [PtCl_3.H_2O]^- \rightleftharpoons [PtCl_3(OH)]^{2-} + H^+ \qquad (4)$$

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.

1.8. 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 1.7). 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 1.8) (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).

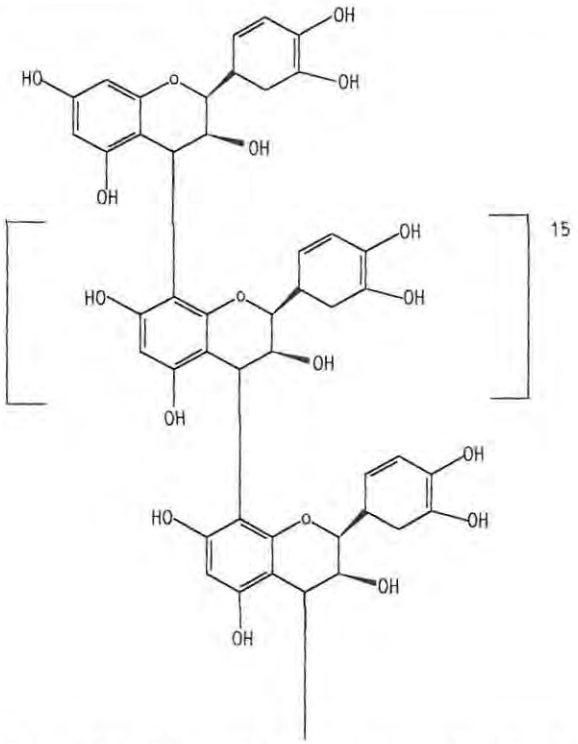


Figure 1.7: Schematic representation of a simple condensed tannin (Sorghum procyanidin).

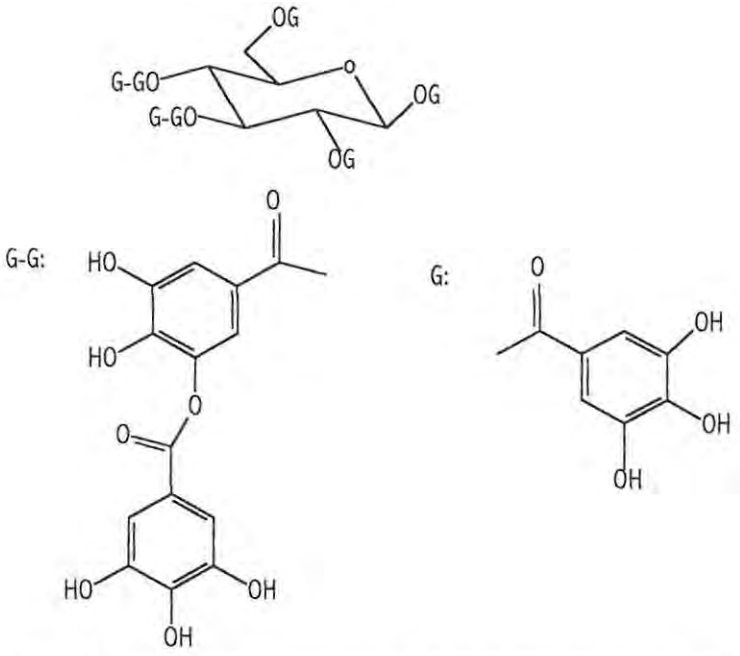


Figure 1.8: Schematic representation of a hydrolysable tannin, gallic acid which is compromised of polygalloyl esters of glucose.

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.

1.9. Scope of the Present Investigation

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

Preliminary studies focused on the removal of gold from dilute solutions under varying parameters of pH, temperature, initial gold concentration and biomass concentration in batch reactors. 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 from solution of another precious metal, platinum. 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.

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.

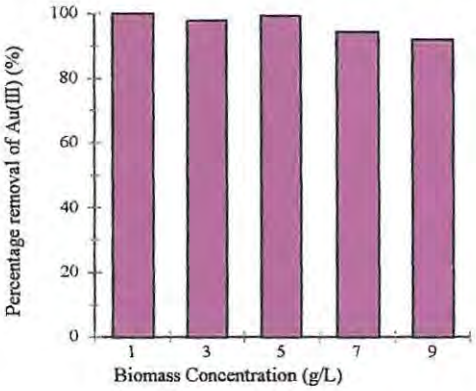


Figure 2.1: The effect of biomass concentrations on the adsorptive capacity of *Azolla filiculoides* with an initial gold concentration of 5 mg/L and pH of 2. The experiment was carried out at room temperature and agitated at a speed of 200 rpm.

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.

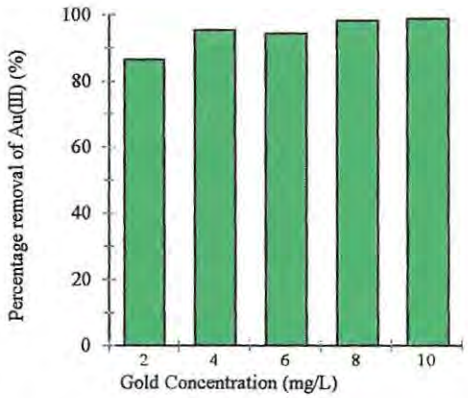


Figure 2.2: The effect of initial gold concentrations on the adsorptive capacity of *Azolla filiculoides* at a biomass concentration of 5 g/L and pH of 2. The experiment was conducted at room temperature and agitated at a speed of 200 rpm.

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.

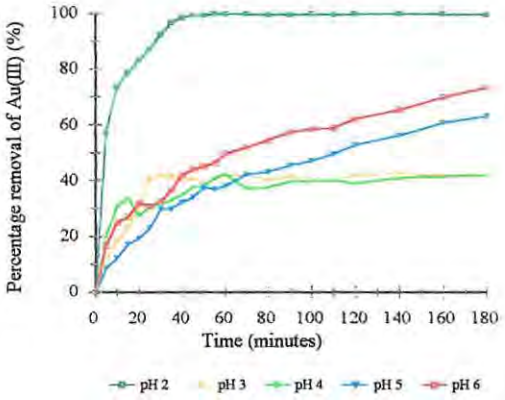


Figure 2.3: The effect of pH on the adsorptive capacity of *Azolla filiculoides* at an initial gold concentration of 8 mg/L and biomass concentration of 5 g/L. The experiment was conducted at room temperature and agitated at 200 rpm.

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.

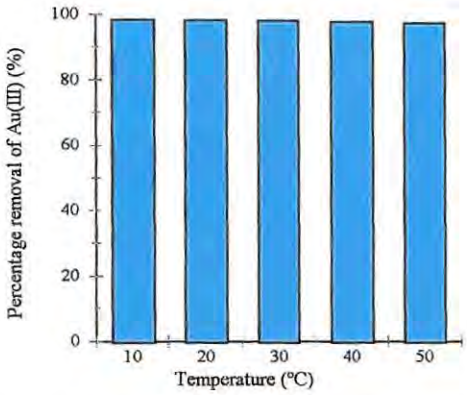


Figure 2.4: Effect of temperature on the adsorptive capacity of *Azolla filiculoides* at an initial gold concentration of 8 mg/L, biomass concentration of 5 g/L and pH 2. The experiment was conducted at room temperature and agitated at 200 rpm.

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.

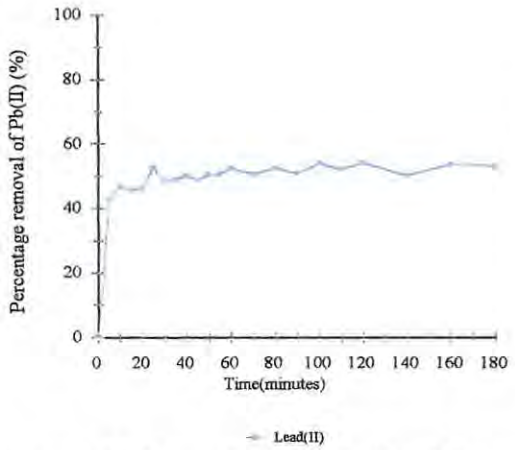


Figure 2.5: Removal of lead(II) (50 μM) from aqueous solution at pH 2. The following parameters were utilised: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was kept constant throughout the experiment.

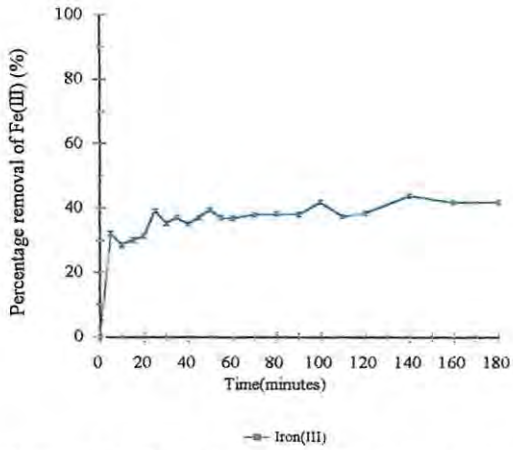


Figure 2.6: Removal of iron(III) (50 μM) from aqueous solution at pH 2. The following parameters were utilised: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was kept constant throughout the experiment.

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.

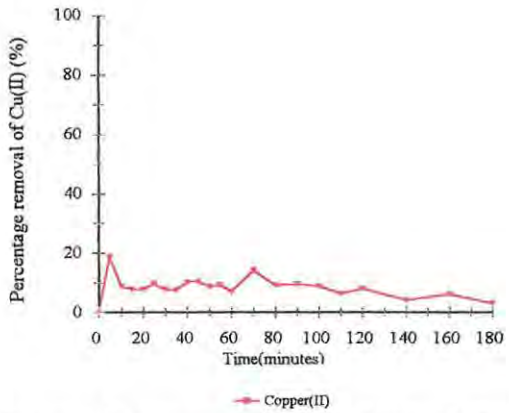


Figure 2.7: Removal of copper(II) (50 μM) from aqueous solution at pH 2. The following parameters were utilised: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was kept constant throughout the experiment.

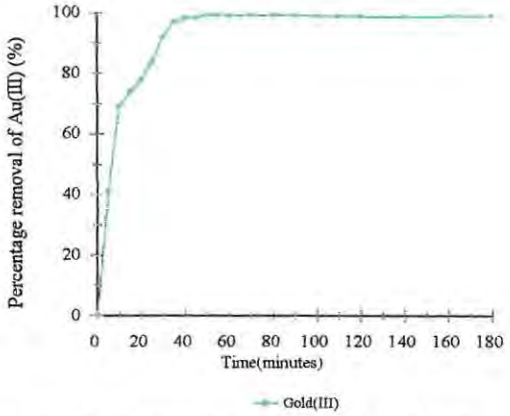


Figure 2.8: Removal of gold(III) (50 μM) from aqueous solution at pH 2. The following parameters were utilised: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was kept constant throughout the experiment.

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.

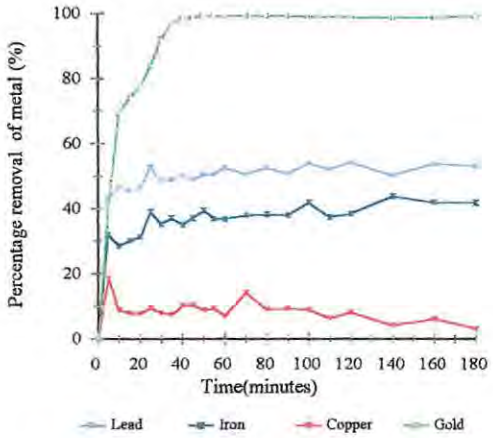


Figure 2.9a: Removal of various metals from aqueous solutions (individual metal studies). Final concentration of all four metals was 50 μM. Parameters included: biomass concentration of 5 g/L, room temperature and agitation speed of 200 rpm. A pH of 2 was maintained throughout the experiment.

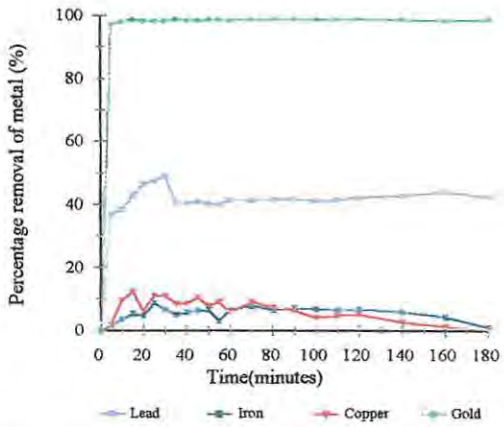


Figure 2.9b: Removal of various metals from an aqueous solution (mixed metal study). Final concentration of all four metals was 50 μM. Parameters included: biomass concentration of 5 g/L, room temperature and agitation speed of 200 rpm. A pH of 2 was maintained throughout the experiment.

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.

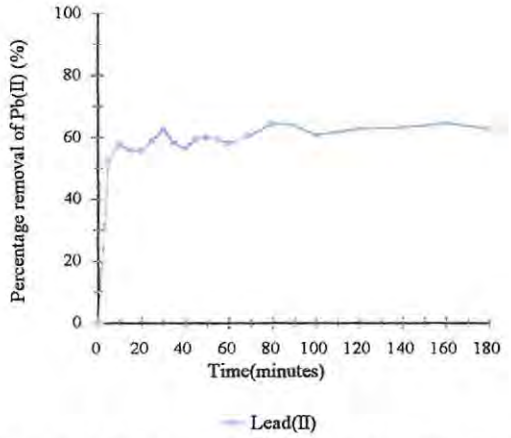


Figure 2.10: Removal of lead (25 μM) from aqueous solution at pH 2. Variables include: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was maintained throughout the experiment.

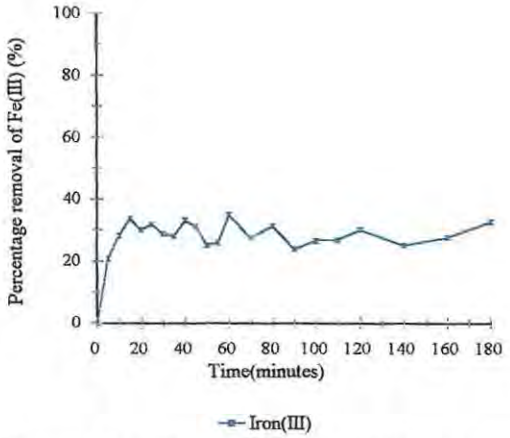


Figure 2.11: Removal of iron (10 μM) from aqueous solution at pH 2. Variables include: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was maintained throughout the experiment.

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.

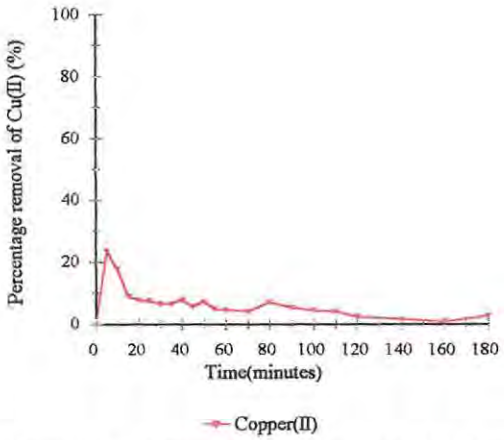


Figure 2.12: Removal of copper (200 μM) from aqueous solution at pH 2. Variables include: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was maintained throughout the experiment.

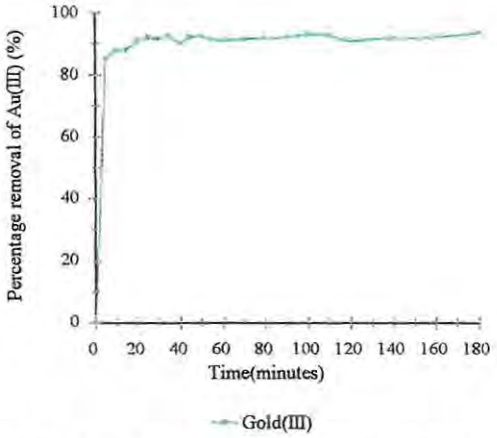


Figure 2.13: Removal of gold (5 μM) from aqueous solution at pH 2. Variables include: room temperature, agitation speed of 200 rpm and biomass concentration of 5 g/L. pH was maintained throughout the experiment.

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.

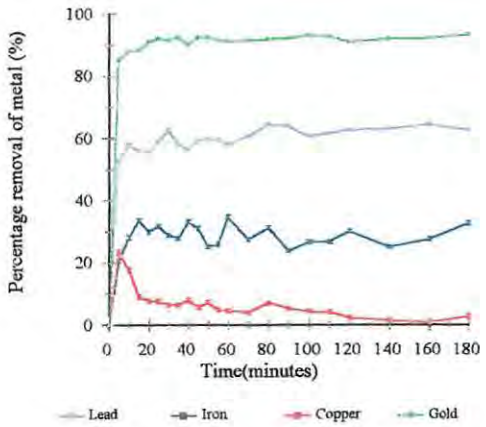


Figure 2.14a: Removal of various metals from aqueous solutions (individual metal studies). Initial effluent concentrations were: lead(II) (25 μM), iron(III) (10 μM), copper(II) (200 μM), and gold(III) (5 μM). The following conditions applied: biomass concentration: 5g/L, room temperature and agitation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

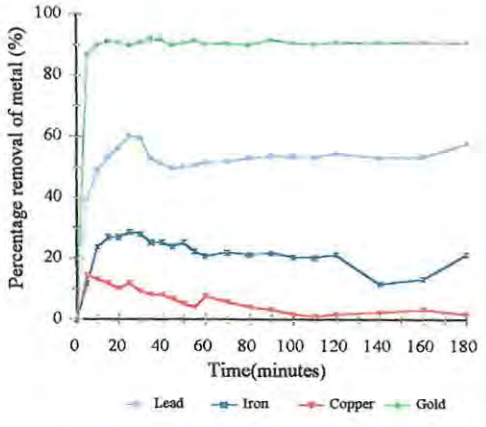


Figure 2.14b: Removal of various metals from an aqueous solution (mixed metal study). Initial effluent concentrations were: lead(II) (25 μM). iron(III) (10 μM), copper(II) (200 μM), and gold(III) (5 μM). The following conditions applied: biomass concentration: 5g/L, room temperature and agitation speed 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: $Cl^- < Br^- < I^-$.

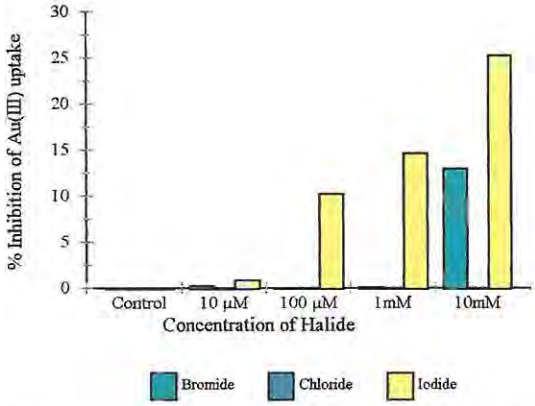


Figure 2.15: The effect of competing halides bromide, chloride and iodide at various concentrations on the removal of gold(III) (40 µM) at pH 2 from solution. The following parameters were utilised: biomass concentration: 5 g/L, agitation speed of 200 rpm and room temperature.

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 β (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*
$AuCl_2^-$	$k_1 = 12.15$, $k_2 = 7.79$, $\beta_2 = 19.94$
$AuBr_2^-$	$k_1 = 11.98$, $k_2 = 8.41$, $\beta_2 = 20.39$
AuI_2^-	$k_1 = 17.1$, $k_2 = 6.7$, $\beta_2 = 23.8$
$Au(CN)_2^-$	$\beta_2 = 33.7$
$Au(NH_3)_2^+$	$k_1 = 10.14$, $k_2 = 8.0$, $\beta_2 = 18.14$
$Au(SCN_2H_4)_2^+$	$\beta_2 = 21.3$
$AuCl_4^-$	$k_1 = 9.26$, $k_2 = 8.31$, $k_3 = 7.31$, $k_4 = 6.16$, $\beta_4 = 26$
$AuBr_4^-$	$\beta_4 = 32$
$Au(CN)_4^-$	$\beta_4 = 56$
$Au(NH_3)_4^{3+}$	$\beta_4 = 30$

k = stepwise formation constant, β = repesents the overall formation constant ($\beta_n = k_1k_2k_3....k_n$)

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.

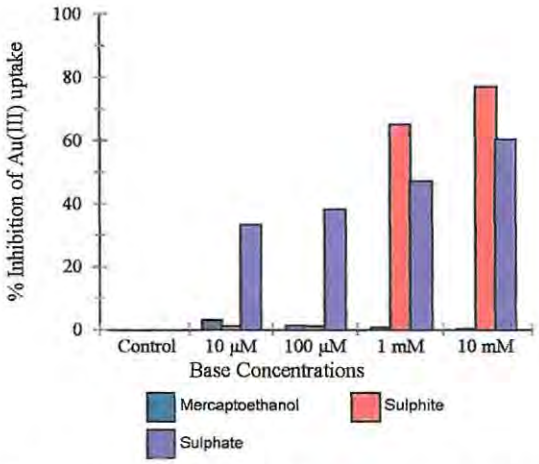


Figure 2.16: Effect of the bases mercaptoethanol, SO_4^{2-} and SO_3^{2-} at various concentrations on the uptake of gold(III) (40 μM) at pH 2 from solution. The following parameters were utilised: biomass concentration: 5g/L, room temperature, agitation speed of 200 rpm.

