Adsorption of trace toxic metals by Azolla filiculoides from aqueous solution

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Adsorption of trace toxic metals by Azolla filiculoides from aqueous solution

Peter J. Lloyd-Jones

A Doctoral Thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

September 2003
Abstract

*Azolla filiculoides* has been evaluated for the adsorption of trace toxic metals from aqueous solution. The adsorption performance of the material was compared with commercial resins and fitted using the Langmuir and Freundlich models. The Freundlich model described the adsorption of copper and cadmium. Whilst the Langmuir isotherm had the better fit of the mercury data. The assumptions of the Freundlich model include multi-layer adsorption and different functional group binding. Conversely the Langmuir model suggests mono-layer adsorption and can infer single group reactivity. The pH effect on the uptake of the metals was investigated and an increase in removal was observed at higher pH with all the metals studied.

The material has been thoroughly characterised using physical methods, such as, scanning electron microscopy X-ray photoelectron spectroscopy and electrophoretic mobility measurements. This enabled conclusions to be made regarding the surface functionality of the solid. Chemical characterisation included direct titrations, revealing a gradual dissociation of acidic groups as the pH increased within the experimental range. Kjeldahl nitrogen and amino acid analysis of several biological materials that have been used in metal sorption experiments showed *A. filiculoides* as having a large proportion of these cell constituents.

The kinetics of metal ion uptake by the biosorbent was investigated and compared with commercially available resins. The kinetics are slower than conventional ion exchange resins and carbon adsorbents but entirely adequate for utilisation in a column process.

The mechanism hypothesised for metal ion removal by the biosorbent is primarily attributed to ionogenic groups exchanging ions for copper and cadmium removal. Mercury on the other hand is said to be predominantly involved in a reduction-precipitation reaction on the surface of the adsorbent.

Regeneration was successfully accomplished for copper and cadmium after minicolumn trials, with greater than 95 % elution of the metals using 0.1M HCl. The mini column trials showed a sharp breakthrough for these metals singularly and a dynamic equilibrium was
observed during multi-metal processing. Mercury removal was much slower and more difficult with the same eluant, achieving a maximum of 50% removal.

A method for a semi-continuous biosorbent process has been evaluated and proven to be successful in processing metal laden solution.

Key words: copper, cadmium, mercury, biosorption, wastewater treatment
Acknowledgements

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1. Introduction

1.1 General Introduction

Water is a necessary human resource, however, it can only be consumed if it is of a reasonable purity. Unpurified natural water used for drinking has often been a cause of outbreaks of intestinal diseases. Only water coming from some underground sources are naturally fit for human consumption. The quantity found in such places in no way satisfies demand, therefore recycling surface waters by treating them to remove toxic compounds and microbial contaminants is of great importance.

With increasing levels of pollution the water treatment industry needs to employ highly effective and efficient systems to produce potable water. This is achieved over several stages, adsorption of micro-pollutants by activated carbons and/or ion exchange resins is normally one of these and is widely used in the water industry.

According to the EPA\(^1\), a vast array of pollutants have been identified in municipal and public water supplies. These often include detergents, dyes, aromatic hydrocarbons, esters and pesticides as well as heavy metals.

1.2 Water Pollution by Metal Ions

Industrialisation has brought with it a dramatic increase in the release of metals to the environment. Attempts are now being made to reverse this trend with increasingly stringent regulations governing discharges to open waters. These new regulations mean it is becoming very important to find a way of treating any effluents prior to discharge by the companies producing pollutants.

The EU Drinking Water Directives (80/778/EEC\(^2\) and 98/83/EC\(^3\)), and Discharge to Inland Waters Directive (76/464/EEC) have established some limits on the concentration of toxic heavy metals, see Table 1.
Table 1 Regulatory limits for certain toxic metals in drinking and wastewater.