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).

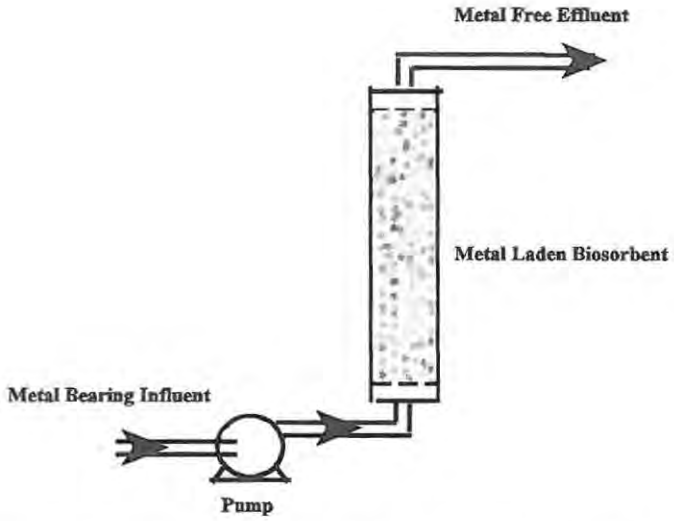


Figure 3.1: Schematic representation of a typical packed bed reactor with an up-flow mode commonly utilised in biosorption studies.

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.

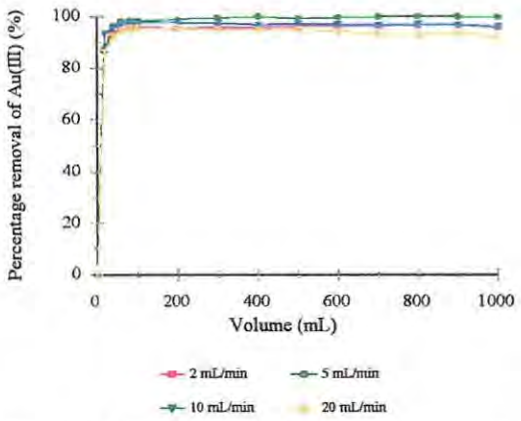


Figure 3.2a: The effect of various flow-rates on the adsorption of gold(III) from solution. Parameters utilised were as follows: pH 2, biomass concentration: 5 g/L, initial gold(III) concentration: 5 mg/L, bed-volume: 49 mL and room temperature.

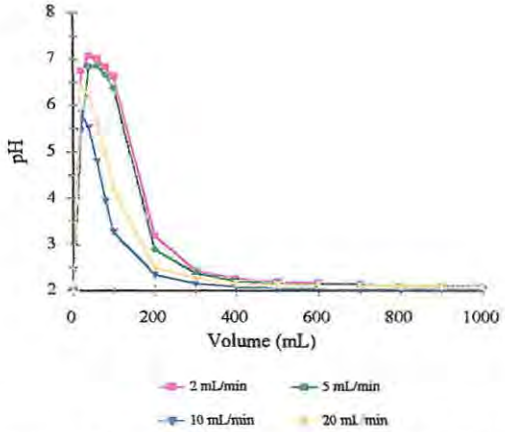


Figure 3.2b: pH profile of a 5 mg/L gold(III) solution at various flow-rates. Parameters utilised were as follows: pH 2, biomass concentration: 5 g/L, gold(III) concentration: 5 mg/L, bed-volume: 49 mL and room temperature.

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).

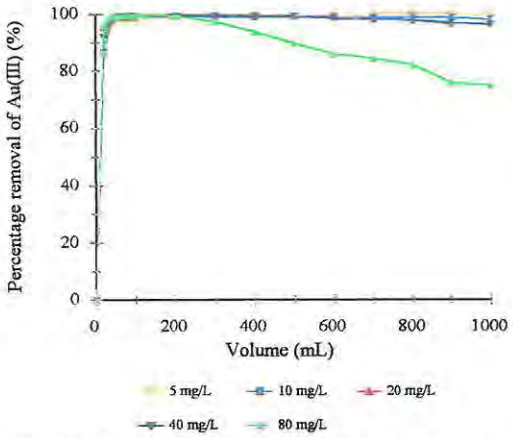


Figure 3.3a: The effect of various initial gold(III) concentrations on the adsorption of gold(III) from solution. The parameters were as follows: initial pH of 2, flow-rate at 5 mL/min, biomass concentration: 5 g/L, room temperature and bed volume: 49 mL.

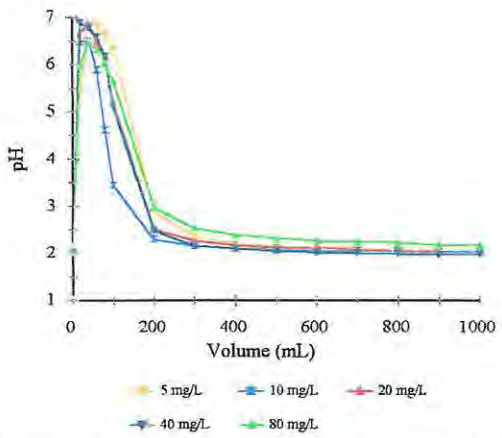


Figure 3.3b: pH profile of various gold(III) concentrations. The parameters were as follows: initial pH of 2, flow-rate at 5 mL/min, biomass concentration: 5 g/L, room temperature and bed volume: 49 mL.

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).

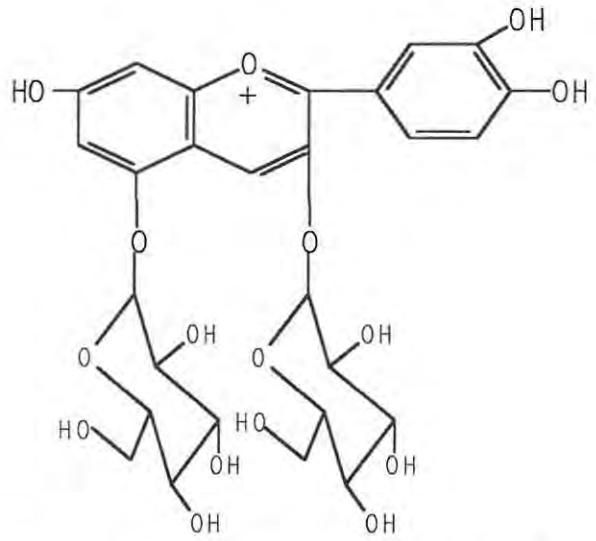


Figure 3.4: Schematic representation of the constituents of a condensed tannin, anthocyanin (Bickley, 1999).

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).

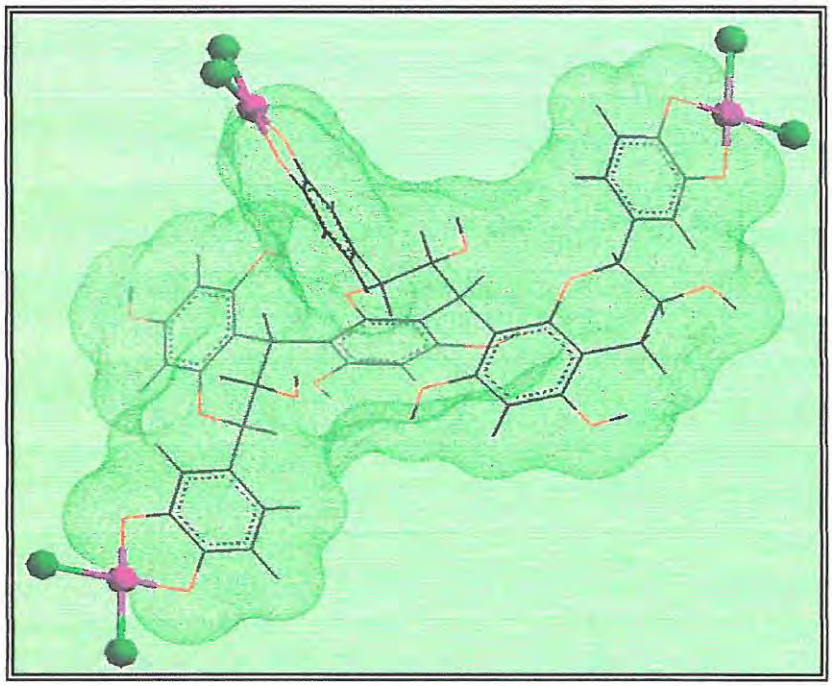


Figure 3.5: Minimised-energy conformation modelling of hydrogen tetrachloroaurate(III) and a typical condensed tannin. The Cerius II™ modelling programme with molecular mechanics (MM) and universal force field (UFF) was utilised.

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.

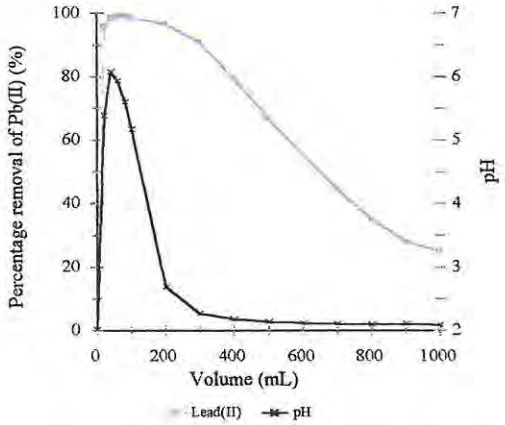


Figure 3.6: Removal of lead(II) (50 μM) from aqueous solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate of 5 mL/min and bed-volume: 49 mL.

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.

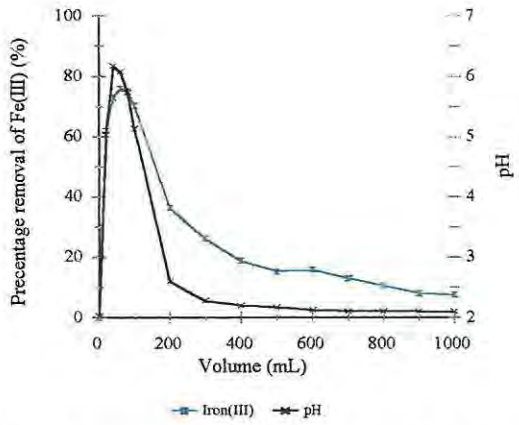


Figure 3.7: Removal of iron(III) (50 μM) from aqueous solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate of 5 mL/min and bed-volume: 49 mL.

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.

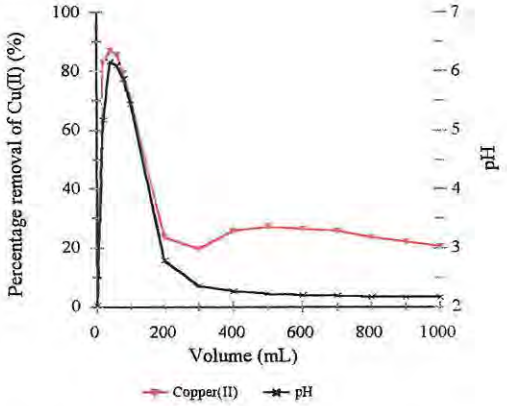


Figure 3.8: Removal of copper(II) (50 µM) from aqueous solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate of 5 mL/min and bed-volume: 49 mL.

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%.

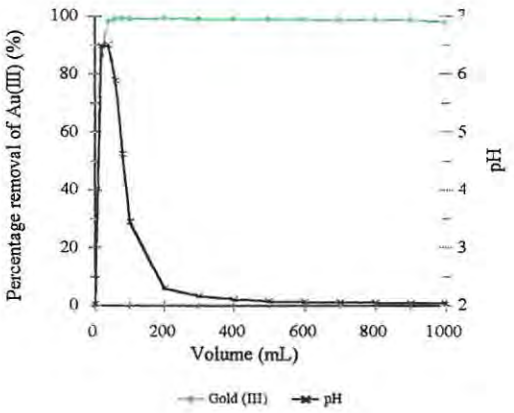


Figure 3.9: Removal of gold(III) (50 μM) from aqueous solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate of 5 mL/min and bed-volume: 49 mL.

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.

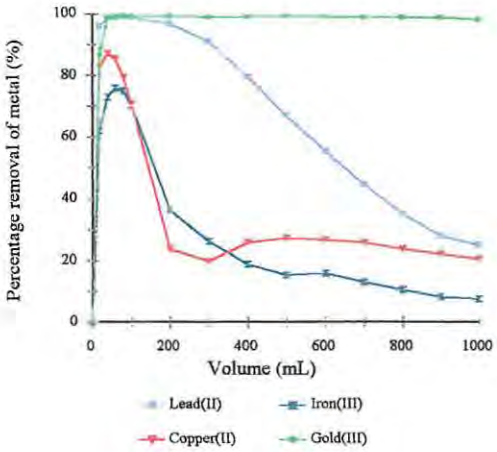


Figure 3.10a: Removal of various metals from aqueous solutions (individual metal studies) at a 50 μM concentration and an influent pH of 2. Parameters utilised were as follows: biomass concentration: 5 g/L, flow-rate: 5 mL/min, room temperature, bed-volume of 49 mL.

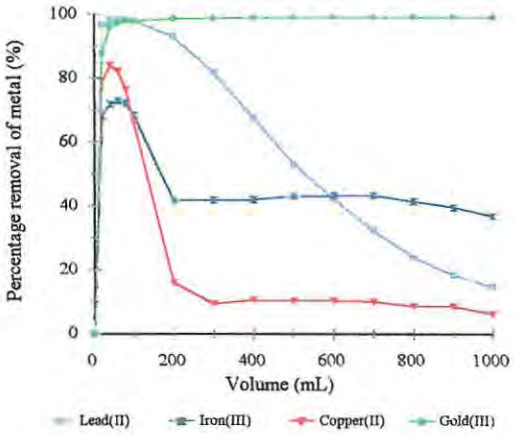


Figure 3.10b: Removal of various metals from a mixed metal solution at a 50 μM concentration and an influent pH of 2. Parameters utilised were as follows: biomass concentration: 5 g/L, flow-rate: 5 mL/min, room temperature, bed-volume of 49 mL.

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.

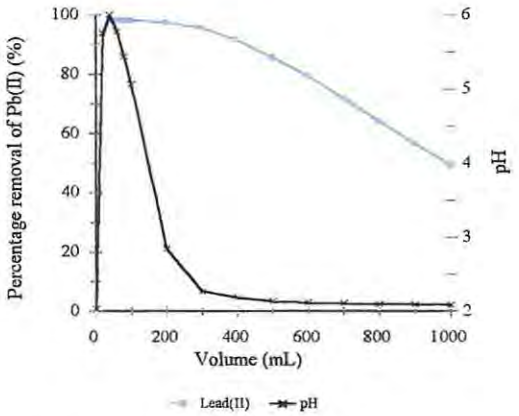


Figure 3.11: Removal of lead(II) (25 μM) from solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

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.

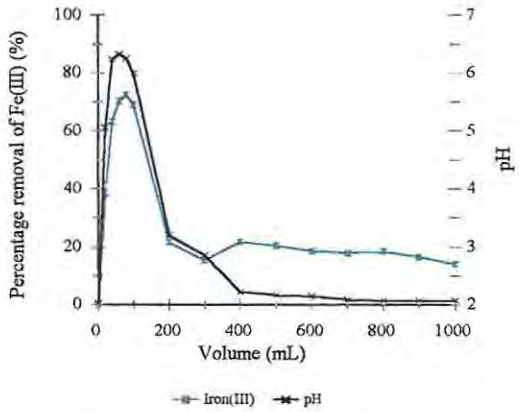


Figure 3.12: Removal of iron(III) (10 μM) from solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

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).

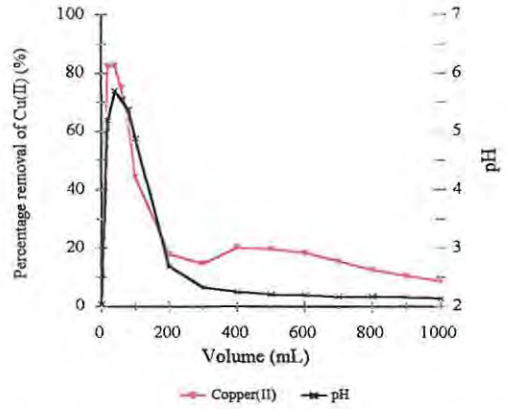


Figure 3.13: Removal of copper(II) (200 μM) from solution accompanied with a pH profile. Parameters were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

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.

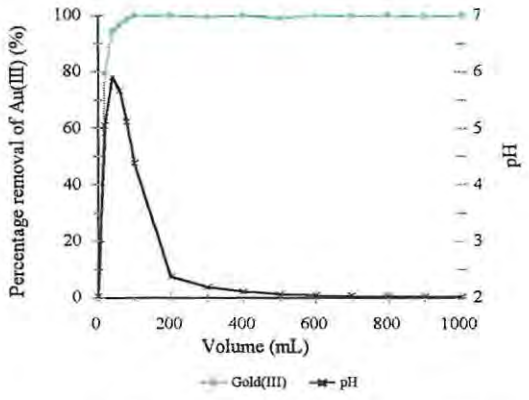


Figure 3.14: Removal of gold(III) (5 μM) from solution accompanied with a pH profile. Parameters utilised were as follows: influent pH of 2, room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

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.

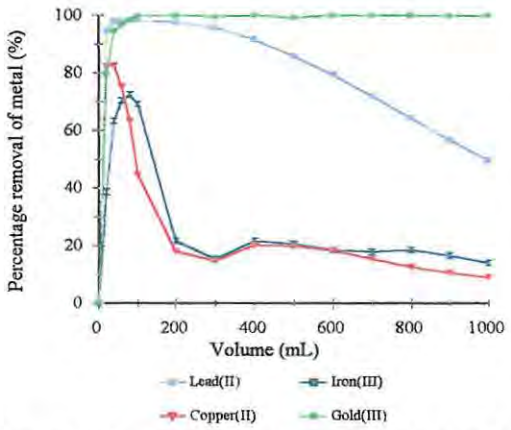


Figure 3.15a: Removal of various metals: lead(II) (25 μM), iron(III) (10 μM), copper(II) (200 μM) and gold(III) (5 μM) from solutions (individual metal studies) at an influent pH of 2. Parameters included: room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

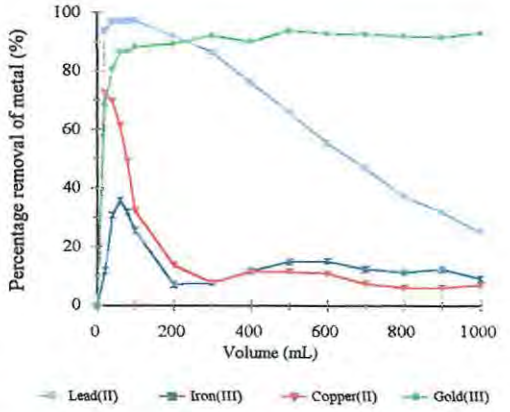


Figure 3.15b: Removal of various metals: lead(II) (25 μM), iron(III) (10 μM), copper(II) (200 μM) and gold(III) (5 μM) from solution at an influent pH of 2. Parameters included: room temperature, biomass concentration: 5 g/L, flow-rate: 5 mL/min and bed volume: 49 mL.

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.

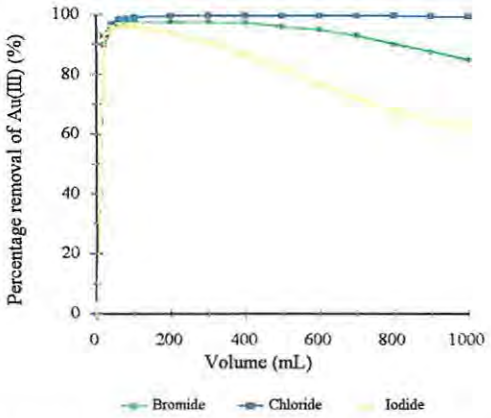


Figure 3.16: The effect of competing halides: bromide, chloride and iodide at 10 mM on the uptake of gold(III) (40 μM) from solution at pH 2. The following parameters were utilised: biomass concentration of 5 g/L, flow-rate of 5 mL/min and room temperature.

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.

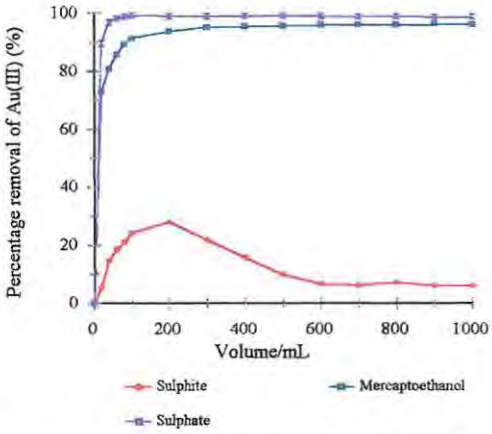


Figure 3.17: The effect of various bases mercaptoethanol, SO_3^{2-} and SO_4^{2-} at 10 mM on the uptake of gold(III) (40 μM) at pH 2 from solution. The following parameters were utilised: biomass concentration: 5 g/L, flow-rate of 5 mL/min and room temperature.

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):

$$Au^{+} + 2CS(NH_2)_2 \rightleftharpoons [Au\{CS(NH_2)_2\}_2]^{+} \quad (5)$$

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 3:1 of 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.

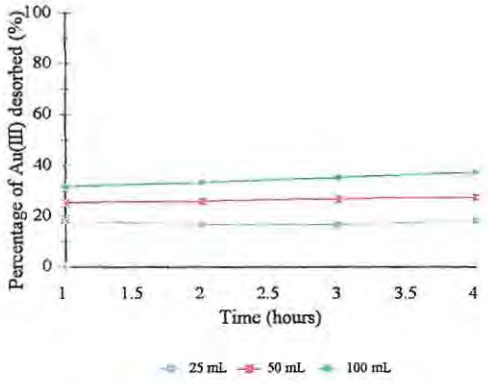


Figure 4.1a: Desorption of gold from *Azolla filiculoides* with various volumes of 0.1 M thiourea at pH 1.7. Desorption followed immediately once adsorption was complete. Parameters utilised included: room temperature, agitation speed of 200 rpm.

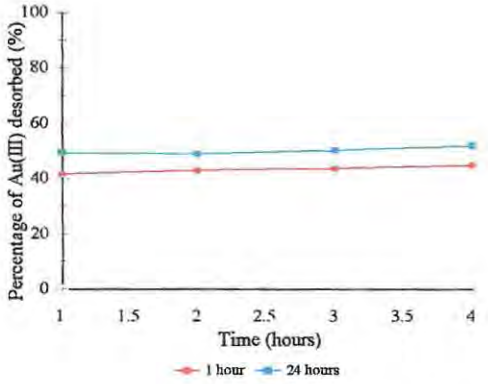


Figure 4.1b: The effect of incubating the plant material for 1 and 24 hours, in the absence of a desorbent. Desorption then proceeded as in Figure 4.1a utilising a ratio of adsorbent:desorbent of 1:1.

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).

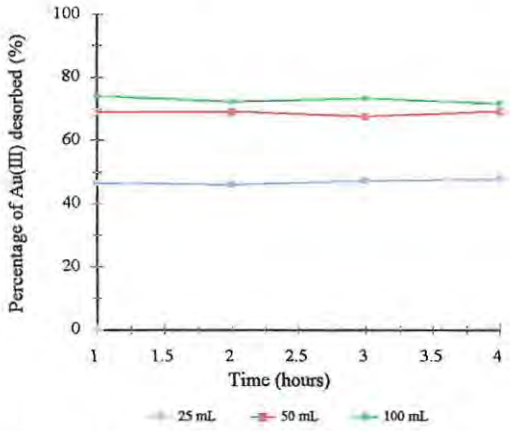


Figure 4.2a: Desorption of gold from *Azolla filiculoides* with various volumes of 8% thiourea, 2% perchloric acid, 0.5% 0.1M HCl at pH 1.4. Desorption followed immediately once adsorption was complete. Parameters utilised included: room temperature, agitation speed of 200 rpm.

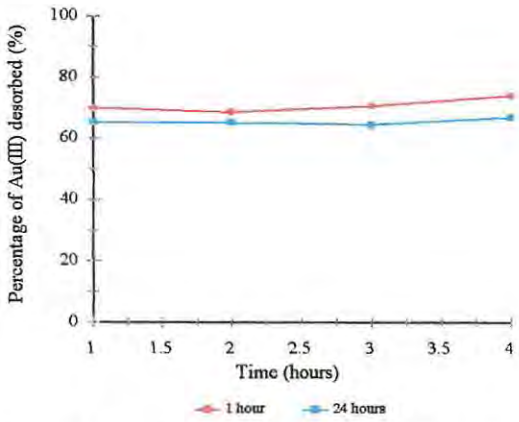


Figure 4.2b: The effect of incubating the plant material for 1 and 24 hours in the absence of a desorbent. Desorption then proceeded as in Figure 4.2a utilising a ratio of adsorbent:desorbent of 1:1.

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).

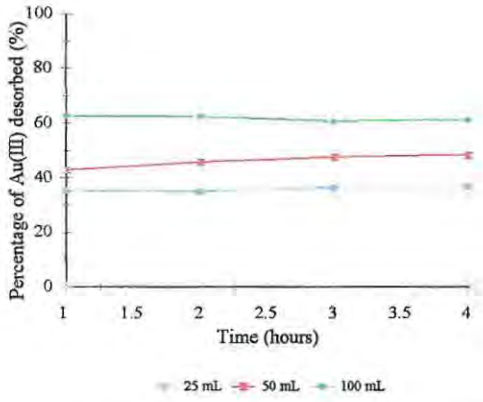


Figure 4.3a: Desorption of gold from *Azolla filiculoides* with various volumes of 0.1 M thiourea and ammonium peroxodisulphate (0.06 M) at pH 2. Desorption followed immediately once adsorption was complete. Parameters utilised included: room temperature, agitation speed of 200 rpm.

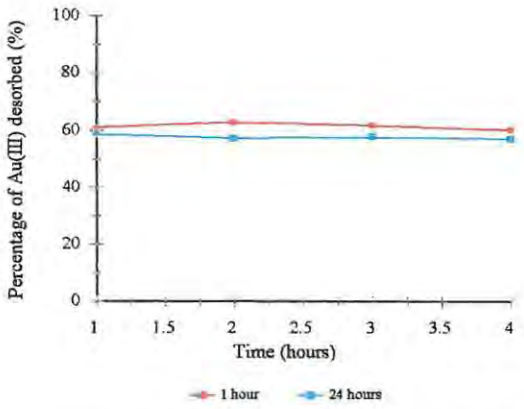


Figure 4.3b: The effect of incubating the plant material for 1 and 24 hours in the absence of a desorbent. Desorption then proceeded as in Figure 4.3a utilising a ratio of adsorbent:desorbent of 1:1.

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.

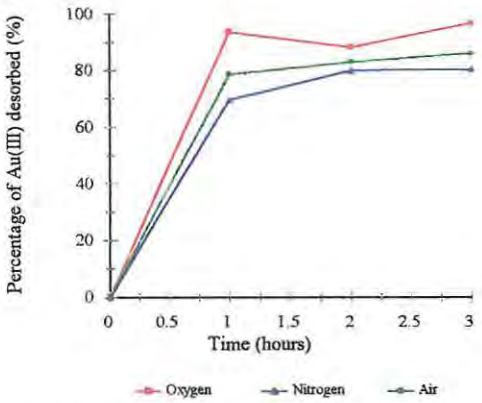


Figure 4.4: Desorption of gold utilising oxygen, air and nitrogen in combination with thiourea. The following parameters were utilised: **Adsorption**: initial gold(III) concentration: 5 mg/L, biomass concentration: 5 g/L, pH 2, agitation speed of 200 rpm and room temperature. **Desorption**: all gases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

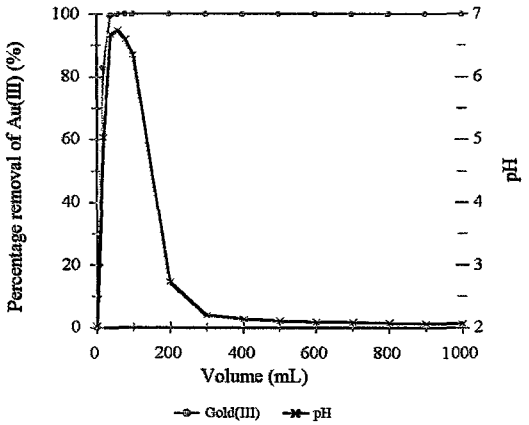


Figure 4.5: Typical adsorption and pH profile obtained when gold(III) is removed from solution by *Azolla filiculoides*. The parameters utilised were: initial gold(III) concentration of 5 mg/L, biomass concentration of 5 g/L, pH 2, flow-rate of 5 mL/min and room temperature.

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.

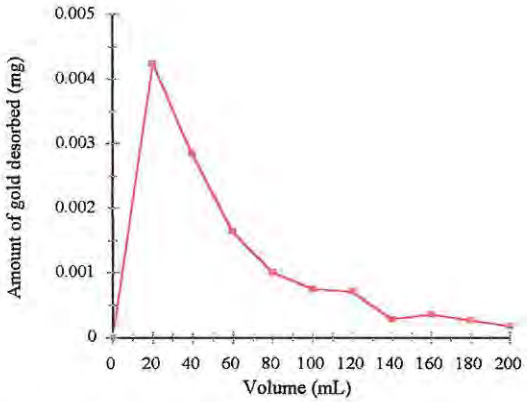


Figure 4.6a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M H_2SO_4. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

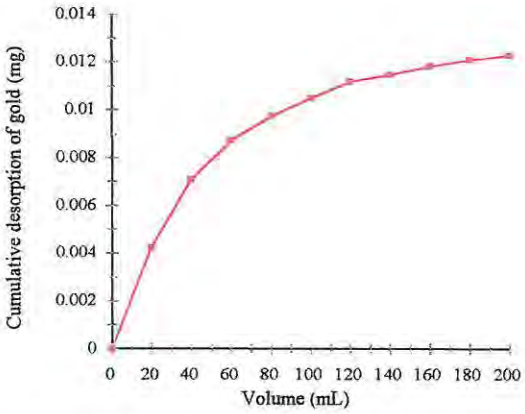


Figure 4.6b: Cumulative desorption of gold utilising 0.1 M H_2SO_4. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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.

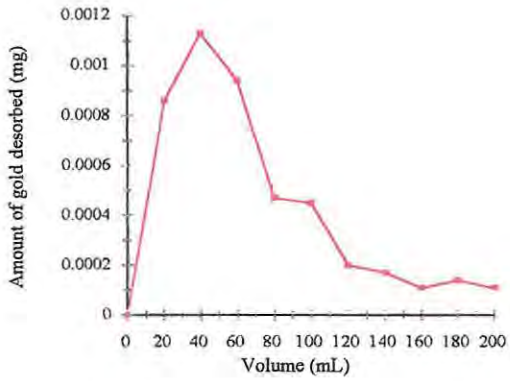


Figure 4.7a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M EDTA. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

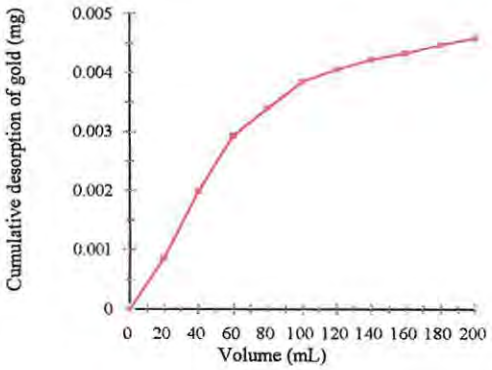


Figure 4.7b: Cumulative desorption of gold utilising 0.1 M EDTA. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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.

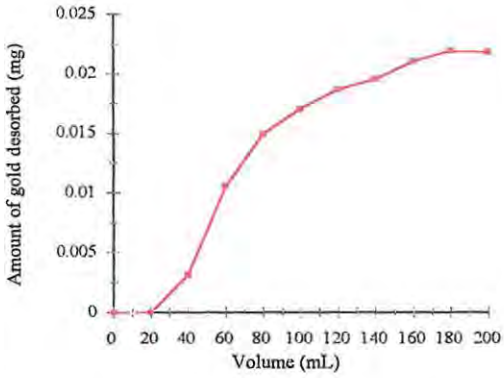


Figure 4.8a: Amount of gold desorbed for every 20 mL fraction utilising 0.5 M mercaptoethanol. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

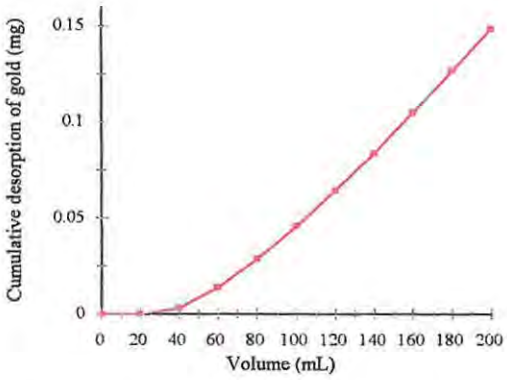


Figure 4.8b: Cumulative desorption of gold utilising 0.5 M mercaptoethanol. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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.

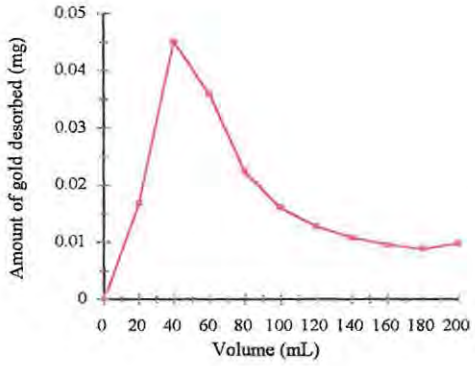


Figure 4.9a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M KOH. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

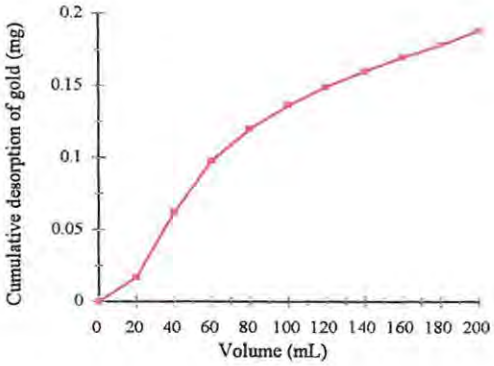


Figure 4.9b: Cumulative desorption of gold utilising 0.1 M KOH. Parameters utilised include the following: biomass concentration: 5 g/L. room temperature, flow-rate: 5 mL/min.

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).

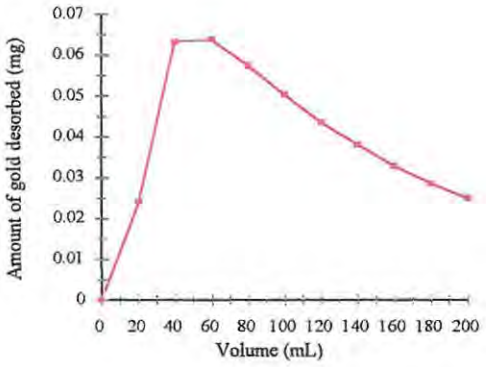


Figure 4.10a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M KBr + 20% EtOH. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

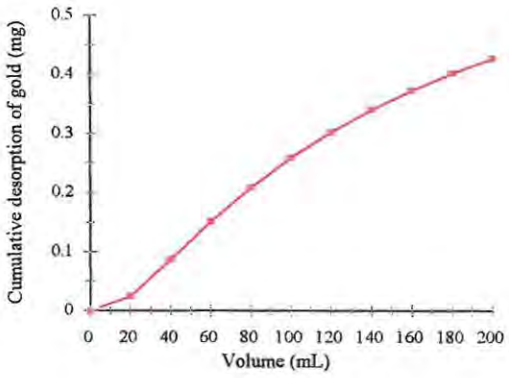


Figure 4.10b: Cumulative desorption of gold utilising 0.1 M KBr + 20% EtOH. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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.

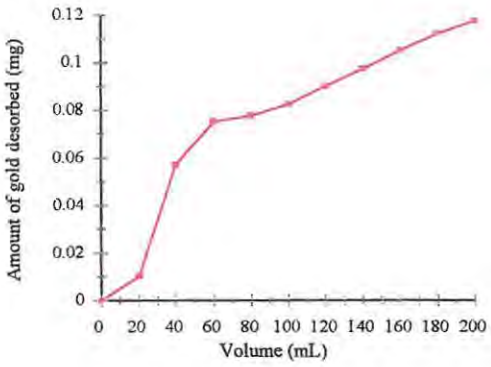


Figure 4.11a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

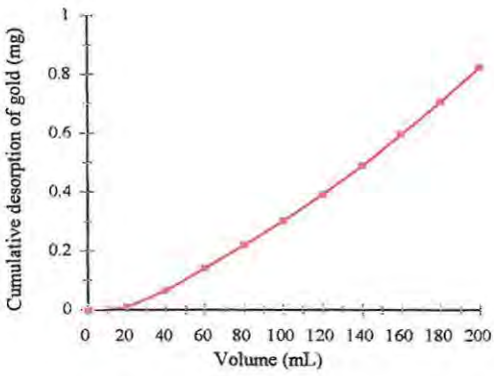


Figure 4.11b: Cumulative desorption of gold utilising 0.1 M thiourea. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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.

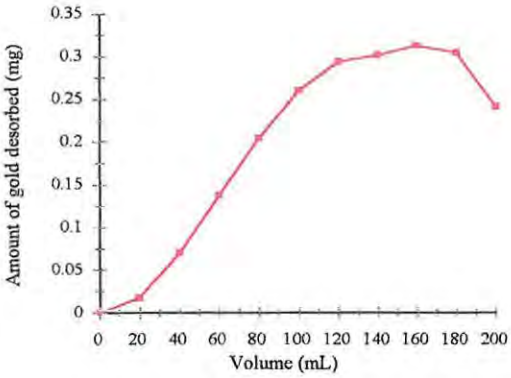


Figure 4.12a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.02 M $NH_4Fe(SO_4)_2$. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

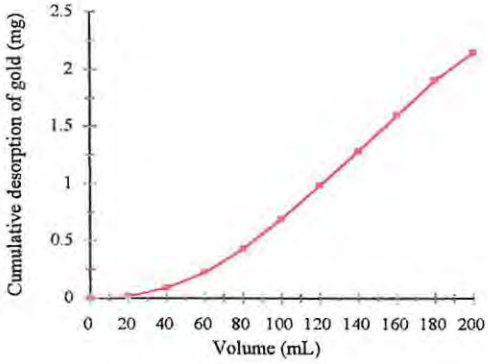


Figure 4.12b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.02 M $NH_4Fe(SO_4)_2$ Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

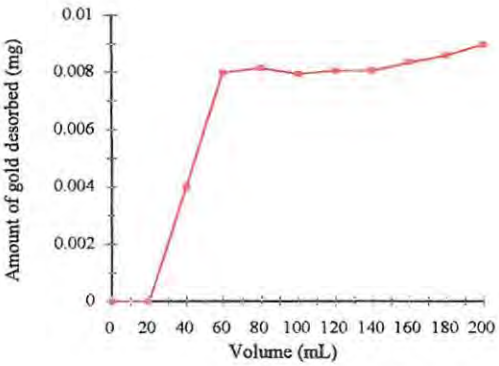


Figure 4.13a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.02 M $(NH_4)_2Fe(SO_4)_2$. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

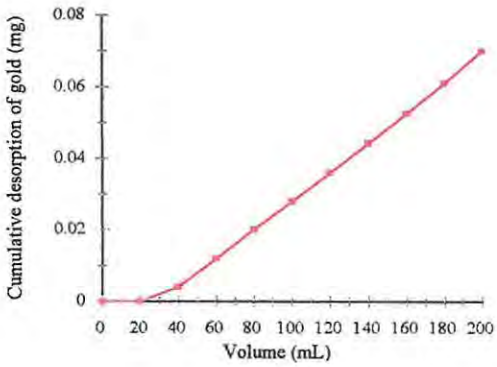


Figure 4.13b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.02 M $(NH_4)_2Fe(SO_4)_2$ Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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.

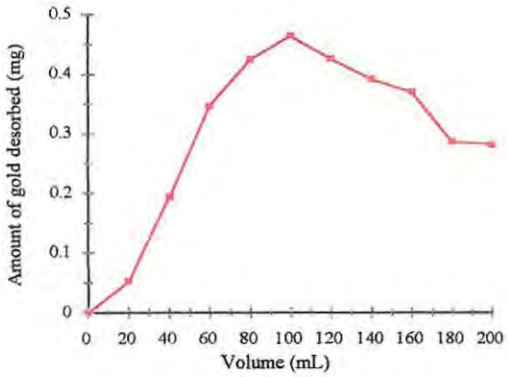


Figure 4.14a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.06 M $NH_4S_2O_8$ at pH 1.46. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

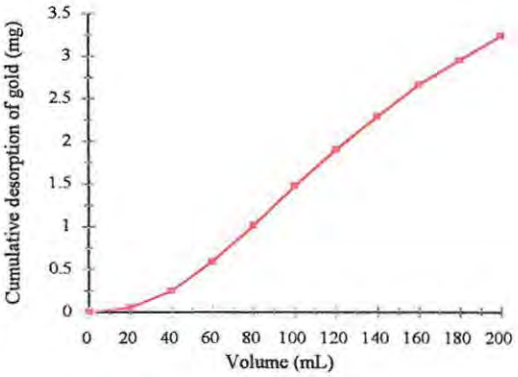


Figure 4.14b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.06 M $NH_4S_2O_8$ at pH 1.46. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

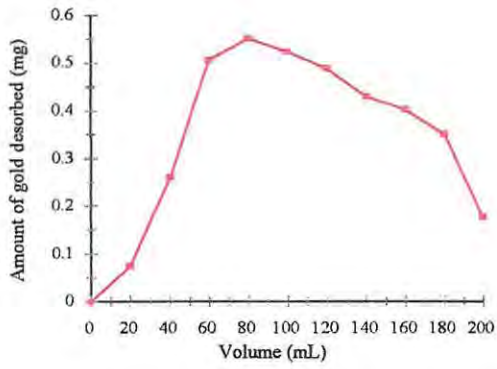


Figure 4.15a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.06 M $NH_4S_2O_8$ at pH 2.46 Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

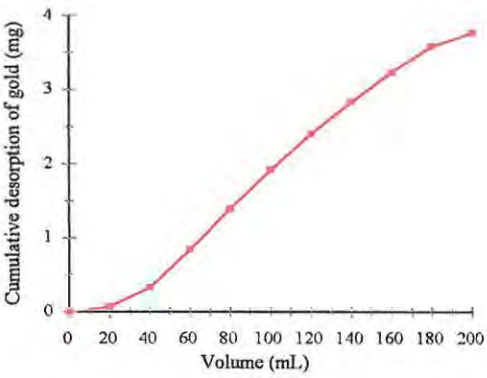


Figure 4.15b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.06 M $NH_4S_2O_8$ at pH 2.46. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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%.