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Regulatory limit (µg/l)</th>
<th>76/464/EEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In effect to 31 December</td>
<td>In effect from 01 January</td>
</tr>
<tr>
<td>Arsenic</td>
<td>50 10</td>
<td></td>
</tr>
<tr>
<td>Boron</td>
<td>2000 1000</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>5 5 200</td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>50 50</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>3000 2000</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>50 10</td>
<td></td>
</tr>
<tr>
<td>Mercury</td>
<td>1 1 5</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>50 20</td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>10 10</td>
<td></td>
</tr>
</tbody>
</table>

1.3 Target metal selection

Three metals were chosen for biosorption experiments, i.e. copper, cadmium and mercury. Copper was chosen due to its prevalence as a pollutant\(^{10}\) and the ability of many materials to adsorb copper\(^{4}\) makes it a useful metal for comparison purposes. Cadmium and mercury were chosen because of their position in the European black list of high toxicity.\(^{5}\) These metals provide a serious challenge for conventional water treatment processes.

1.3.1 Copper

The main source of copper into the environment is from the extraction and working of the metal. The total flux of copper to the atmosphere is approximately 75000 metric tons per year, of which 5000-13000 tons are deposited into the ocean.\(^{6}\) Air emissions are the major source of copper into the aquatic environment and approximately 75%
of atmospheric emissions are from anthropogenic sources. Production of non ferrous metals is the largest single emission source, followed by wood combustion and iron/steel production. Soil contamination is also quite common but results in a lower rate of infiltration to the hydrocycle. Soil discharges of copper total approximately 86000 metric tons per year. Most of this in the form of solid copper waste from mine tailings and fly ash. Examples of industrial processes resulting in high content copper effluents are smelters, the plating industry, coating and refinishing processes and the textile industry.\textsuperscript{7}

Metal bio-availability is a much more important factor, in terms of environmental impact, than the total metal concentration.\textsuperscript{8} In aquatic environments, copper can exist in three broad categories: particulate, colloidal and soluble. This speciation is determined by the physico-chemical, hydrodynamic and biological characteristics of the water. Copper is most toxic in the free ion form prevalent at pH<6.3. Copper may become less available to living organisms due to the copper binding with particulates such as clay minerals. This is true whether it is a natural environment or effluent from an industrial process. In mining effluents for example, much of the particulate copper is trapped inside a particle matrix, preventing recovery. Using an efficient extraction process there should be relatively little metal left that can become available for uptake by organisms.

\textbf{1.3.1.1 Copper toxicity}

Copper is highly toxic to most aquatic plants. Inhibition of growth generally occurs at ≤0.1mg/L, regardless of test conditions and species; other effects such as reduced carbon uptake can occur at much lower concentrations. Copper is highly toxic to most freshwater and marine animals. LC\textsubscript{50}' s are generally less than 1 mg/L , again chronic sub-lethal doses reduce survival, growth and reproduction. The only metal which is consistently more toxic to aquatic plants than copper is mercury.

Copper is an essential element to the human body and the adult daily requirement has been estimated at 2.0mg. Large oral doses may, however, induce emesis and with prolonged exposure cause irreversible liver damage. Copper is not acutely toxic to
humans due to the intermediate coordinate character of copper between hard and soft acids. Hence copper seldom interferes with sulphur-containing proteins. There is no indication that copper is carcinogenic or mutagenic to humans.

1.3.2 Cadmium

Cadmium can enter the environment as a by-product of the primary non ferrous metal industry from impurities in the metal ores. It is also released into the environment by volatilisation of impurities present in fuels used in smelting and refining. The EC have set a drinking water limit of 5ppb (see Table 1) and a maximum permissible discharge limit of 200 ppb. The problems of incinerating waste containing cadmium can be solved using existing best available technology to capture more than 99% of incinerator fume emissions. When possible, rather than disposing of it as a waste, engineers have been able to utilise its unique properties for many important industrial applications e.g. paints, corrosion resistant coatings, alloys and above all (almost 70% of its use) in rechargeable nickel-cadmium batteries.