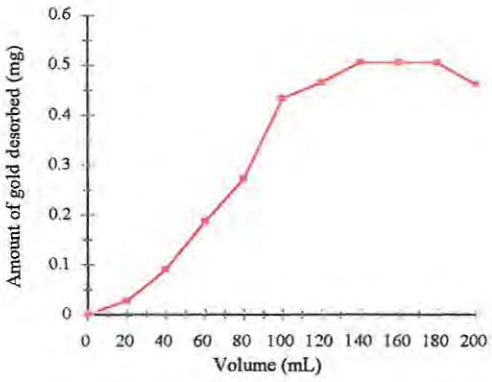


Figure 4.16a: Amount of gold desorbed for every 20 mL fraction utilising 0.1 M thiourea + 0.02 M $FeCl_3$. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

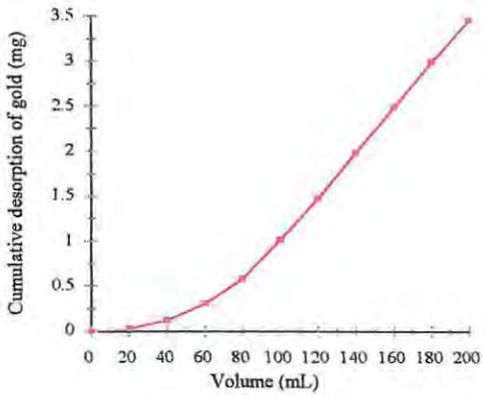


Figure 4.16b: Cumulative desorption of gold utilising 0.1 M thiourea + 0.02 M $FeCl_3$. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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.

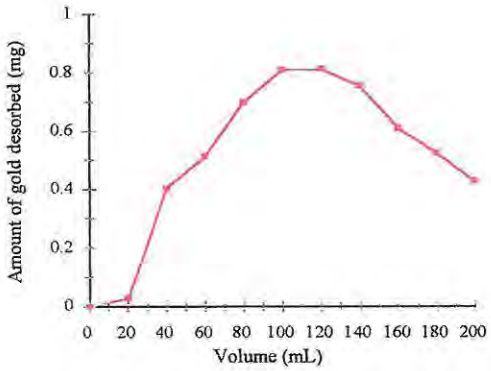


Figure 4.17a: Amount of gold desorbed for every 20 mL fraction utilising 8% thiourea, 2% $HClO_4$ and 0.5% 0.1 M HCl. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

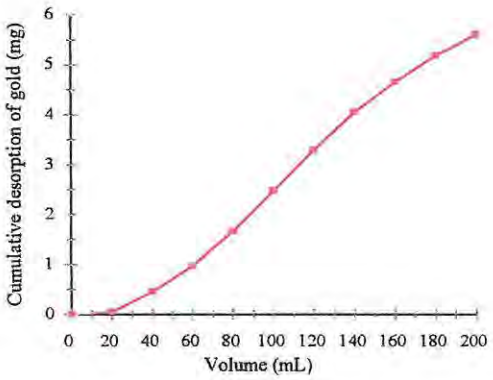


Figure 4.17b: Cumulative desorption of gold utilising 8% thiourea, 2% $HClO_4$ and 0.5% 0.1 M HCl. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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.

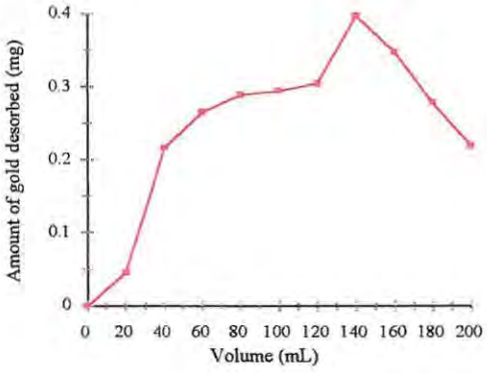


Figure 4.18a: Amount of gold desorbed for every 20 mL fraction utilising 8% thiourea, 0.06 M HNO_3 and 0.5% 0.1 M HCl. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

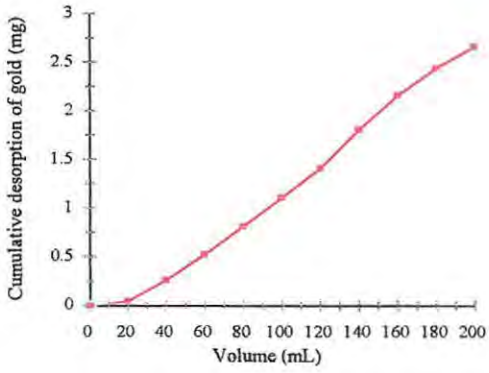


Figure 4.18b: Cumulative desorption of gold utilising 8% thiourea, 0.06 M HNO_3 and 0.5% 0.1 M HCl. Parameters utilised include the following: biomass concentration: 5 g/L, room temperature, flow-rate: 5 mL/min.

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

RECOVERY FROM MIXED METAL SOLUTIONS

5.1. INTRODUCTION

It is important to note the type of effluent that is to be dealt with, since there are effluents with high as well as low metal contents. Metals co-exist with other anionic pollutants which are also toxic to various organisms. High flow-rates with a short contact time need to be considered with high volume treatment. Other factors, considered to be important, for recovery are the oxidation state of the metal, pH, as well as the presence of possible metal binding ligands (Macaskie and Dean, 1990). Studies in Chapter 3 have shown that excess metals such as lead and copper may affect the adsorption of gold, and anionic species such as sulphate (a hard base) do not interfere with gold adsorption, while sulphite (a borderline base) inhibits approximately 70% of gold(III) adsorption.

If the metal is able to exist in various oxidation states and if the adsorbent displays reducing properties, adsorption complexities may occur (Macaskie and Dean, 1990). This was found with gold and *Azolla filiculoides*. The presence of tannins on the plant material may be responsible for gold precipitation through the binding of gold(III) to the hydroxyl groups on the tannins (Chapter 3).

Copper and lead were chosen for the mixed metal studies in this investigation as they represent the highest concentration of metals found in the effluent from Mine A (Appendix I). The demand for the selective removal of metals from aqueous waste solutions is increasingly important from an environmental and technological point of view. Copper and lead pose a contamination threat in terms of their toxicity which ultimately endangers human life, while gold recovery is important because of its high value in today's economic market (Volesky, 1990). An opportunity thus exists for adsorption and recovery of the metals, lead, copper and gold. The omission of iron from this particular study was due to the presence of excess iron on the plant material. An equimolar

concentration of metals were employed to compare the adsorption and desorption characteristics of each metal. A pH of ± 4.00 was utilised since this was an average of all pH optimums for the removal of these metals from solution, i.e., lead(II) at pH 4.5, copper(II) at pH 5.5, and gold(III) at pH 2. As a preliminary study, the utilisation of the non-viable biomass in the treatment of carbon-in-pulp (CIP) residue effluent solution from Mine B was also undertaken.

5.2. OPTIMISATION STUDIES

5.2.1. Effect of pH on Desorption

The particular study was aimed at determining the desorption profile for each of the metals at various pH's utilising 0.1 M HNO_3.

5.2.1.1. Materials and Method

5.2.1.1.1. 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 which was obtained from Merck, Germany. Aqueous gold(III) solutions were prepared from hydrogen tetrachloroaurate(III) {$H[AuCl_4]$}, whilst copper and lead solutions were prepared from copper(II) chloride ($CuCl_2$) and lead(II) chloride ($PbCl_2$) and dissolved in deionised water until a desired final concentration of 50 μM was achieved. Sodium hydroxide was utilised to adjust the pH of 0.1 M HNO_3. Atomic absorption spectrometric standards were prepared from 1000 mg/L lead, 1000 mg/L copper and 1000 mg/L gold solutions (Wirsam, South Africa) and diluted with deionised water until the desired concentration was obtained.

5.2.1.1.2. Method

All experimental work was conducted in duplicate. A solution (1 litre), containing lead(II),

copper(II), and gold(III) at an equimolar concentration (50 μM), was pumped through a packed column in an up-flow mode containing 5 g of whole *Azolla filiculoides* (bed volume of 49 mL). The solution (pH ± 4) was pumped at a flow-rate of 5 mL/min. 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 (Pharmacia, South Africa). For desorption, nitric acid {0.1 M HNO_3 (100 mL)} was employed since it was a stronger oxidising agent than hydrochloric acid. The metals, lead(II) and copper(II), were sequentially desorbed, under gradient-flow, with the various prepared pH's (7-1). Gold desorption followed acid desorption, with thiourea (8%), perchloric acid (2%) and 0.1 M HCl (0.5%) (100 mL, under gradient-flow) being utilised. The samples were collected and analysed for lead, copper and gold using an AA spectrophotometer. The results were expressed as percentage removal of lead, copper and gold from solution or as percentage removal from the biomass for the adsorption studies and desorption studies respectively.

5.2.1.2. Results and Discussion

For lead(II) and gold(III) an adsorption of 100% occurred (Figure 5.1). Copper(II) however, had a maximum adsorption of 96% gradually decreasing to 84%. This may be due to the fact that as a borderline hard acid its binding is mainly electrostatic. The decrease in removal of copper(II) with increasing volume may indicate that there may be a change in the equilibrium species adsorbed or a change in the oxidation state of copper(II) to copper(I) or 0. The initial binding of copper(II) as a softer acid may be stronger and as the oxidation state of the metal is transformed, becoming harder, binding may be less favourable. Another factor may be that the binding sites for copper may be saturated. Results shown in Figure 5.1 compare well with those obtained by Darnall *et al.* (1986) who demonstrated that little to no binding occurred at pH 2 for lead(II) and copper(II), while maximum removal occurred as the pH was raised to 5 or 6.

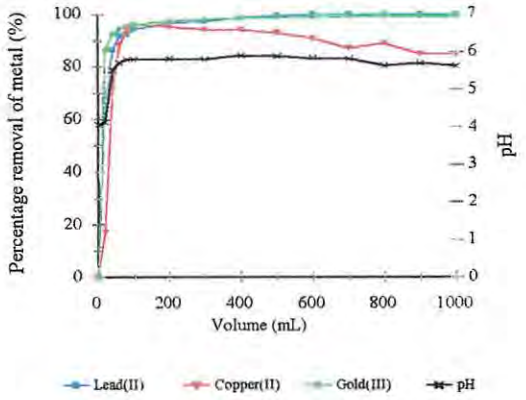


Figure 5.1: Removal of lead(II), copper(II) and gold(III) from an aqueous solution at 50 μM, pH ± 4.00. The following adsorption parameters were utilised: flow-rate: 5 mL/min, biomass concentration: 5 g/L and room temperature.

The main aim of the present study was to determine the desorption characteristics of each metal initially utilising nitric acid (0.1 M) and finally utilising thiourea (8%), perchloric acid (2%) and 0.1 M HCl (0.5%) for gold removal (the desorbent which demonstrated optimal removal for gold in Section 4.3.1.2.11). The data in Figure 5.2 demonstrates the pH sensitivity in the desorption of copper(II) and lead(II) which suggests that binding may largely be electrostatic. Gold binding is not reversed by the lowering of the pH of the acid, however, on exposure to thiourea in combination with the oxidising agent, the metal is effectively desorbed from the plant surface. Gold(III) exhibits 99% removal from the biomass with the desorbent employed (Figures 5.2 and 5.3), thus indicating that exposure, in oxidising conditions, to complexing agents containing sulphur ligands may be necessary for total removal.

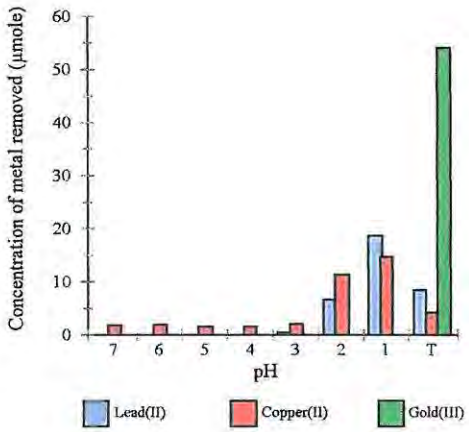


Figure 5.2: Sequential pH-dependent removal of lead(II), copper(II) and gold(III) from the biomass utilising 0.1 M HNO_3. Abbreviation utilised: **T:** Thiourea (8%), perchloric acid (2%) and 0.1 M hydrochloric acid (0.5%). The following parameters were utilised: biomass concentration of 5 g/L, room temperature and desorption was under gradient-flow conditions.

A total removal of 80% of adsorbed copper and 74% of adsorbed lead from the biomass was achieved by sequential pH reduction (Figure 5.3). The desorption of copper(II) and lead(II) may be a result of a competitive effect whereby the hydrogen ions compete for binding with the metal eventually leading to displacement. The recovery may be improved by decreasing the flow-rate or increasing the volume of the desorbent. Additional desorption of copper(II) and lead(II) by thiourea (pH 1.3) is ascribed to the acidity of this solution.

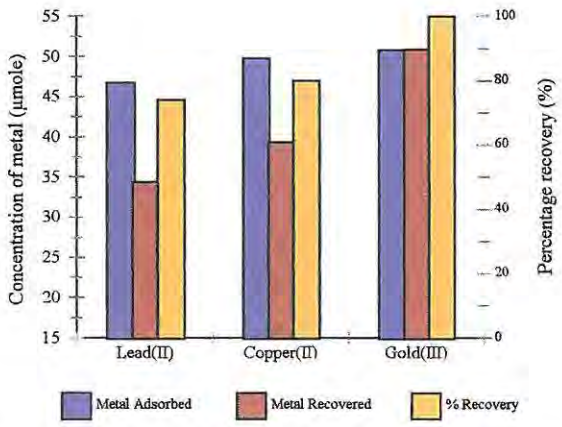


Figure 5.3: Cumulative removal of lead(II), copper(II) and gold(III) of 50 µM.

5.2.2. Effect of Nitric Acid Concentration and Recycling

5.2.2.1. Materials and Method

5.2.2.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 5.2.1.1.1.

5.2.2.1.2. Method

The adsorption procedure was followed as described in Section 5.2.1.1.2. Various concentrations of nitric acid were utilised (0.1 M, 0.3 M and 0.5 M). Once adsorption was complete, the extraction of the metals from the biomass utilising nitric acid (100 mL) followed. The elution of lead and copper with acid was carried out under gradient-flow. The solution was recycled a total of one, three or six times for each acid concentration utilising the same volume. The same procedure for gold desorption was followed as described in Section 5.2.1.1.2. Once the final cycle was complete, lead, copper and gold concentrations were analysed utilising an AA spectrophotometer. Results were expressed as percentage metal removal from aqueous solution for adsorption and percentage metal removal from the biomass for desorption for each of the metals studied.

5.2.2.2. Results and Discussion

The adsorption at pH 4 for each of the metals, in the mixed metal solution, was 100% for lead (II), 88% for copper(II) and 98% for gold(III). Table 5.1. shows the results for the total removal obtained for the desorption experiments at various acid concentrations once the third cycle was complete. Desorption of the metals, lead(II) and copper(II), varied for the different acid concentrations. Gold(III) removal from the biomass was constant at 98% using the thiourea, perchloric and hydrochloric acid solution.

Table 5.1. Total desorption values for lead and copper obtained for the various nitric acid concentrations.

Nitric Acid Concentration	*Metal Recovered (%)*	
	Lead	**Copper**
0.1 M	68.16	80.27
0.3 M	82.73	70.03
0.5 M	91.55	76.17

Although 0.5 M HNO_3 resulted in a higher desorption, under gradient-flow conditions, an intermediate concentration of 0.3 M HNO_3 was selected for further desorption studies. The reason for this was that a larger volume of water (greater than 500 mL water as compared with 300 mL for 0.3 M HNO_3) was required to remove the acid from the biomass before subsequent adsorption cycles could be carried out if the biomass was to be recycled.

The aim of a further experiment was to determine whether recycling once, three or six times would be optimal. For the adsorption studies a removal of 100% of lead(II), greater than 81% for copper(II), and greater than 97% for gold(III) occurred. The total desorption for lead, copper and gold improved with each cycle with the first cycle showing less than 1-7% removal as compared with recycling three times. The results from Table 5.2 indicate that recycling six times under gradient-flow was optimal for the removal of the three metals concerned.

Table 5.2. Summary of the results obtained for the total desorption of metals under gradient-flow.

Recycling	*Metal Recovered (%)*		
	Lead	**Copper**	**Gold**
1 x	80.52	71.02	96.22
3 x	85.17	78.90	97.14
6 x	91.25	84.94	100.00

5.2.3 Effect of Pre-Washing the Biosorbent

5.2.3.1. Materials and Method

5.2.3.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 5.2.1.1.1.

5.2.3.1.2. Method

The adsorption procedure was followed as described in Section 5.2.1.1.2. The first study involved leaving the plant material in its natural state as has been done with the previous experiments. The second study involved firstly pumping deionised water (300 mL) at 15 mL/min through a packed column in an up-flow mode. For both studies, adsorption was followed as described in Section 5.2.1.1.2. Desorption of the metals utilised 0.3 M HNO_3 (100 mL) under gradient-flow. The same volume of acid was recycled six times. Desorption of gold followed the acid desorption utilising thiourea in combination with perchloric and hydrochloric acid (100 mL), also recycled six times under gradient-flow. Once the final cycle was complete, concentrations for lead, copper and gold after adsorption and desorption were measured as before. Results were expressed as percentage metal removal from aqueous solution for adsorption and as percentage metal removal from the biomass for desorption for each of the metals studied.

5.2.3.2. Results and Discussion

The adsorption results, utilising the plant material in its natural state showed a removal from solution of 100% lead(II), 73% copper(II), and 95% gold(III). In pre-washing the plant material adsorption was 100% for lead(II), 85% for copper(II), and 97% for gold(III). An improved adsorption of copper(II), 12%, was found from pre-washing the plant material with water. Minor improvement in the desorption of lead and copper was found (7% and 5% respectively) while gold recovery remained 100% (Table 5.3).

Table 5.3. Summary of the results obtained for total desorption of metals under gradient-flow.

Study	*Metal Recovered (%)*		
	Lead	**Copper**	**Gold**
Natural state	93.54	86.20	100.00
Pre-wash/Rinse	100.00	91.40	100.00

Rinsing the plant material with deionised water may possibly remove salts which impede the binding of certain metals to the biomass and their subsequent recovery.

5.2.4. Recovery of Metals Under Pump Conditions

Although gradient-flow resulted in improved recovery of all three metals, the studies demonstrated that channelling occurred in the packed-column under these conditions (data not shown). Thus pumping the acid in an up-flow mode to eliminate channelling was explored.

5.2.4.1. Materials and Method

5.2.4.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 5.2.1.1.1.

5.2.4.1.2. Method

The procedure was followed as described in Section 5.2.1.1.2. The plant material was pre-washed by pumping deionised water (300 mL) at 15 mL/min through a packed column in an up-flow mode. For both studies, adsorption followed as detailed in Section 5.2.1.1.2. Desorption of the metals utilised 0.3 M HNO_3 (100 mL, 15 mL/min) in an up-flow mode. The acid was recycled three or six times depending on the study. Desorption of gold with thiourea in combination with perchloric and hydrochloric acid occurred in the same manner as the acid desorption. Once the

final cycle was complete, the concentrations of lead, copper and gold after adsorption and desorption were measured as before. Results were expressed as percentage metal removal from aqueous solution for adsorption and as percentage metal removal from the biomass for desorption for each of the metals studied.

5.2.4.2. Results and Discussion

Lead, copper and gold adsorption was 100%, 95% and 99% respectively. An improvement in the recovery of the metals as compared with the gradient-flow studies (Table 5.2) was found. A 5% and 8% increase for copper and lead desorption was achieved respectively for the recycling of the acid six times (Table 5.4). Gold removal remained the same (100%).

Table 5.4. Total desorption values obtained for lead, copper and gold under pump conditions with recycling.

Recycling	*Metal Recovered (%)*		
	Lead	**Copper**	**Gold**
3x	92.00	94.54	100.00
6x	100.00	100.00	100.00

For subsequent studies pumping the acid for the desorption of copper, lead and gold was utilised. Although no difference was found in gold desorption for each recycling, the same procedure was followed as for lead and copper desorption.

5.2.5. Cost Analysis of Gold Recovery from Azolla filiculoides

Studies in Chapter 4 demonstrated that the removal of gold from the biomass was possible in batch and column studies. A five times concentration factor, i.e., one litre of adsorbent concentrated in less than 200 mL of desorbent was utilised, since a larger volume would be uneconomical for disposal. A comparison of the cost analysis for gold recovery between desorbing gold from the biomass versus ashing the plant material (5 g) was investigated. Table

5.5 demonstrates that employing thiourea (technical grade), perchloric acid and hydrochloric acid as a desorbent for gold costs approximately R 0.89/100 mL, while running a furnace at 1000°C for 3 hours, which will ash the plant material would cost approximately R 0.80c. It should be noted that the cost of using a furnace would be further reduced with the use of a larger mass of biomass (furnace capacity utilised was 500 g) while disposal costs of the desorbent would also be factored. For the final mixed metal desorption studies the furnace was utilised for gold recovery since it was cheaper.

Table 5.5. Comparison of costs involved in recovering gold from *Azolla filiculoides*.

	Treatment	
Cost/	**Desorbent**	**Furnace**
1 litre of adsorption solution	R 0.89 (100 mL)	R 0.80 (3 hours)
1 g biomass	R 0.18	R 0.16

5.3. ADSORPTION AND DESORPTION STUDIES OVER 5 CYCLES

The following studies were conducted to determine which process would be the most suited for adsorption and desorption for lead(II), copper(II) and gold(III) for a sequence of 5 successive cycles.

5.3.1. Materials and Method

5.3.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 5.2.1.1.1.

5.3.1.2. Method

The experimental protocol was followed as described in Section 5.2.1.1.2. This investigation involved two studies. The first part of the study, washing the plant material with 300 mL

deionised water in an up-flow mode (15 mL/min), followed by adsorbing the metals in an up-flow mode, namely lead(II), copper(II) and gold(III) (50μM, pH 4, 1 litre, and a flow-rate of 5 mL/min), and again washing the biomass with deionised water (300 mL, flow-rate of 15 mL/min, up-flow mode) at each stage once adsorption was complete. This procedure was repeated a further four times. On completion of the final fifth cumulative cycle, the metals, lead(II) and copper(II), were desorbed with 0.3 M HNO_3 (100 mL), the same volume recycled six times at a flow-rate of 15 mL/min (up-flow mode). The plant material was then rinsed with deionised water (300 mL) to remove any residue metal and acid. Since ashing was cheaper and a less labour-intensive method of gold recovery, the biomass was ashed in a furnace at 1100°C for three hours.

The second part of the study consisted of washing the plant material with deionised water (300 mL, 15 mL/min, up-flow mode) adsorbing the metals lead(II), copper(II) and gold(III) in an up-flow mode (50 μM, pH 4, 1 litre, and a flow-rate of 5 mL/min). This was followed by immediate desorbing of lead(II) and copper(II) with 0.3 M HNO_3 (100 mL, 15 mL/min, up-flow mode), recycling the same volume six times. Rinsing of the biomass with deionised water (300 mL, 15 mL/min, up-flow mode) followed. Once desorption and the washing cycle were completed, adsorption was followed with desorption and rinsing again. The same procedure was repeated a further three times and the biomass ashed in a furnace for 2-3 hours at 1100°C for the recovery of gold after the final fifth cycle was complete. The metal concentrations of lead, copper and gold were analysed utilising an AA spectrophotometer. Results were expressed as percentage removal of metal from solution or μmole of metal adsorbed, as well as μmole metal or total percentage of metal recovered from the biomass.

5.3.2. Results and Discussion

The first part of this study involved the adsorption of the three metals at pH 4 with washing of the biomass before the adsorption of metals in the next cycle. Acid desorption of lead and copper was not carried out before the five adsorption cycles were complete to determine whether it was in fact necessary for optimum cumulative adsorption, principally, of gold and secondly of lead and copper. The results in Figure 5.4 show that over the period of the five cycles investigated, lead(II)

showed a sequential decrease in adsorption after the first two cycles, gradually decreasing from 100% in the first cycle to 58% in the fifth cycle. Copper(II) had an even greater sensitivity decreasing from 100% in the first cycle to 48% at the end of the fifth adsorption cycle. Clearly both lead(II) and copper(II) require regeneration of the biomass to maintain its adsorptive capacity, or the decrease in adsorption could be as a result of the binding sites for copper and lead being saturated. Greater than 90% removal of gold from solution occurred for the five adsorption cycles. This indicates that regeneration of the biomass may not be necessary for the adsorption of gold.

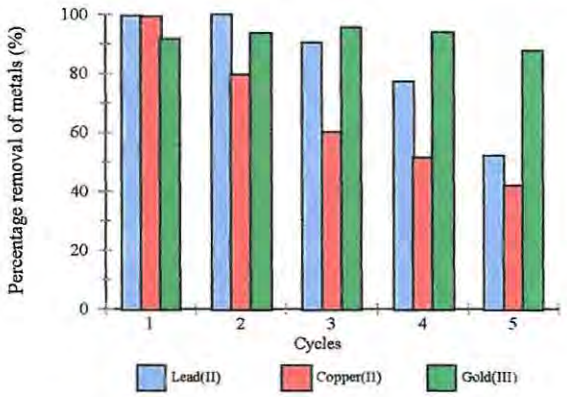


Figure 5.4: Percentage removal of lead(II), copper(II) and gold(III) from solution over a period of successive cycles, i.e., 1-5. The following parameters were utilised: biomass concentration of 5 g/L, metal concentration of 50 μM, ± pH 4.1, flow-rate of 5 mL/min and room temperature. In between cycles, the biomass was rinsed with deionised water before the next adsorption cycle.

In the second part of this study, once the five adsorption cycles were completed, an elutant at optimal conditions (0.3 M HNO_3) was passed through the biomass to recover the non-precious metals. Total desorption values for lead(II) and copper(II) were found to be 93% and 83% respectively (Figure 5.5). Ashing the plant material as an alternative process of gold recovery was attempted. The results from the ashing demonstrated a total recovery of 94 %, thus indicating that it is a viable recovery method for gold, however, trace metals associated with the plant material would probably contaminate the metal. The final decision for the process recovery selected would ultimately depend on the overall feasibility study of the process on site.

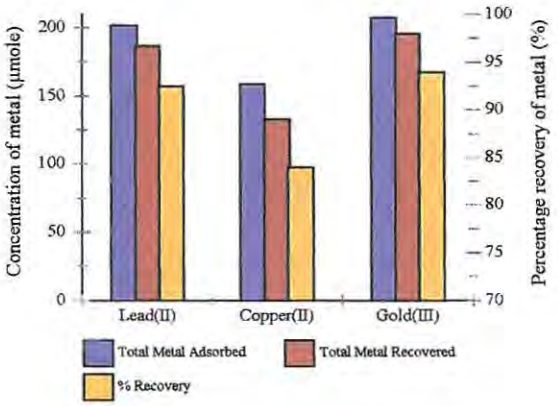


Figure 5.5: Total adsorption of lead(II), copper(II) and gold(III) and the percentage recovery for each metal.

The second study involved desorption and the regeneration of the biomass before re-adsorption in each successive cycle. The results in Figure 5.6, indicate the adsorptive capacity for lead(II) remained the same over the five cycles investigated, exhibiting greater than 95% removal from solution. Copper(II) demonstrated a maximum removal of not less than 80% over the five cycle period. These results indicate that the nitric acid was probably able to regenerate the biomass sufficiently for a better removal than those results obtained without regeneration (Figure 5.4). Although desorption released the metal from the biomass and thus freed binding sites for further adsorption, it may not be the sole reason for the high adsorption over the subsequent cycles. There are probably two main reasons for the release of the metal from the biomass: (1) most ligands are usually only able to chelate metals when ionised, thus a change in the pH results in the destruction of the stable metal-ligand complex, and (2) the ionic strength of the elutant, an increase in the ionic strength weakens the stability of metal-ligand complex (Huber *et al.*, 1990). Sanyahumbi (1998) demonstrated that the maximum adsorptive capacity of the same biomass for lead(II) is 100 mg Pb/g biomass, thus its adsorptive capacity had not been reached. A similar conclusion for copper(II) adsorption is also probable. Gold(III) adsorption was not affected by the acid desorption and rinsing of the biomass and continued to exhibit not less than 95% removal from solution in each cycle.

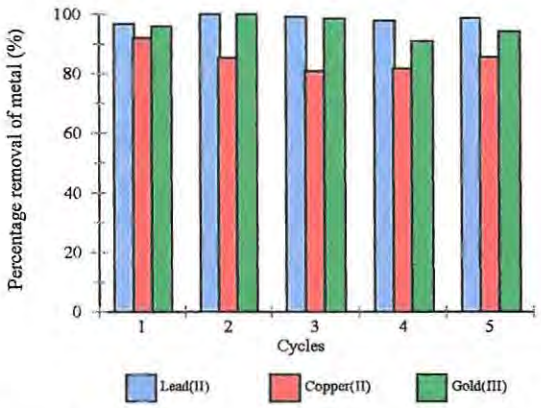


Figure 5.6: Percentage removal of lead(II), copper(II) and gold(III) from solution over a period of successive cycles, i.e., 1-5. The following parameters were utilised: biomass concentration of 5 g/L, metal concentration of 50 μM, ± pH 4.1, flow-rate of 5 mL/min and room temperature. In between cycles, the metals were desorbed with acid (0.3 M HNO_3) and rinsed with deionised water before the next adsorption cycle.

The repetitive adsorption of lead(II) and copper(II) was followed by immediate desorption. The recovery of the lead(II) was more than 82% after the first recovery, thereafter the elution of the metal improved with not less than 92% recovery occurring at the end of each cycle. The total recovery value for this metal was 92%. Copper(II) desorption was 86% at the end of the first desorption cycle, and in subsequent cycles improved to greater than 96% removal. The final total recovery of copper was 96% (Figure 5.7). Desorption with nitric acid had an insignificant effect on the removal of gold from the biomass. Once all the adsorption and desorption cycles were concluded, the plant material was ashed and the gold recovery was found to be 97%. Again the value of the recovery of this precious metal plays an important role in deciding which process is utilised.

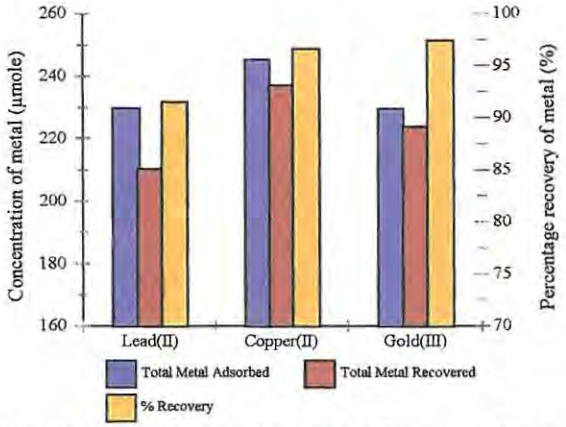


Figure 5.7: Total adsorption of lead(II), copper(II) and gold(III) and the percentage recovery for each metal.

5.4. GOLD EFFLUENT STUDIES

Although Mine A was initially utilised for a detailed investigation into the binding characteristics of the metals (Chapters 2 and 3), another effluent, Mine B, was obtained and utilised since precipitation of the metals from Mine A had occurred during storage. The aim of these studies was to evaluate the extent of removal of the various metals, including gold, present in the effluent.

5.4.1. Batch Studies

5.4.1.1. Materials and Method

5.4.1.1.1. Materials

The plant material was obtained and prepared as described in Section 2.2.1.1. The effluent, a carbon-in-pulp (CIP) residue filtrate, obtained from Mine B, was utilised unmodified.

5.4.1.1.2. Method

All experimental work was conducted in duplicate. A volume of 100 mL of effluent and a biomass

concentration (5 g/L) 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 nylon filters (25 mm diameter, 0.45μM pore size). The filtrate was then analysed for the various non-precious metals using AA spectrophotometry. The gold concentration was determined utilising inductive coupled plasma mass spectrometry (ICP-MS) (Perkin-Elmer) due to the low level present in the effluent (less than 0.1 mg/L). Cyanide and sulphate levels were measured with a Spectraquant® cyanide and sulphate test kit (Appendix II and III respectively) (Merck, Germany). The results were expressed as percentage removal from solution for each of the metals.

5.4.1.2. Results and Discussion

The results indicate that iron and copper were not adsorbed by the biomass, while nickel showed a 46% removal (Table 5.6). Iron(III) and copper(II) are hard and borderline hard acids respectively, thus binding is pH-dependent. This suggests that either the pH of 9.55 is not optimal for binding or that there may be some competition between these two metals and nickel (showing a higher removal). Most important of all, 100% removal of gold was found. The biomass removed 14% of cyanide and 15 % of sulphate from solution. The pH decreased by 2.65 units suggesting that the adsorption is followed by the release of hydrogen ions in limited amounts.

Table 5.6. Percentage removal of metal from the waste water of Mine B in batch studies.

Metal	*Pre-Treatment (mg/L)*	*Post-Treatment (mg/L)*	*Percentage Removal (%)*	**Max. Specifications (mg/L)*
Iron (Fe)	0.24	0.24	0	9.00
Zinc (Zn)	0	0	-	0.70
Lead (Pb)	0	0	-	0.10
Copper (Cu)	6.40	6.40	0	0.10
Nickel (Ni)	0.24	0.13	45.83	1.14
Gold (Au)	0.0422	0	100.00	-
Cyanide (CN^-)	0.28	0.24	14.29	NA
Sulphate (SO_4^{2-})	508.00	431.00	15.15	NA
pH	9.55	6.90	-	9.00

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

5.4.2. Column Studies

5.4.2.1. Materials and Method

5.4.2.1.1. Materials

The plant material was obtained and prepared as described in Section 3.2.1.1. The effluent, a carbon-in-pulp (CIP) residue filtrate, obtained from Mine B was utilised unmodified.

5.4.2.1.2. Method

All experimental work was conducted in duplicate. A volume (1 litre) of waste water was 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 a flow-rate of 5 mL/min (optimal). Samples were collected at regular intervals using a fraction collector and were analysed for the various non-

precious metals using an AA spectrophotometer. Gold content was analysed utilising ICP-MS (Perkin-Elmer) due to the low level present in the effluent. Cyanide and sulphate levels were measured with a Spectraquant® cyanide and sulphate test kit (Appendix II and III respectively) (Merck, Germany). The results were expressed as percentage removal (effluent concentration relative to influent gold concentration) from solution for each of the metals.

5.4.2.2. Results and Discussion

The results in Table 5.7 indicate that no iron or lead was adsorbed this suggests that the effluent (Mine B) is vastly different to the effluent studied in Chapter 3 (Mine A). As in the batch studies, 46% of nickel was removed. Since nickel, is a borderline acid its likelihood of binding pH-independently is relatively high, thus both batch and column studies are suitable for nickel removal. In contrast to the batch studies (showing a 100% removal) only 4% of gold was removed from solution. This suggests that a longer residence time, such as in the batch studies, is more suited for the removal of gold from this effluent. No cyanide and 18% of sulphate was removed from solution. The pH decreased by 3.15 units.

Table 5.7. Percentage removal of metal from the waste water of Mine B in column studies.

Metal	*Pre-Treatment (mg/L)*	*Post-Treatment (mg/L)*	*Percentage Removal (%)*	**Max. Specifications (mg/L)*
Iron (Fe)	0.12	0.12	0	9.00
Zinc (Zn)	0	0	0	0.70
Lead (Pb)	0	0	0	0.10
Copper (Cu)	7.02	5.82	17.09	0.10
Nickel (Ni)	0.24	0.13	45.83	1.14
Gold (Au)	0.0500	0.0479	4.20	-
Cyanide (CN)	0.27	0.27	0	NA
Sulphate (SO_4^{2-})	510.00	420.00	17.65	NA
pH	9.55	6.40	-	9.00

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

5.5. SUMMARY

The primary concern for any process design for metal bioremediation is the ease with which the metals may be recovered from the biomass with a minimal amount of eluant. The following aspects therefore need to be considered: the cost of the biosorbent, efficiency of the desorbent in terms of concentrating the final metal solution, the loss of efficiency of the biomass for metal removal for regeneration cycles and the lifespan of the adsorbent. The biomass, *Azolla filiculoides* meets most of the requirements for utilisation in successive adsorption and desorption cycles.

An investigation into a mixed-metal adsorption and recovery for lead(II), copper(II) and gold(III) was undertaken. Once adsorption of lead(II), copper(II) and gold(III) had occurred, 0.1 M HNO_3 at various pH's was utilised to desorb lead(II) and copper(II). Adsorption of 100% for lead(II) and gold(III) and 84% for copper(II) from solution occurred. Copper(II) demonstrated the weakest binding since elution occurred at all pH's from 7 to1. Lead(II) and copper(II) were eluted at pH 2 and 1. Both metals were also eluted to a certain extent with the gold desorbent mainly due to its acidic pH. Lead and copper exhibited a total final recovery value of 74% and 80% respectively. A recovery of 100% of gold occurred from the biomass utilising thiourea, perchloric acid and hydrochloric acid as a desorbent.

Studies on the optimisation of the acid desorbent for lead(II) and copper(II) were carried out. A concentration of 0.3 M HNO_3 was found to be suitable. Using this system, a study into the desorption under gravity with recycling 1, 3 and 6 times was undertaken with the data indicating that six times recycling was optimal for removal of all three metals (91% for lead(II), 85% for copper(II), and 100% for gold(III)). Rinsing of the plant material with water prior to adsorption, increased the adsorption of copper(II) by 12%, while the recovery of lead(II) and copper(II) increased by 7% and 5% respectively. Since gradient-flow demonstrated channelling in the column studies, pumping and recycling the acid six times in an up-flow mode was found to be optimal. A cost analysis comparing the desorption of gold versus ashing the plant material, before the re-usability of the plant material was explored, showed the latter to be a better economical option and was subsequently utilised for further studies.

Utilising the biomass in successive adsorption and desorption cycles were carried out in two experiments. The first involved 5 adsorption cycles with only rinsing of the biomass with water in between cycles. Acid desorption of lead(II) and copper(II) was carried out only once the final fifth adsorption cycle had occurred. Gold was recovered by ashing the plant material. For lead(II) 100% removal from solution occurred for the first 2 cycles, while copper(II) removal was 100% for the first cycle with both metals gradually decreasing to 58% and 48% respectively at the final cycle. Gold(III) demonstrated greater than 93% adsorption for all five adsorption cycles. The total recovery of lead(II) and copper(II) from the biomass was found to be 93% and 83% respectively. Gold recovery was 94% by ashing. Ashing of the plant material was found to be the most economical with the added benefit of low disposal volumes. The second part of the study involved 5 adsorption and desorption cycles with adsorption followed by acid desorption (0.3 M HNO_3) and rinsing of the biomass with water at each stage. Gold was recovered once the final adsorption and desorption cycles were complete. Lead(II) exhibited > 95% removal from solution for all five cycles, while copper(II) removal was not less than 80%, and gold(III) 95% from solution. The final total recovery after desorption was 92% for lead(II), 96% for copper(II), and 97% for gold(III) (the latter by ashing).

Both waste water studies were undertaken in batch and column conditions to determine which would be the most feasible. In the batch studies no adsorption occurred for copper and iron. Nickel removal was 46%, while 100% of gold was adsorbed. Cyanide and sulphate levels decreased greater than14% each. In the column studies no iron was removed, while 17% and 46% of copper and nickel was removed respectively. Sulphate levels decreased by 18%. For both the batch and column studies a decrease in pH accompanied the adsorption of metals by the biomass suggesting that an ion-exchange process may be involved.

Although extensive studies have been conducted on the effect of various anions on gold removal (Chapters 2 and 3), the application of these studies is difficult since the effluent concentrations of the various metals and anions vary daily. The biomass is able to selectively adsorbed the metal of interest, gold, from the effluent with relative ease in batch systems. Although it has been

established that sulphate does not affect the binding of gold in column studies, the presence of high concentrations of sulphate may influence the binding of the other metals present in the effluent.

The utilisation of *Azolla* in the treatment of effluent from Mine B provided an indication of its feasibility in the treatment of mine effluents, this was shown by the selective removal in the batch and column studies of gold, nickel and sulphate.

CHAPTER 6

CHARACTERISATION STUDIES

6.1. INTRODUCTION

Viable and non-viable biological material has been utilised for the recovery and concentration of metals from solution (Drake *et al.*, 1996). As reported in Chapters 2 to 4, the biomass *Azolla filiculoides* has a high affinity for the metals investigated, namely: lead(II), copper(II) and gold(III). The metals are taken up by a physiochemical adsorption process at the surface of the biomass as the biomass is non-viable. The binding of the metals is believed to occur through a variety of functional groups present on the surface of the biomass and these are characteristic of the biomass. These functional groups may include amines, amides, thiols, hydroxyls, carboxylates, phosphates, lipids and carbohydrates (Darnall *et al.*, 1986; Drake *et al.*, 1996; Nakajima *et al.*, 1981). It is important to establish which functional groups are responsible for the metal binding observed in order to understand the characteristics of adsorption of the metal. Determination of the functional groups responsible for metal binding may be achieved by the modification of different functional groups and investigating the influence of change on the metal adsorption. Very few such studies have been reported, particularly for gold binding.