1.3.2.1 Cadmium in the aquatic environment

When present in soil or aquatic environments, cadmium can bioaccumulate. Industrial emissions are now tightly controlled due to the significant improvement in pollution control technology and to strict regulation and legislation, particularly in the metals industry. With regard to end-of-life disposal of products containing cadmium, it should be emphasised that, in many of its applications, cadmium is embedded in a product matrix and hence not directly bioavailable. In the very long term, the limited traces of cadmium eventually released from waste products will transform to a stable chemical form (oxide or sulphide) and so return to the original state found in nature.
1.3.2.2 Human effects

Cadmium mainly accumulates in the kidneys. At high levels it can reach a critical threshold and can lead to serious kidney failure. However, the most recent studies have shown that these effects are reversible, at least at low exposures, once the exposure to cadmium is reduced. Cadmium is present in cigarettes and therefore can be inhaled. This along with ingestion are the major routes for cadmium intake. Dermal exposure is also possible, the toxicity via this route appears to be relatively low. Problems occur when the size of the toxin is too large to filter through the kidney and it is then forced back into the liver, heart or lungs. If the liver is saturated, the toxins cycle elsewhere. Cadmium causes bone and joint aches and pains. These symptoms were first described in Japan, as part of the itai-itai ("ouch-ouch") disease. The disease was associated with weak bones that led to deformities, especially of the spine. Long term exposure can also strip calcium ions (Ca$^{2+}$) from the bones, weakening them greatly.\textsuperscript{10}

1.3.3 Mercury

Mercury occurs naturally in the environment. Approximately 2,700 to 6,000 tons of mercury are released annually into the atmosphere naturally by degassing from the Earth's crust and oceans. Another 2,000 to 3,000 tons are released annually into the atmosphere by human activities, primarily from burning household and industrial wastes, and especially from fossil fuels such as coal. Inorganic mercury enters the air from mining ore deposits, burning coal and waste, and from manufacturing plants. Mercury vapour is easily transported in the atmosphere, deposited on land and water, and then, in part, released again to the atmosphere. Metallic mercury is used to produce chlorine gas and caustic soda and also used in thermometers, dental fillings, and batteries. Mercury enters the water or soil from natural deposits, disposal of wastes, and volcanic activity. It is highly persistent in water, with a half-life greater than 200 days. Mercury from both natural and anthropogenic sources tends to bioaccumulate with increasing trophic levels.
1.3.3.1 Toxokinetics

Mercury in its ionic form (Hg$^{2+}$) is not an aggressive toxin. However, it tends to form methylated derivatives. Methyl mercury (MM) is the most toxic of the mercury species.$^{11}$ It tends to interact with SH and S-S groups and since these groups are ubiquitous and are crucial to the structure of proteins, this accounts for much of the toxicity of Hg.

Oral absorption of MM is nearly complete. Once absorbed MM can be converted to inorganic mercury, specifically the divalent cation, which then enters the oxidation-reduction cycle. Subsequently glutathione is believed to play a role in its reduction to metallic mercury. This oxidation of MM to the inorganic form in the brain can result in longer retention. The kidney has been identified as the organ with the highest mercury accumulation. The distribution of organic mercury involves the formation of complexes with proteins in the body. MM associates with water-soluble molecules (e.g. proteins) or thiol-containing amino acids because of the high affinity of the methylmercuric cation for sulfhydryl groups. Complexes of MM with cysteine or glutathione have been identified in blood, liver and bile.

Mercury, at high levels, may damage the brain, kidneys, and developing foetus. The nervous system is very sensitive to all forms of mercury. Methylmercury and metal vapours are more harmful than other forms, because more mercury in these forms reaches the brain. Exposure to high levels of metallic, inorganic, or organic mercury can permanently damage the brain, kidneys, and developing fetus. Short-term exposure to high levels of metallic mercury vapors may cause effects including lung damage, nausea, vomiting, diarrhoea, increases in blood pressure or heart rate, skin rashes, and eye irritation. The Environmental Protection Agency (EPA) has determined that mercuric chloride and methyl mercury are possible human carcinogens.

The average intake of mercury varies with location and diet. It may range from 10 µg to more than 500 µg, mainly depending on air contamination. Industrial cities and heavily sprayed farmland have the highest levels. The average overall daily intake is probably about 30-50 µg. Most humans can process at least that much daily without
any problems. Blood levels of mercury should be below 0.02 ppm, while hair levels may be higher, up to about 3-5 ppm. More than 5 ppm becomes a concern.

The three metals are viewed very differently by various regulating bodies as to their toxicity, see Table 2.