Studies employing the treatment of the biomass *Chlorella vulgaris* with an sulphydral blocking reagent, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), have shown that the thiol groups may be involved in the binding of silver (Darnall *et al.*, 1986). Results from Greene *et al.* (1986a) using DTNB modification, suggest that amine groups present on the *Chlorella vulgaris* are responsible for much of the binding of gold(III) to the biomass. Chemical modification of algae cells with acidic methanol suggests that the esterified carboxyl groups also determine the extent of gold(III) uptake by the biomass (Gardea-Torresdey *et al.*, 1990). Understanding the fundamental properties of the biosorbent can be beneficial in both modelling and predicting the binding mode, as well as assisting in attempts to improve its binding characteristics by chemical modification. For the latter, costs involved in the modification procedure also need to be taken into consideration.

The main aim of this study was to attempt to elucidate the binding mechanism of gold(III) to the biomass surface of *Azolla* by determining which functional groups were responsible for its binding. While attempts to characterise the functional groups present on the *Azolla filiculoides* were made by chemical modification, these groups may not be solely responsible for the binding of the metals of interest. Further experiments are therefore necessary to understand the detailed mechanisms responsible for the binding.

6.2. CHARACTERISATION STUDIES

6.2.1. Chemically Treated Biomass

6.2.1.1. Materials and Method

6.2.1.1.1 Materials

The plant material and reagents were obtained and prepared as described in Section 2.2.1.1. Sodium hydroxide, potassium hydroxide, hydrochloric acid, methanol and chloroform were obtained from Saarchem, South Africa. Atomic absorption standards were prepared utilising a gold atomic absorption solution (1000 mg/L) (Wirsam, South Africa). All solutions were diluted with deionised water until the desired concentrations were achieved.

6.2.1.1.2. Method

Boiling Water-Treated Biomass

The plant material (3 g) was treated with deionised water at 100°C under reflux for a period of 1 hour, thereafter the plant material was filtered under vacuum, dried in an oven at 30°C, and weighed.

Dilute Alkali (0.01 N NaOH)

The plant material (3 g) was placed with 0.01 N NaOH (500 mL) and stirred continuously for a period of 1 hour, thereafter the biomass was filtered under vacuum, thoroughly washed with water, dried at 30°C, and weighed.

Dilute (0.01 N) and Concentrated (0.6 N) Hydrochloric Acid(HCl)

The plant material (3 g) was placed with 0.01 N (500 mL) and stirred continuously for a period of 1 hour. Once complete, the biomass was filtered under vacuum, thoroughly washed with water, dried at 30°C, and weighed. The same procedure ensued with 0.6 N HCl.

Methanol

The plant material (3 g) was placed in methanol (500 mL) and stirred continuously for a period of 1 hour. The biomass was then filtered under vacuum, allowed to evaporate, and weighed.

Chloroform-Methanol

The plant material (2 g) was placed with methanol (96% v/v, 100 mL) at 65°C under reflux for 10 minutes and cooled at room temperature. Chloroform (200 mL) was added to the methanol and stirred for 20 minutes. After filtration, the plant material was retreated with the chloroform:methanol (2:1, 100 mL) for a further 20 minutes, allowed to evaporate and weighed.

Chloroform-Methanol-Concentrated Alkali

The biomass was treated with chloroform-methanol (2:1) mixture as described above. Once complete, the plant material was further treated with 24% KOH (100 mL) solution at room temperature under nitrogen for a period of 2 hours with occasional agitation. The biomass was filtered and retreated with KOH for another period of 2 hours. After thoroughly washing the

filtrate with deionised water, the biomass was dried at 30°C and weighed.

Uptake Experiments

All experiments were conducted in duplicate. The plant material, once treatment was complete, was subjected to uptake studies with gold(III) in the form of hydrogen tetrachloroaurate(III) at a concentration of 50 μM and pH of 2. A sample of 50 mL of gold(III) solution was placed with 0.25 g of biomass in an Erlenmeyer flask (100 mL) and agitated at a speed of 200 rpm at room temperature. Samples (5 mL) were taken at regular intervals of 10 minutes for a period of 90 minutes and filtered (cellulose-acetate, 25 mm diameter, 0.45 μM pore size). The gold concentration was analysed with an AA spectrophotometer. The results were expressed as percentage uptake of gold(III) from solution after 90 minutes.

6.2.1.2. Results and Discussion

The effect of the modification on *Azolla filiculoides* and on gold(III) uptake is summarised in Table 6.1 below. The results were compared with a control which demonstrated a 100% uptake of gold(III) from solution.

Table 6.1. Effect of various treatments on the biomass and on the uptake of gold(III) from solution.

Treatment	*% Biomass Loss*	*% Uptake of Gold(III)*	*Reference*
Boiling Water	31.67	97.40	A and B
0.01 N NaOH	25.73	83.97	A and B
0.01 N HCl	23.13	99.04	A
0.6 N HCl	28.32	98.20	A
Methanol	23.60	98.50	A
Chloroform-Methanol	8.29	95.87	A and B
Chloroform-Methanol-Concentrated Alkali	25.45	91.53	A and B

A: Kuyucak and Volesky, 1989b; **B:** Nakajima *et al.*, 1981

The treatment of the plant material with boiling water allows for the extraction of water soluble oligosaccharides, polysaccharides and proteins. The results presented in Table 6.1 indicate that the removal of the water soluble oligosaccharides, polysaccharides, and proteins did not significantly affect gold binding and that these sites are not primarily responsible for the removal of gold(III) from solution as the value of 97% uptake corresponds closely to the control.

Treatment with dilute alkali (0.01 N NaOH) solutions allows for the extraction of polysaccharides and proteins which are soluble at higher pH's. The uptake of the gold(III) from solution was reduced (84%) as compared with the control (Table 6.1). The plant material acquired a paste-like appearance once it had been treated with the dilute alkali. This suggests that NaOH is able to breakdown the integrity of the plant material, this may be as a result of the extraction of the polysaccharides and proteins from the biomass.

Treatment of the plant material with dilute or concentrated acid solution allows for the extraction of tannins. In Table 6.1 the results indicate that the effect of 0.01 N and 0.6 N HCl on the uptake of gold(III) from solution was minimal as compared to the control. The biomass now has an overall positive charge due to the acid treatment, an attraction between the biomass and the negatively charged gold(III) complex may exist.

The treatment of plant material with methanol will extract the more polar organic substances. The results in Table 6.1 demonstrate that the methanol treatment of plant material had no effect on the uptake of gold(III) when compared to the control. An uptake of 99% suggests that the more polar organic substances may not be responsible for gold(III) removal from solution.

The treatment with chloroform and methanol is responsible for the extraction of lipids from the plant material. The data presented in Table 6.1 indicates that 96% of gold(III) was removed from solution and thus suggests that the lipids are not responsible for significant gold(III) uptake.

The treatment with chloroform-methanol-concentrated alkali extracts cellulose from the plant material. The data presented in Table 6.1 indicates that 92% of gold was removed. This suggests

that the cellulose may play a small role in the adsorption of gold(III). The appearance of the biomass on the treatment with the alkali did not result in a paste-like formation as previously described.

6.2.2. Functional Group Modification

6.2.2.1. Materials and Method

6.2.2.1.1. Materials

The plant material and reagents were obtained and prepared as described in Section 2.2.1.1. Sodium carbonate, analytical grade, was obtained from Saarchem, South Africa. Succinic anhydride, hydroxylamine and DTNB (Ellman's Reagent) were purchased from Sigma, St. Louis, USA. Deionised water with minimal sodium hydroxide was utilised to prepare the DTNB. Atomic absorption standards were prepared utilising a gold atomic absorption solution (1000 mg/L) (Wirsam, South Africa). All solutions were dissolved and diluted with deionised water (Milli Q, Millipore).

6.2.2.1.2. Method

Amine Modification (Succinic Anhydride)

The plant material (2 g) was placed with 1 M sodium carbonate (250 mL, pH 8) and succinylated by the addition of 3.6 g of solid succinic anhydride for a period of 10 minutes. The acetylated biomass was washed with deionised water and resuspended in 1 M hydroxylamine (pH 8) for 10 minutes to remove the *o*-acetyl groups. The biomass was then thoroughly washed with deionised water, dried at 30°C and weighed.

Sulphydral Modification (DTNB)

Azolla (3 g) was placed with DTNB (200 mL) at various concentrations (0.2 mM, 0.4 mM, 0.8 mM and 1.6 mM) for a period of 2 hours to determine if a concentration effect determined the extent of gold(III) uptake. The biomass was filtered under vacuum and thoroughly washed with deionised water. The plant material was dried at 30°C and weighed.

Uptake Experiments

All experimental work was conducted in duplicate. The plant material, once treatment was complete, was subjected to uptake studies with gold(III) in the form of hydrogen tetrachloroaurate(III) at a concentration of 50 μM (pH 2). A sample of 50 mL of gold(III) solution was placed with 0.25 g of biomass in an Erlenmeyer flask (100 mL) and agitated at a speed of 200 rpm at room temperature. Samples (5 mL) were taken at regular intervals of 10 minutes for a period of 90 minutes and filtered (cellulose-acetate, 25 mm diameter, 0.45 μM pore size). The gold concentration was analysed with an AA spectrophotometer. The results were expressed as percentage uptake of gold(III) from solution after 90 minutes.

6.2.2.2. Results and Discussion

Succinic anhydride ($C_4H_4O_3$) reacts preferentially with amine groups, forming an amide linkage and a carboxylate group at pH 8 according to the following reaction (Doyle *et al.*, 1980):

$$R\text{-}NH_3^+ + C_4H_4O_3 \rightarrow R\text{-}NHCOCH_2CH_2COO^-$$

The modification of the plant material with succinic anhydride had no considerable effect on the uptake of gold(III) from solution (96%) (Table 6.2). This then suggests that amine functional groups are not responsible for the principle adsorption process.

Table 6.2. Effect of succinic anhydride treatment on the uptake of gold(III).

Treatment	*% Biomass Loss*	*% Uptake of Gold(III)*
Succinic Anhydride	22.83	95.76

Thiols, as soft bases, and gold(III) as a soft acid, have a strong tendency to bind to one another. Thus a sulphydral blocking reagent, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), was utilised to determine whether this functional group may be responsible for gold(III) uptake (Darnall *et al.*, 1986). This particular reagent reacts with aliphatic thiol groups to form a mixed disulfide at pH 7-8 in the following manner (Ellman, 1959; Kuwata *et al.*, 1982; Riddles *et al.*, 1979):

$$RSH + R'SSR' \rightarrow RSSR' + R'SH$$ (R= *Azolla* substrate, R'SSR'= DTNB)

5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) dissolves in a minimal amount of sodium hydroxide, however, a pH of 7 should not be exceeded as the stability of the compound in water decreases over time. The treatment of the biomass resulted in an average of 22% weight loss which remained more or less constant over the range of DTNB concentrations employed (Table 6.3).

Table 6.3. Effect of DTNB treatment on the biomass.

DTNB Concentration (mM)	*Percentage Weight Loss (%)*
0	22.43
0.2	25.00
0.4	19.67
0.8	22.93
1.6	21.93

The treatment of the biomass with increased concentrations of DTNB resulted in an increase in the reaction kinetics (Figure 6.1). Equilibration of gold(III) uptake was reached at end of the incubation period for all the DTNB concentrations, each concentration displaying greater than 93% removal except for the lowest concentration of DTNB (0.2 mM) where only 89% of

gold(III) was removed. This indicates that the thiol functional group is not the principle site responsible for the uptake of gold(III).

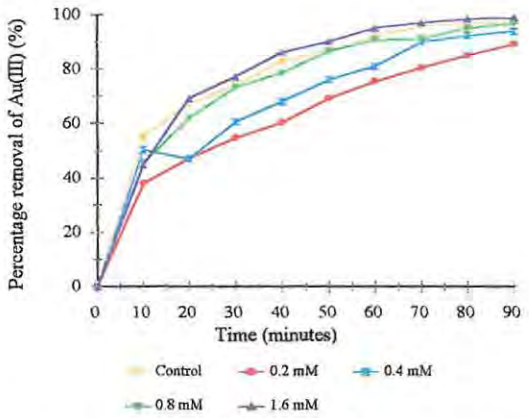


Figure 6.1: Effect of DTNB at various concentrations ranging from 0-1.6 mM on the uptake of gold(III) (50 μM). The following parameters were utilised: biomass concentration of 5 g/L, pH 2, room temperature and agitation speed of 200 rpm.

6.2.3. Infra-Red Analysis of Modified Azolla filiculoides

6.2.3.1. Materials and Method

6.2.3.1.1. Materials

The plant material, after modification, was placed in an oven, at 30°C, to be kept dry. Potassium bromide was obtained from Saarchem, South Africa.

6.2.3.1.2. Method

The mid-infrared (4000-400 cm^{-1}) of the particular modified plant material was run as a KBr disk on a Perkin-Elmer 2000 Fourier-Transform Infrared (FT-IR) spectrometer.

6.2.3.2. Results and Discussion

Characteristic group frequencies are presented in Tables 6.4. Representative spectra are given in Figure 6.2. The control study is characterised by a broad O-H stretch (ν) at 3373 cm^{-1}, aliphatic C-H stretches at 2953, 2918, and 2851 cm^{-1}. The broad shoulder at 1732 cm^{-1} may be assigned to either the N-H bend (δ) or O-H bending vibrations. The very strong broad band at 1651 cm^{-1} is typical of a carbonyl stretch. On chemical modification the three bands of the C-H stretches and the 1732 cm^{-1} bend (the δN-H or δO-H) are little affected. Treatment of the plant material with dilute alkali, methanol, chloroform:methanol (2:1), chloroform:methanol-concentrated alkali, succinic anhydride and DTNB resulted in no significant spectral changes apart from shifts in the broad O-H stretch.

In contrast, biomass treated with concentrated HCl (0.6 N) and on adsorption of gold(III) resulted in significant spectral changes. Protonation of the O-H group results in a sharper stretch at 3414 cm^{-1} and the appearance of two additional very strong and sharp O-H stretches of higher frequency (3350 and 3470 cm^{-1} respectively). The very broad hydrogen bonded O-H stretch in the control masks possible N-H stretches. However, as a result of increased hydrogen bond formation on protonation (treatment with acid or adsorption of gold(III)) the N-H stretch is shifted from under the broad νO-H to appear at 3240 cm^{-1}. The carbonyl band is split and shifts to a lower frequency, suggesting protonation or coordination. The region below 1500 cm^{-1} in any infrared spectrum is normally complex due to the appearance of C-H bends, C-H, O-H and N-H scissors rocks, twists and wags, as well as the appearance of C-O, S-O, C-C, C-S stretches and their corresponding bends. However, there are two regions below 1500 cm^{-1} for *Azolla* which are sensitive to chemical modification. The first involves the disappearance of a broad band of medium intensity at 1322 cm^{-1}. The second region showing change (800-450 cm^{-1}) involves three weak to medium intensity bands (at 772, 671, and 618 cm^{-1} respectively) superimposed on a very broad band of weak to medium intensity. These are masked by the appearance of two prominent bands of medium to strong intensity at approximately 620 and 473 cm^{-1} on treatment with acid or adsorption of gold(III). These two bands are probably identified as the protonated O-H rocking and wagging vibrations, respectively.

Table 6.4. The effect of various treatments on the functional groups of *Azolla filiculoides*.

Treatment	*Wavenumbers (cm^{-1})*								
	νO - H				**νN -H**	**νC - H**			**δN-H or δO-H**
Control	-	-	-	3373 *vsbr*	-	2953 *wsh*	2918 *vs*	2851 *vs*	1732 *wsh*
Dilute Alkali (0.01 N NaOH)	-	-	3423 *vs*	-	-	2957 *wsh*	2920 *vs*	2853 *s*	-
Dilute Acid (0.01 N HCl)	-	-	3426 *vsbr*	-	-	2955 *wsh*	2919 *vs*	2852 *s*	1726 *wsh*
Concentrated Acid (0.6 N HCl)	3550 *vs*	3470 *vs*	3414 *vs*	-	3240 *ssh*	2953 *msh*	2918 *s*	2851 *s*	1732 *mbr*
Methanol	-	-	3413 *vsbr*	-	-	2953 *msh*	2919 *s*	2852 *ms*	1729 *msh*
Chloroform-Methanol	-	-	-	3352 *vsbr*	-	2955 *msh*	2928 s	2850 *msh*	1729 *msh*
Chloroform-Methanol-Conc. Alkali	-	-	3433 *vsbr*	-	-	2957 *wsh*	2925 *s*	2854 *wsh*	-
Succinic Anhydride	-	-	3406 *vsbr*	-	-	2953 *wsh*	2919 *vs*	2852 *s*	1732 *msh*
DTNB	-	-	-	3335 *vsbr*	-	2957 *ssh*	2921 *vs*	2852 *s*	1732 *msh*
Gold and *Azolla*	3545 *vs*	3470 *vs*	3414 *vs*	-	3239 *vsh*	2954 *msh*	2918 *s*	2851 *s*	1732 *mbr*

Abbreviations:

v: *very,* ***m:*** *medium*

s: *strong,* ***w:*** *weak*

br: *broad,* ***sh:*** *shoulder*

Table 6.4. (Continued).

Treatment	*Wavenumber (cm^{-1})*							
	νC=O			**1350-1300**	**800-450**			
Control	1651 *vsbr*	-	-	1322 *m*	772 *wbr*	671 *wsh*	618 *m*	-
Dilute Alkali (0.01 N NaOH)	1638 *sbr*	-	-	1325 *wbr*	-	-	-	-
Dilute Acid (0.01 N HCl)	1638 *sbr*	-	-	1321 *wsh*	-	-	-	-
Concentrated Acid (0.6 N HCl)	1651 *ssh*	1638 *vs*	1619 *vs*	-	-	-	621 *msbr*	473 *msbr*
Methanol	1644 *sbr*	-	-	1321 *m*	780 *w*	667 *wbr*	617 *m*	-
Chloroform-Methanol	1653 *sbr*	-	-	1323 *mbr*	769 *mbr*	667 *mbr*	618 *ms*	-
Chloroform-Methanol-Conc. Alkali	1633 *sbr*	-	-	-	-	-	-	-
Succinic Anhydride	1652 *vsbr*	-	-	-	-	-	-	-
DTNB	1652 *vs*	-	-	-	778 *wsh*	667*msh*	618 *m*	-
Gold and *Azolla*	-	1639 *vs*	1618 *vs*	-	-	-	616 *msbr*	473 *msbr*

Abbreviations:

v: *very,* ***m:*** *medium*

s: *strong,* ***w:*** *weak*

br: *broad,* ***sh:*** *shoulder*

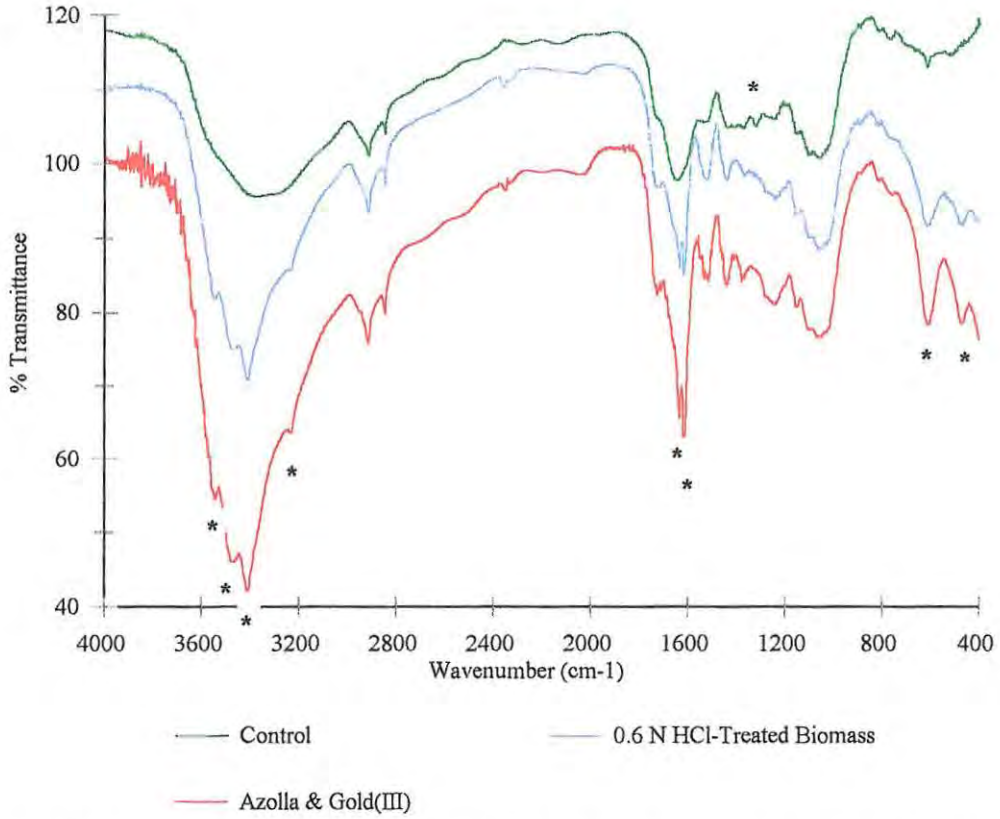


Figure 6.2: Representative mid-infrared spectra (4000-400 cm^{-1}). Similar spectra are obtained for the 0.6 N HCl-treated *Azolla* and the *Azolla* containing gold once adsorption has taken place. The spectra suggests the biomass is protonated due to the acidity of the gold solution. * denotes characteristic absorbances.

6.2.4. Representative Extracted Compounds and Gold Studies

6.2.4.1. Materials and Method

6.2.4.1.1. Materials

Chitin, bovine serum albumin and fibrous cellulose were obtained from Sigma, U.S.A. Lanolin (lipid) was obtained from ISTT, Grahamstown.

6.2.4.1.2. Method

All experiments were conducted in duplicate. The reagents, chitin (polysaccharide), bovine serum albumin (BSA) (protein), cellulose (polysaccharide) and lanolin (lipid) were placed in deionised water. Gold(III) was added to the mixture at a final concentration of 100 mg/L. All reagents were utilised to mimic the extracted compounds of the previous treatment experiments (Section 6.2.1) and all were insoluble except for BSA. The solutions were vortexed and allowed to stand for a period for 24 hours. The concentration of gold in the solution was measured utilising an AA spectrophotometer. The results were expressed as percentage removal of gold(III) from solution. The samples were lyophilised and kept in a dessicator prior to infra-red analysis. The procedure of infra-red analysis was followed as described in Section 6.2.3.1.2.

6.2.4.2. Results and Discussion

Having established the spectral changes on chemical modification of the plant material and upon gold adsorption, several uptake experiments were conducted using representative compounds typically found in biomass. The addition of gold(III) (100 mg/L) to chitin, fibrous cellulose, protein (BSA) and lipid, resulted in no removal of gold from solution over the 24 hour period. Upon lyophilisation of the samples it was noted that the infra-red spectra of each of the polysaccharides did not significantly change upon the addition of gold(III). This suggests that these sites are not responsible for the primary binding of gold(III) to the plant material, although

they may still play a role in binding to any reduced species of the metal subsequent to initial binding. Upon lyophilisation of the lipid and protein it was noted that there was a change in the infra-red spectra on the addition of gold (Figures 6.3a and 6.3b respectively). For the lipid there is a moderate shift of 10 cm^{-1} to a higher frequency of the two medium to strong bands at 1250 and 1116 cm^{-1}, a shift of 33 cm^{-1} to lower frequency of the medium broad band at 1065 cm^{-1}, the loss of the weak to medium band at 950 cm^{-1} and the appearance of a new sharp triplet band of medium intensity at 800 cm^{-1}. The band at 1261 cm^{-1} in the protein is tentatively assigned to the amide III vibration, and the 1250 cm^{-1} in the lipid is possibly the C=O symmetric stretch which would be coupled to the C-O-C stretch expected between 1150 and 1060 cm^{-1}. However, all three vibrations would be strongly coupled. While no vibrational assignment for Au_2O_3 has been found in the literature, the appearance of the band at approximately 800 cm^{-1} in both the lipid and the protein spectra on addition of gold is similar to other metal oxides involving abridged oxygen, as is found in the structure for Au_2O_3. It is thus possible that the species bound to the lipids and protein may be gold oxide or various oxo-gold compounds. The addition of gold(III) to the BSA results in the appearance of the sharp, medium to strong, 1261 cm^{-1} band and the medium triplet band at 800 cm^{-1} observed in the lipid-gold spectrum. These results suggest lipids and protein are possible binding sites for the gold. It is not possible to determine their overall contribution due to the solubility of the complexes.

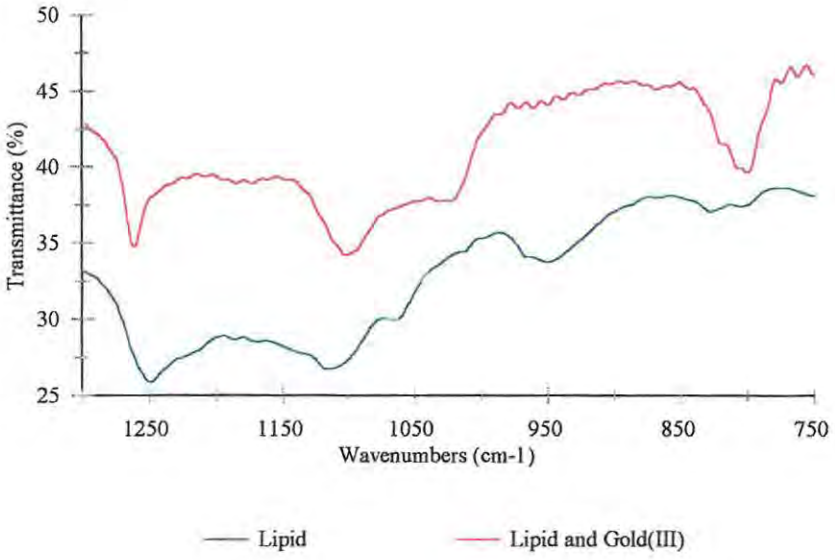


Figure 6.3a: Representative infra-red spectra of lipid and lipid in combination with gold(III).

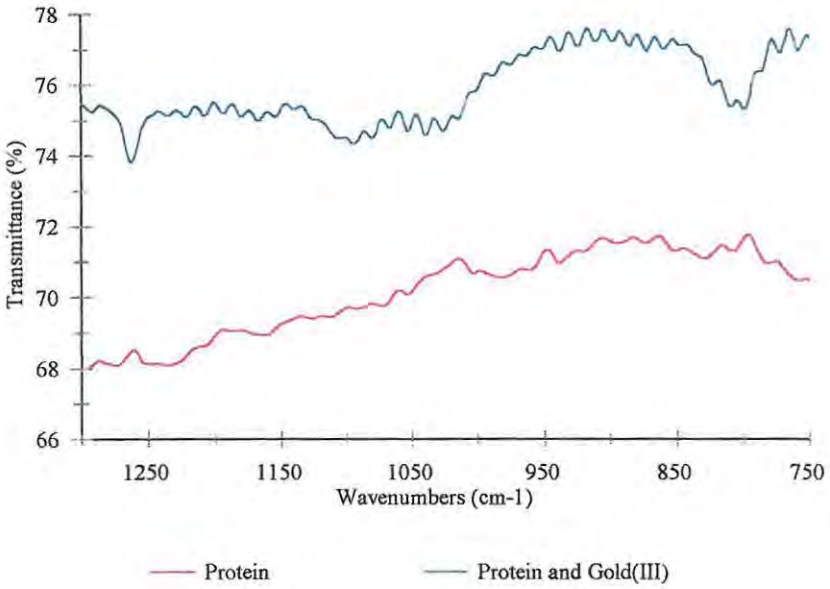


Figure 6.3b: Representative infra-red spectra of protein (BSA) and protein in combination with gold(III).

6.3. TANNIN STUDIES

6.3.1. Precipitation Studies

As described in Chapter 1, tannins are divided into two groups: hydrolysable, which are composed of gallic acid or ellagic acid esterified to a sugar moiety; and condensed, also known as proanthocyanidins, which are flavonol-based tannins (Mole and Waterman, 1987). Studies by McDonald *et al.* (1996) and Mole and Waterman (1987) demonstrated that metal precipitation with tannins occurred. Since precipitation was found to occur with high gold(III) concentrations through the possible interaction of tannins on *Azolla filiculoides* (Chapter 3), an attempt was made to determine whether tannins (pure phenols, hydrolysable and condensed) are responsible for the precipitation of gold(III).

6.3.1.1. Materials and Method

6.3.1.1.1. Materials

Mimosa ME and FS, trupotan MT, and quebracho (all condensed tannins) were obtained from ISTT, Grahamstown. Tannic acid (a hydrolysable tannin), gallic acid and catechol (pure phenols) were obtained from Sigma, St. Louis, USA. All tannins were dissolved in deionised water (Milli Q, Millipore). Any sediment was removed by filtration. Hydrogen tetrachloroaurate(III) was obtained from Saarchem, South Africa, and dissolved in deionised water. Atomic absorption standards were prepared from a 1000 mg/L gold atomic absorption solution (Wirsam, South Africa) and diluted with deionised water.

6.3.1.1.2. Method

All experimental work was conducted in duplicate. Tannins of various concentrations (1, 2, 3, 5, 7 and 9 g/L) were dissolved in deionised water until the desired concentration was achieved. A final gold(III) (100 mg/L) and tannin, of a specific concentration, were agitated together. The solution was immediately vortexed. Once precipitation was complete, the solution was analysed for gold utilising an AA spectrophotometer. The results were expressed as gold(III) concentration (mg/L) in solution.

6.3.1.2. Results and Discussion

The tannins used in industry rely on their complexation with metal ions which result in precipitation e.g., ferric chloride and tannins to produce ink. Thus the formation of a precipitate between tannins and gold(III) would indicate that a complex has been formed. Catechol, and gallic acid (pure phenols), tannic acid (hydrolysable tannin), trupotan MT and mimosa ME (condensed tannins) were all able to precipitate gold as demonstrated by the low concentration of gold (0-2 mg/L) remaining in solution (indicating 98-99% removal, Figure 6.4). Quebracho, containing a mixture of condensed and hydrolysable tannins, resulted in a 7 mg/L residue (93% removal).

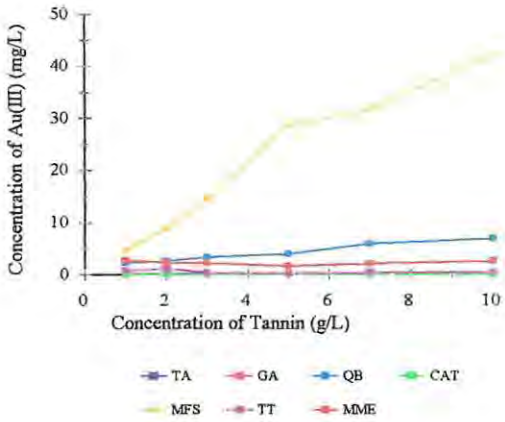


Figure 6.4: Effect of various tannins at various concentrations on gold(III) in solution. The following abbreviations are as follows: **TA**: Tannic Acid, **GA**: Gallic Acid, **QB**: Quebracho, **CAT**: Catechol, **MFS**: Mimosa FS, **TT**: Trupotan MT, and **MME:** Mimosa ME.

Functional groups of mimosa FS have been modified from phenolic to sulphite ($R\text{-}OH \rightarrow R\text{-}SO_3^{2-}$). This resulted in a precipitation of only 58% of gold(III) from solution as compared with mimosa ME which showed 95% precipitation. The results in Figure 6.4 suggest that the phenolic functional groups play an important role in the complexation of gold(III) which ultimately results in precipitation (Chapter 3, Figure 3.5).

Once precipitation had occurred, samples were filtered and subjected to a visual analysis. This gives an indication of the particle size and rate of complex formation. Large complexes form a black precipitate whereas smaller, colloidal structures yield the indicative Purple of Cassius. With gallic acid, precipitation was observed at higher concentrations of tannin, 5-10 g/L. Whilst for tannic acid, catechol and quebracho, a black precipitate was observed from 1-10 g/L. For mimosa FS, at low concentrations, 0.5 -1.0 g/L, gold flakes were observed in the precipitate suggesting that reduction had occurred, whilst at 2-3 g/L colloidal gold was present, and at 5-10 g/L a black precipitate (Figure 6.5). This demonstrates the rate of formation of colloidal or black precipitated gold with respect to the concentrations of tannin utilised. The gold flakes found in Mimosa FS suggests that sulphite modification assists in the reduction of the gold(III) to gold(0).

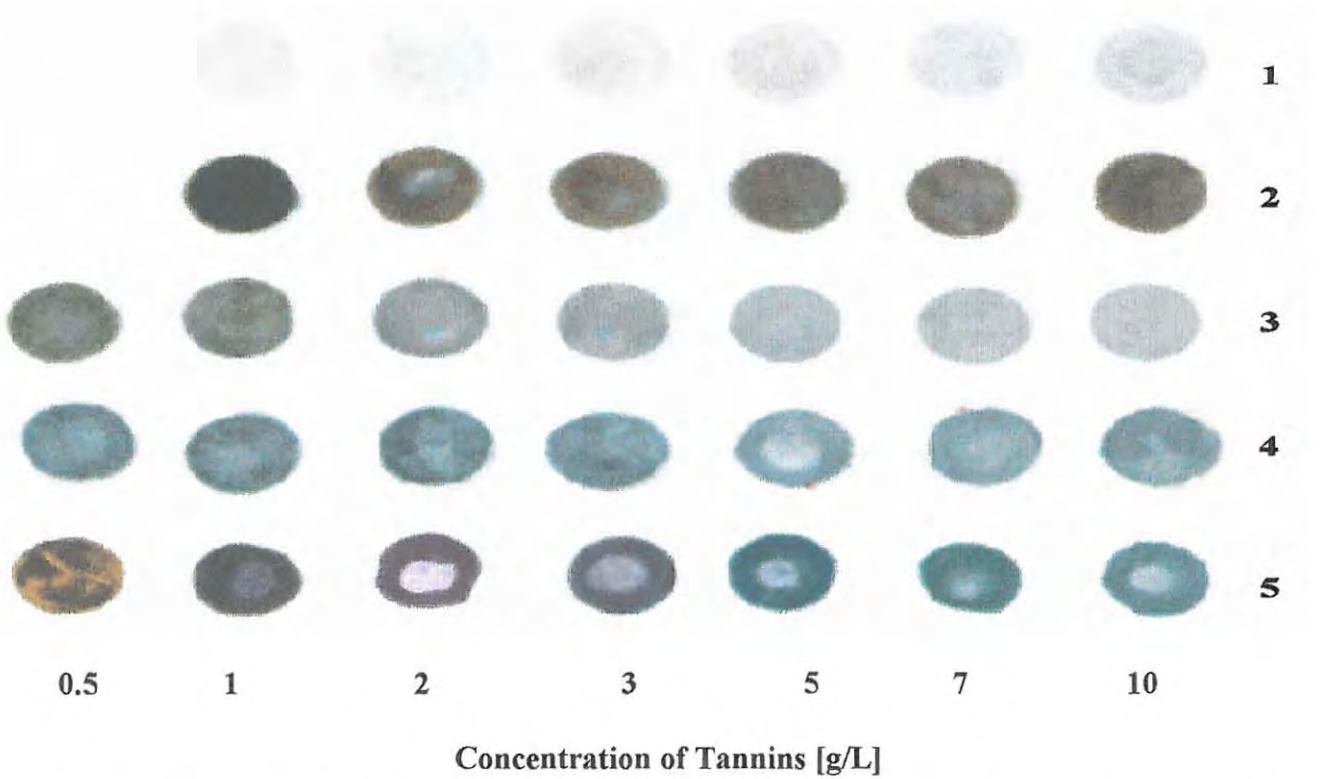


Figure 6.5: Representative colour schematics for the reactions observed for selected tannins. The following abbreviations occurs as follows: **1:** Gallic acid, **2:** Tannic acid, **3:** Catechol, **4:** Quebracho, **5:** Mimosa FS.

6.3.2. Infra-Red Analysis of Tannin Precipitates

6.3.2.1. Materials and Method

6.3.2.1.1. Materials

The precipitation studies were repeated as described in Section 6.3.1.1.2 and allowed to evaporate to complete dryness. Analytical grade potassium bromide was obtained from Saarchem, South Africa.

6.3.2.1.2. Method

The mid-infrared (4000-400 cm^{-1}) of the particular tannin, and gold and tannin precipitate was run as a KBr disk on a Perkin-Elmer 2000 Fourier-Transform Infrared (FT-IR) spectrometer. Representative spectra are given in Figures 6.6a-c.

6.3.2.2. Results and Discussion

All the tannins are characterised by an extremely broad O-H stretch at about 3380 cm^{-1}, and broad strong C-O stretches with an additional broad strong band at 1714 cm^{-1}. On the addition of gold(III) to the various tannins, most of the spectra resemble one another and show the characteristic band pattern observed for the *Azolla* with gold. Consequently only one example of a gold and tannin complex is shown in Figures 6.6a-c. The precipitate showed the resolution of three sharper strong O-H stretches suggesting that either protonation occurs or that on coordination to the gold the tannins become more ordered. The broad carbonyl at 1651 cm^{-1} is split while the band at 1714 cm^{-1} (where present) is reduced in intensity both of which suggests either protonation or coordination. The bands at 621and 473 cm^{-1} may be ascribed respectively to the rocking and wagging vibrations of the protonated hydroxyl groups. These results suggest that the infra-red spectra of the acid-treated biomass and the biomass exposed to a gold solution are similar. This indicates that the biosorbent is most likely to be protonated due to the acidity of the gold solution and that may aid the binding of the gold anion complex initially to the biomass.

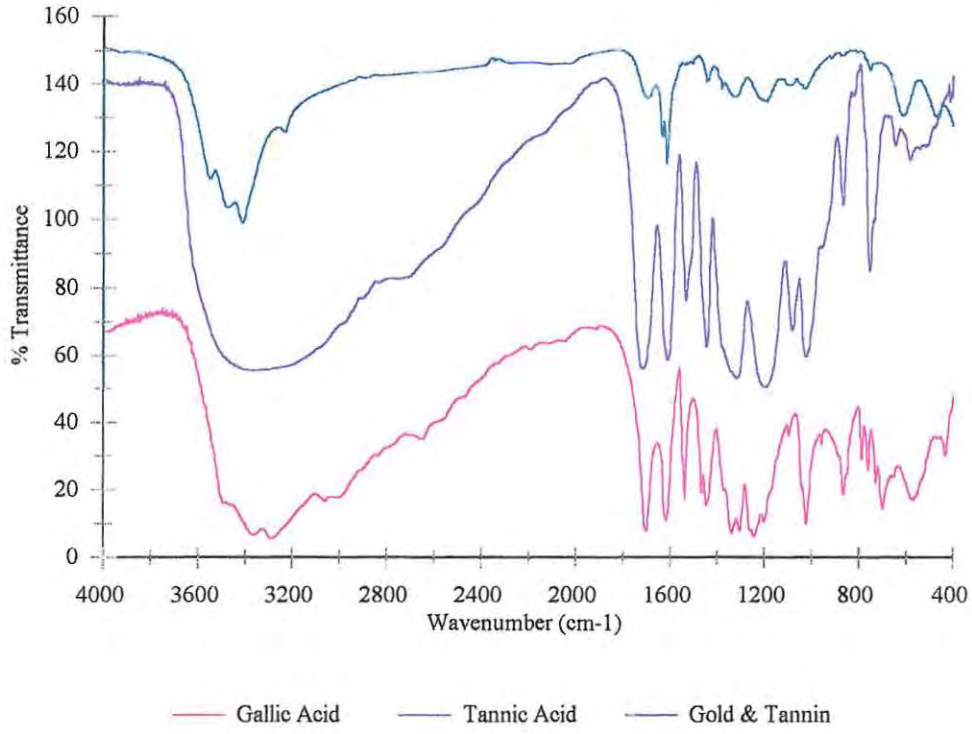


Figure 6.6a: Representative FT-IR spectra of a pure phenol, gallic acid; hydrolysable tannin, tannic acid; and a representative gold and tannin precipitate.

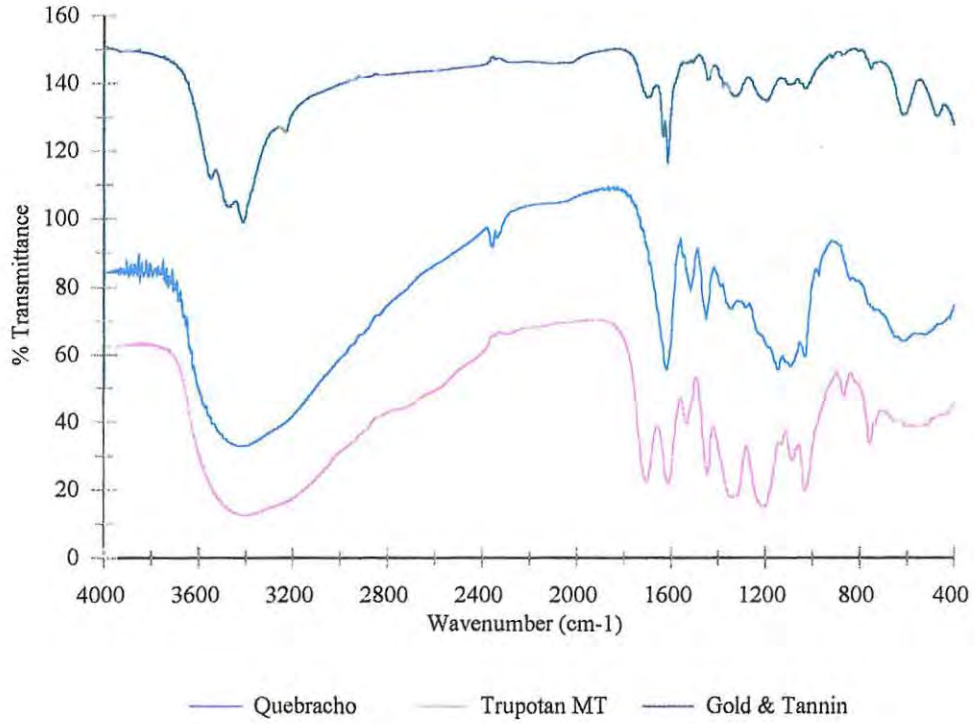


Figure 6.6b: Representative FT-IR spectra of condensed tannins: Quebracho and Trupotan MT, and a representative spectra of gold and a tannin precipitate.