**Table 2 Regulatory Information**

<table>
<thead>
<tr>
<th>Agency</th>
<th>Parameter</th>
<th>Parameter</th>
<th>Cu</th>
<th>Cd</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO</td>
<td>Adult tolerable mean uptake (mg/kg body weight/day)&lt;sup&gt;12&lt;/sup&gt;</td>
<td>0.5</td>
<td>0.001</td>
<td>0.00047</td>
<td></td>
</tr>
<tr>
<td>FDA</td>
<td>Food additives permitted for direct addition&lt;sup&gt;13&lt;/sup&gt;</td>
<td>-</td>
<td>0.1 ppm</td>
<td>0.05 ppm</td>
<td></td>
</tr>
<tr>
<td>IBWA</td>
<td>Permissible level in bottled water&lt;sup&gt;14&lt;/sup&gt;</td>
<td>-</td>
<td>0.01 mg/L</td>
<td>0.001 mg/L</td>
<td></td>
</tr>
</tbody>
</table>

### 1.4 Wastewater treatment processes

Methods of wastewater treatment were first developed in response to the realisation of the toxicological effect of metal pollution in the environment. Early wastewater treatment 1900-1980 was concerned with removal of suspended material, biodegradable organics and pathogenic organisms.<sup>15</sup> Since 1980, due to the increase in scientific knowledge, several other toxic compounds were targeted for treatment including heavy metals. As a consequence while the early treatment objectives remain valid the required degree of treatment has increased significantly. There are several different industrial unit operations that can be applied to a wastewater stream in order to remove polluting metals depending on the quantity and type of pollutant and the aqueous streams chemistry.

### 1.4.1 Membrane processes

Membrane technologies can remove heavy metals such as zinc, copper, arsenic and antimony to a level that is allowable for discharge while adding the ability to reduce the total volume of water to be discharged. Much of the water that is reclaimed will have a purity high enough to be re-used in the industrial process. Membrane
processes involve the flow of a permeate through a porous media. Modern membranes even incorporate functional material or an electric current to enhance the separation of metals from solution. A membrane is involved in the reverse osmosis purification of water, however this can be a costly option.

1.4.2 Chemical Precipitation

Methods involving chemical precipitation are some of the easiest to perform and most effective for the removal of metallic species from aqueous waste. Most heavy metal cations form sparingly soluble hydroxides or carbonates when subjected to elevated pH conditions by the addition of caustic soda (NaOH) or soda ash (Na₂CO₃). Flocculant aided sedimentation followed by filtration can then be performed to remove the precipitated metal-bearing waste from the aqueous effluent. However, this method does not ensure total compliance for the various metals present in the waste stream, since all metal hydroxides do not completely precipitate at a single pH.

Table 3 Typical concentration levels obtainable through chemical precipitation

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Minimum achievable concentration [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.3</td>
</tr>
<tr>
<td>Chromium(VI)</td>
<td>0.05</td>
</tr>
<tr>
<td>Chromium, total</td>
<td>0.5</td>
</tr>
<tr>
<td>Copper</td>
<td>0.5</td>
</tr>
<tr>
<td>Iron</td>
<td>1.0</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.5</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The concentration of metal ions in solution needs to be of a significant level (>100ppm) for chemical precipitation and filtration to be an efficient method of removal. The most generally used method of removing metal contaminants is lime treatment to precipitate metals as hydroxides. However this method means that the metal is not normally recovered resulting in the need for sludge disposal. There are
many commercial chemical precipitants available that are designed to be suitable for a certain combination of process conditions.

A comparison of the minimum possible concentration attainable by precipitation (see Table 3) to the EU limits on drinking water (see Table 1) reveal that precipitation cannot in its own right render water suitable for human consumption. A secondary or polishing step would be required to further lower the concentration to the EU levels.

1.4.3 Electrochemical techniques

A process that decomposes a chemical compound into its elements or produces a new compound by the action of an electrical current. The electrical current is passed through an electrolytic cell so that reactions occur at the electrodes, e.g., a metal can be electroplated by electrolysis. The metal ions deposited on the cathode can be dissolved anodically to form a concentrated solution of metal ions, which can then be used for further processing to recover metals. This may be an attractive option for more valuable metals as recovery/recycling becomes an option.