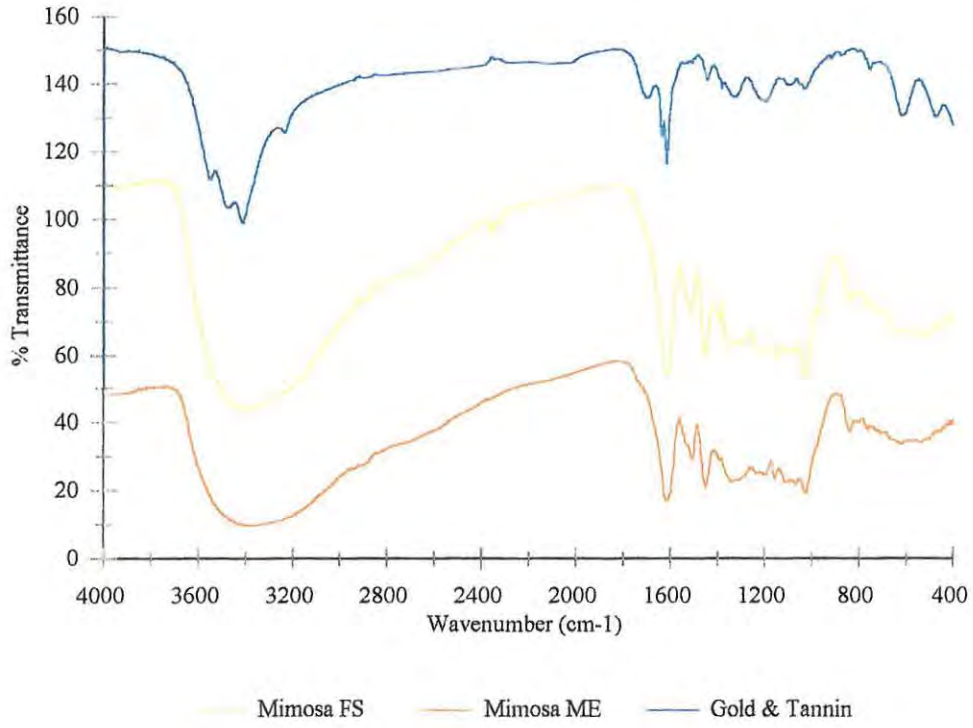


Figure 6.6c: Representative FT-IR spectra of modified condensed tannins: Mimosa FS and Mimosa ME, and a representative spectra of gold and a tannin precipitate.

6.4. SUMMARY

An understanding of the underlying mechanism involved in the adsorption process of gold(III) to *Azolla filiculoides* allows for the ability to maximise the plant material's ability to bind not only gold but other metals found in a waste solution. The functional groups present on the surface of the biomass, especially the non-viable biomass, play an important role in the adsorption of the metal. The main aim of this chapter was to attempt to elucidate the binding mechanism of gold(III) to *Azolla filiculoides*.

The moderate decrease in gold uptake of 16% with alkali treatment and 8.5% with chloroform-methanol (2:1) and alkali, may be due to the contribution of polysaccharide and proteins as well as cellulose to the binding of Au(III) to the biomass. However, the overall loss of integrity of the plant material on the addition of the alkali may be ascribed as the cause of the moderately reduced efficiency in Au(III) uptake (Table 6.5). Modification of the functional groups present in the plant material by treatment with boiling water, dilute and concentrated acid, methanol, chloroform and methanol (2:1), and DTNB did not have a significant effect on the gold uptake and failed to establish the major binding group.

Table 6.5. The effect of various treatments on the uptake of gold(III) from solution.

Treatment	*Extraction*	*Percentage Uptake (%)*
Boiling Water	Polysaccharides, Proteins(H_2O soluble)	97.4
Dilute Alkali(NaOH)	Polysaccharides, Proteins(higher pH- soluble)	84.0
Dilute and Concentrated Acid (HCl)	Tannins	99.0 & 98.2
Methanol	Polar organics	98.5
Chloroform:Methanol	Lipids	95.9
Chloroform:Methanol-Conc. Alkali	Cellulose	91.5
DTNB	Sulphydral	>89%
Succinic Anhydride	Amine	95.8

Consequently, an alternate strategy was adopted to compare the changes in the mid-infrared spectra of the modified biomass on Au(III) adsorption with spectroscopic changes in the isolated biomass components on addition to a gold(III) solution.

No significant changes were observed with the treatment of the biomass by the various chemicals compared with the control except for the dilute and concentrated acid (HCl), and the biomass treated with gold. This suggests that the acidity of the gold(III) solution (pH 2), is responsible for obtaining a similar spectra to that of the acid-treated biomass. The splitting and sharpening of the O-H stretch, the splitting and shift to lower frequency of the carbonyl stretch and the appearance of the hydrogen bonded N-H stretch shows that protonation of the biomass occurs at all three functional groups. Consequently, the infra-red studies have shown that the initial mode of binding of $[AuCl_4]^-$ to protonated groups on the biomass is likely to be ionic. This may occur very rapidly, and allows for subsequent displacement of a chloride ion which probably initiates covalent binding to the biomass.

Polysaccharides were eliminated as possible binding sites on the biomass. Lipids and proteins show the possibility of coordination as evidenced by shifts of several bands in the region of 1270 - 1150 cm^{-1}. The appearance of a multiple band at 800 cm^{-1} in the infra-red spectrum suggests that adsorption for these sites is in the form of an oxo-gold complex or the oxide.

An attempt to determine if tannins found on the *Azolla,* were principally responsible for the initial gold(III) binding was undertaken. Pure phenols, hydrolysable as well as condensed tannins (polyphenols) are able to precipitate the gold(III) complex out of solution. This was shown by the greater than 93% removal from solution for all the tannins investigated except for mimosa FS (modified functional group, RSO_3^-) which demonstrated 50% removal. The infra-red spectra showed the protonation of the O-H stretch as well as the better definition of the C-O stretch, this suggests that functional groups, such as the phenols, on the tannins now become protonated due to the acidity of the gold(III) solution. The protonation of the hydroxyl groups present on the tannins may be largely responsible for initial adsorption of gold, and in some cases be responsible for the reduction and/or precipitation of the metal if present at suitable concentrations.

CHAPTER 7

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

7.1. 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.

7.2. BATCH OPTIMISATION STUDIES

7.2.1. Materials and Method

7.2.1.1. 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.

7.2.1.2. 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.

7.2.2. 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.

7.2.2.1. Effect of biomass concentration

The results in Figure 7.1 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.

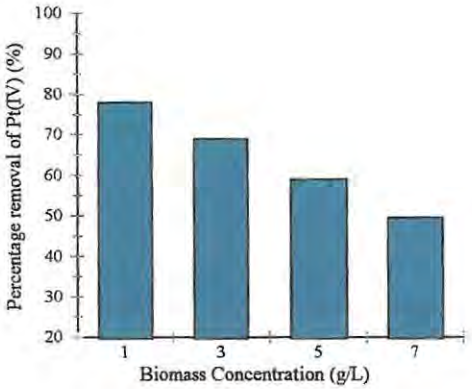


Figure 7.1: Effect of biomass concentrations on the adsorption of platinum(IV) by *Azolla filiculoides*. The following parameters were utilised: room temperature, agitation speed of 200 rpm, platinum(IV) concentration of 15 mg/L and pH 2.

7.2.2.2. 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 7.2 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.

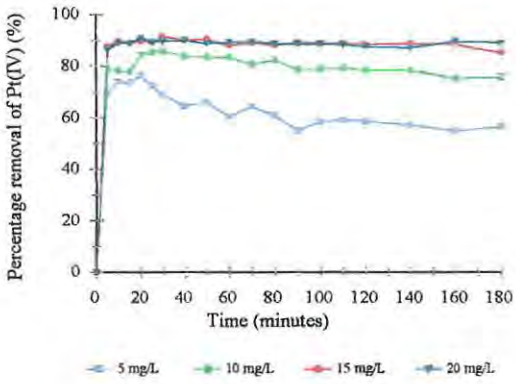


Figure 7.2: Effect of initial platinum(IV) concentration on the adsorption of platinum(IV) by *Azolla filiculoides*. The following parameters were utilised: room temperature, agitation speed of 200 rpm, biomass concentration of 1 g/L and pH 2.

7.2.2.3. 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 7.3 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 7.3) is faster than gold(III) but is not as efficient. A pH of 2 was utilised for further experiments.

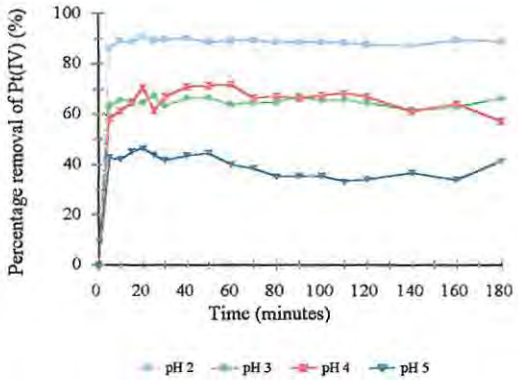


Figure 7.3: Effect of pH on the adsorption of platinum(IV) by *Azolla filiculoides*. The following parameters were utilised: room temperature, agitation speed of 200 rpm, platinum(IV) concentration of 20 mg/L and biomass concentration of 1 g/L.

7.2.2.4. 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 7.4). The removal remained fairly constant over the temperature range investigated suggesting that the adsorption process is energy-independent.

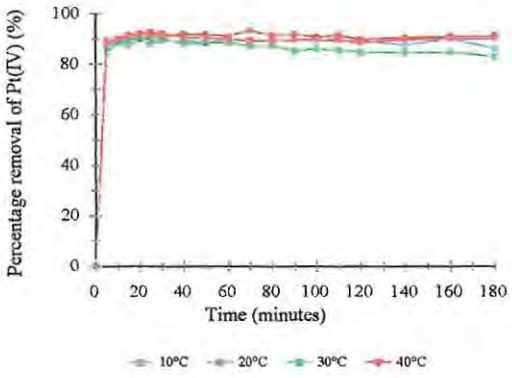


Figure 7.4: Effect of temperature on the adsorption of platinum(IV) by *Azolla filiculoides*. The following parameters were utilised: agitation speed of 200 rpm, platinum(IV) concentration of 20 mg/L, biomass concentration of 1 g/L and pH 2.

7.3. COLUMN OPTIMISATION STUDIES

7.3.1. Materials and Method

7.3.1.1. 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.

7.3.1.2. 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.

7.3.2. Results and Discussion

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

Batch studies demonstrated that pH 2 was optimal for platinum(IV) adsorption (Figure 7.3). 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 7.5. 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.

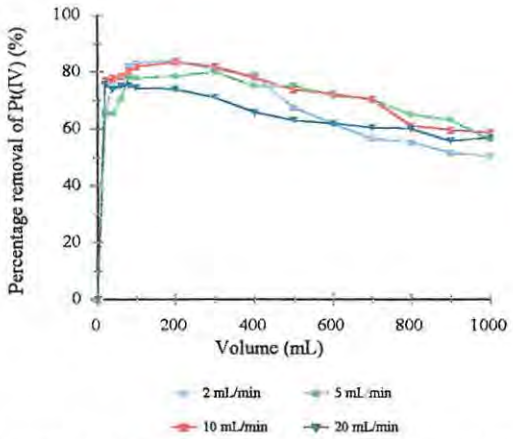


Figure 7.5: Effect of flow-rate on the removal of platinum(IV) from solution by *Azolla filiculoides*. The following parameters were utilised: biomass concentration: 1 g/L, platinum(IV) concentration of 20 mg/L, pH 2 and room temperature.

7.3.2.2. 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 7.6). 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).

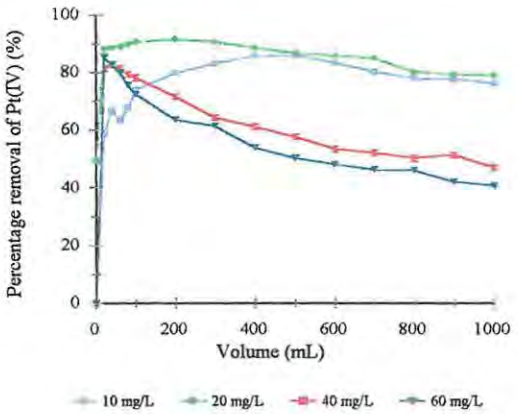


Figure 7.6: Effect of initial platinum(IV) concentration on the removal of Pt(IV) from solution by *Azolla filiculoides*. The following parameters were utilised: biomass concentration: 1 g/L, pH 2, flow-rate of 10 mL/min and room temperature.

7.4. PLATINUM EFFLUENT STUDIES

Sections 7.2 and 7.3 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.

7.4.1 Batch Studies

7.4.1.1. Materials and Method

7.4.1.1.1. 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 7.1. depict the highest concentrations found in the effluent.

7.4.1.1.2. 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 III and IV respectively) (Merck, Germany). The results were expressed as percentage removal from solution for each of the metals studied.

7.4.1.2. 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 7.1. 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.

7.4.2. Column Studies

7.4.2.1. Materials and Method

7.4.2.1.1. 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 7.4.1.1.1 and is represented in Table 7.2.

7.4.2.1.2. 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.

7.4.2.2. 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 7.2) 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 7.2. 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.

7.5. 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 8

GENERAL DISCUSSION

Escalating industrialisation and decreasing precious metal ore reserves, such as gold and platinum, has focused 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. In this particular study, plant material, *Azolla filiculoides,* was utilised in the removal and recovery of gold from solution. Preliminary studies into the adsorption characteristics of platinum removal from solution were also carried out.

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.

In the mixed metal desorption and recovery studies at various pH's (1-7) of 0.1M HNO_3, copper(II) was found to show the weakest electrostatic binding, with a maximum removal of both copper(II) and lead(II) occurring at pH 1. Maximum recovery of gold occurred with thiourea, perchloric acid, and hydrochloric acid. The optimal system for lead(II) and copper(II) recovery was: to pre-wash the plant material with water, an acid concentration of 0.3M HNO_3 pumped in an up-flow mode, and to recycle the desorbent six times. For the final recovery, optimisation of the selective removal was carried out, however cost analysis for gold recovery from the biomass was compared indicating ashing of the plant material to be more economical than desorption with thiourea in combination with perchloric and hydrochloric acid. Studies to determine the optimal cumulative adsorption and desorption of the three metals of interest, over five successive cycles were carried out. It was found that rinsing of the biomass prior to desorption but after adsorption had occurred, resulted in a gradual decrease in adsorption of lead(II) and copper(II) over the 5 cycles suggesting that regeneration of the biomass was necessary, however, gold adsorption was greater than 90% for all cycles. A total recovery of adsorbed metal of 93%, 83% and 94% for lead, copper and gold respectively was found at the end of the fifth cycle. The desorption of lead and copper from the biomass after each adsorption cycle, and prior to rinsing of the plant material

resulted in a greater than 80%, 95% and 95% adsorption for lead, copper and gold in each cycle respectively over the five cycles. Final values for desorption were 92% for lead, 96% for copper, and 97% for gold. Improved lead and copper adsorption appears to occur with the regeneration of the biomass for subsequent adsorption and desorption cycles. This allows for a more efficient process of metal removal and recovery. The criteria for the utilisation of the biomass in a cost-effective process has been satisfied. The low cost due to the abundance of the adsorbent, high mechanical strength and the large surface area for adsorption of *Azolla filiculoides* allows for the rapid uptake of various metals and all these factors contribute to its utilisation (Banks, 1997; Garnham, 1997; Volesky, 1990).

Waste water studies were undertaken on mine effluent in batch and column conditions to determine which would be the most feasible. In the batch studies no adsorption occurred for most metals except for nickel which displayed a 46% removal while 100% of gold was adsorbed. Cyanide and sulphate levels decreased by approximately 14% each. In the column studies, 17% and 46% of copper and nickel was removed respectively. Sulphate levels decreased by 18%. For both the batch and column studies a decrease in pH accompanied the adsorption of metals by the biomass suggesting that an ion-exchange process may be involved. Batch studies is the optimal system for the removal of gold, although not necessarily feasible on a large scale.

Understanding the underlying mechanism involved in the adsorption process of gold(III) to *Azolla filiculoides* is important to maximise the plant material's ability to bind not only gold but the various metals found in waste solutions. Studies towards such an understanding showed that the various treatments of the plant material did not significantly reduce the uptake of gold(III). A slight reduction in the gold(III) uptake as result of the extraction of polysaccharides, proteins and cellulose was found suggesting these to be minor sites involved in gold(III) adsorption. Infra-red spectroscopic studies allowed a more detailed examination of gold binding and a preliminary determination of the principle sites of gold. In these studies, no spectral changes were observed for the modifications with the exception of the dilute and concentrated acid treatment, and the gold-adsorbed biomass. This suggests that the low pH of the solution resulted in the protonation of the biomass as evidenced by changes in the O-H, N-H and carbonyl stretches, as well as in the

appearance of the O-H rock and wagging vibrations. Studies with representative cellulose and polysaccharide solutions in combination with gold(III), showed no changes in the infra-red spectra of the polysaccharide, while the protein and lipid studies indicated to be possible binding sites for gold(III). The binding mechanism can then be understood as initially involving ionic attraction between these protonated sites and the anionic $[AuCl_4]^-$ complex, which occurs very rapidly, leading to the interaction of the gold complex and the biomass. Displacement of the labile chloride ligand ensues with an initiation of the covalent binding of the gold complex to the biomass. Column studies in Chapter 3 suggested the possibility of gold precipitation during biosorption at high gold concentrations. It is well-known that tannins, hydrolysable or condensed, precipitate metals and are known to be associated with the plant material, and for this reason a study into its ability to chelate and possibly precipitate gold was examined. All the tannins readily precipitated gold (>93%) except for the sulphited mimosa FS (only 50%). It seems that the phenolic functional groups are important for complexation and precipitation to occur. The protonated hydroxyl groups present on the tannins are likely to chelate gold(III), and play a role in the adsorption of gold onto the biomass. However, if conditions are favourable gold precipitation at high metal concentrations may also occur.

Preliminary batch studies on the removal of Pt(IV) by *Azolla filiculoides* were also conducted. A range of biomass concentrations (1 to 7 g/L) demonstrated that platinum removal decreased as the concentration increased showing that there may be limited binding sites. At initial platinum(IV) concentrations of 5 to 20 mg/L removal increased (from 56% to 89%) as the metal concentration increased. pH had a marked effect on the adsorptive capacity of *Azolla filiculoides,* decreasing from 89% to 41% removal between pH 2 and 5. For the column studies a pH of 2 was utilised for optimal removal. Flow-rates had little effect on platinum removal. At initial platinum concentration studies, removal was rapid for all concentrations except for the initial concentration of 10 mg/L. At the 40 and 60 mg/L initial concentrations an immediate decrease in removal was observed which suggests that saturation of the biomass had been achieved.

For the platinum effluent, no significant removal of iron, zinc, nickel and copper occurred, except for lead. Platinum removal was found to be 33%. No significant adsorption of iron, lead, nickel

and copper was found to occur in the column studies, while zinc and platinum removal was found to be 7% and 22% respectively. As with the gold effluent, the process of adsorption was accompanied by H^+ release. There is no effect on the removal of platinum from solution utilising *Azolla* in batch or column studies, however, the treatment of larger volumes would be most suited to a column process.

The economic factor, abundant availability of *Azolla* in South Africa and the ease with which it may be cultured provides an incentive for the employment of *Azolla* as a biosorbent in metal recovery. The utilisation of *Azolla filiculoides* in the recovery from synthetic solutions at pH 2 has demonstrated the feasibility of this system for the removal of gold and platinum from solution and the potential recovery of metals from the biomass. Further characterisation studies, however, need to be undertaken to determine the feasibility for metal recovery in waste water with various anions present. Ultimately a pilot scale study would be required to fully validate the use of *Azolla filiculoides* for the biosorption and recovery of gold and platinum.

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: CYANIDE DETERMINATION
(Merck Spectraquant® Cyanide Test Kit: 1.14800)

Cyanide ions react with a chlorinating agent to form cyanogen chloride which in turn reacts with 1,3-dimethylbarbituric acid to form a violet dye (pyridine-free König's reaction) that is determined photometrically.

Method

The pH range of the sample should be between 2-8. 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 (5 mL) is added into to a test tube. One green microspoonful of CN-1A is added to the sample and dissolved. Thereafter one green microspoonful of CN-2A is added and vortexed. This is followed with the addition of 3 drops of CN-3A and vortexed. Leave for a period of 5 minutes. The absorbance is read at 606 nm utilising a spectrophotometer. In the blank, Milli Q water replaces the sample.

APPENDIX III: 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 IV: 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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