1.4.4 Ion exchange

Ion exchange involves the ions in solution changing places with those of a similar charge associated with the solid. This process is reversible and is used extensively in removing metals from aqueous streams. Because the saturated resin can be regenerated a cascade of ion-exchange beds can be used in a continuous process. One of the disadvantages is the high equipment cost. Another is that it would require a secondary process in order to recover any precious metals. An ion exchange process would be recommended for streams of low metal concentration (<1000ppm) and with small quantities of competing ions to make it successful.

1.4.5 Adsorption

Adsorption is also a technology which has been examined for removing heavy metals. The use of solids for removing substances from either gaseous or liquid solutions has
been widely used since biblical times. This process, known as adsorption, involves nothing more than the preferential partitioning of substances from the gaseous or liquid phase onto the surface of a solid substrate. From biblical days when Moses in Marah enabled his followers to drink as “The waters were made sweet”\(^{18}\), to today’s thousands of applications, the adsorption phenomenon has become a useful tool for purification and separation.

The most popularly employed adsorbent has been granular activated carbon (GAC). Commercially produced carbons and those produced from waste products have both been proven to adsorb heavy metals. They are not, however, ideal in every situation as they have sometimes had limited affinity for certain pollutants and are an expensive adsorption process. With environmental concern growing in developing countries, there has been considerable research interest in alternative low-cost adsorbents, that might be considered as one-use materials. There is an extensive list of materials that have been tested and include: seaweeds, tyres, tea leaves and immobilised microbes.
2 History of Biosorption

2.1 Introduction

Biosorption describes the passive accumulation of a solute by inactive, dead, biomass. It differs from the active metabolic uptake of pollutants known as bioaccumulation. Many different researchers have shown that natural biomass can be utilised to remove metal ions from solution. This phenomenon has been attributed to several different mechanisms and processes, including ion exchange with functional groups present within the biomass structure. In particular, polysaccharides bearing carboxyl functional groups have been identified as dominant metal sorption sites. A range of materials have been evaluated for their metal sorption qualities either by direct use, or after processing steps. This chapter is a review of previous pertinent research.

2.2 Bacteria used in Biosorption

The realisation that bacteria can be used to bio-remediate metal polluted sources has opened up a large field of research. Bacteria are useful for a number of reasons, including their size, ubiquity, resilience to harsh environments and the ability to cultivate them under controlled conditions.

2.2.1 Bacterial Cell Wall

Bacteria maintain their shape and protect themselves from the exterior world through the cell envelope. This cell envelope is central to diffusion based sorption processes. Bacteria carry an envelope of water around their periphery, due to viscous forces, so that any substance in solution will first come into contact with the cell envelope. This envelope can be made of several different types of structures, the most important being the cell wall. The cell wall functionality is based upon its cellular make up and can include a wide variety of compounds and functional groups, e.g. lipids, lipoproteins, peptidoglycan, lipopolysaccharide, various proteins, phospholipids (which provide most of the negative charge at the surface of gram-negative bacteria).
sulphated polysaccharides and glycoproteins. These compounds contain, amongst others, groups such as phosphoryl and carboxyl.\(^{26,27}\)

Nuclear magnetic resonance experiments (NMR) have been conducted on the isolated outer membrane of *Escherichia coli* K12.\(^{28}\) This study conclusively proved that metal interaction (manganese, europium) occurs primarily with the phosphate groups present as part of the phospholipids and lipopolysaccharide. Interestingly only one of the three carboxyl groups in the lipopolysaccharide is actually available for reaction. Bacterial cell charge is strongly affected by pH, because the different ionogenic groups at the cell surface are susceptible to protonation/de-protonation reactions. Four different zeta-potential curves are observed:

- **Type 1** shows a linear increase in negative charge as the pH increases. This is attributed to adsorption of anions and/or desorption of protons from uncharged surface with increase in pH.
- **Type 2** display an exponential increase in negative charge with increase in pH. It is suggested that this reflects an anionically charged surface with carboxyl, sulphate and/or phosphate groups.
- **Type 3** indicate the surface is dominated by positive charge from acid to neutral pH then the surface rapidly changes to negative in alkaline pH. This is said to typify an amino dominated surface.
- **Type 4** show positive charge at low pH and change linearly to negative charge as the pH rises to 5. Above this value the negative charge stays almost constant until pH 8 when a sharp drop in negativity is observed at pH >9. A heterogeneous surface of amino and carboxyl groups is thought to be responsible for this observation.

A large quantity of information has been collected in the previous three decades on the sorption of metal ions by bacterial cells and suggestions of the mechanism responsible have been proposed. Both dead and alive bacterial cultures have been examined and non-viable biomass was found to sequester a greater quantity of metal ions on a weight basis.\(^{29,30,31}\) It has been suggested that active metabolising cells with
energised plasma membranes generate H⁺ ions that can compete with the metal cations. 29

2.2.2 Selectivity

Selectivity has been observed, whereby certain metals have been preferentially adsorbed over others by the outer membrane. Some metals are used in physicochemical processes, for example Ca²⁺ and Mg²⁺ 28 and so they may be expected to have preference as there are already active sites present designed to utilise them. The same researchers also postulated that there were three fundamental ideas to explain differences in metal selectivity by bacteria:

1. Binding depends on the free energy difference between the site bound cation and cation-water interactions.
2. The free energy of interaction from electrostatic forces.
3. The principal electrostatic forces were coulomb forces.

2.2.3 Mechanisms

Beveridge and Murray 26 proposed a two phase process. The first step is stoichiometric adsorption on the active site. The second is crystallisation/precipitation at the neutralised chemical site. This idea is supported by the evidence of mineral nucleation that has been shown by Shultze-Lam et al, 32 where the microbial cells actually lower the activation energy that normally retards spontaneous crystal nucleation.

Geesey and Jang 33 conducted research that showed the carboxyl group to be the predominant electron donor in polysaccharides. The lone pair of electrons on the oxygen atom of carboxyl groups are free to interact with metal ions to give the molecule charge neutrality. The oxygen atoms present in ether and hydroxyl groups can also form links with metal cations however they are much weaker. 34 It is also possible for coordination of metal cations by hydroxyl and oxyanion groups of an uncharged polysaccharide molecule. This mechanism decrees that there will be a reduction in affinity with increase in ionic radii of the metal.
The metal binding sites present in many bacteria are found to be the same, however their performance in metal cationic removal vary greatly as does the mechanism. The conformation of the molecule is another crucial parameter affecting metal adsorption properties and the flexibility of active site carrying molecules to “wrap” around target cations.

2.3 Fungi as biosorbents

The potential of fungal biomass in removing metal ions from wastewater was first recognised by Zajic and Chiu in 1972. Fungal biomass actively uptake trace amounts of several metals that are required for growth. They can also, however, accumulate large quantities of other heavy metals that are not macro-nutrients. Fungi and yeast are used in a wide variety of industrial fermentation processes and as such are available as cheap by-products for wastewater treatment and the recovery of precious metals.

There are two main modes of metal ion uptake by fungi. Active uptake or bio-accumulation occurs in living cells and is dependent on cell metabolism. The second mode is passive adsorption of metal ions onto the cell walls and extra cellular material.

Metal uptake is also facilitated by the production of metal binding proteins such as metallothionein and the presence of chitin and chitosan. Living and dead cells have been evaluated for their metal removal ability and dead cells have proportionally higher uptake. The absence of toxicity limits plays a large part in the superiority. The pre-treatment of the dead cells can change the cell surface characteristics and result in an increase in metal uptake.

A particularly successful use of fungi in heavy metal removal was realised by Tsezos et al who removed uranium from wastewater with immobilised particles of *Rhizopus arrhizus* and maintained 100% uptake (0.21mmol/g) after several cycles of adsorption and desorption.
Yan and Viraraghavan\textsuperscript{37} studied the uptake of \textit{Mucor rouxii} following several different pre-treatments. One treatment involved the formation of non-viable biomass by autoclaving the material. When compared with the live material it was shown to adsorb less of the metal ions studied. This was attributed to loss of intracellular (active) uptake. A similar reduction in cadmium uptake by dead biomass was demonstrated with \textit{Rhizopus oryzae}.\textsuperscript{38} These results contradict the vast majority of biosorption research, however there are several possible explanations: active transport maybe crucial to the sorption mechanism. The processing of the material might mean that it incurs denaturation either by heat, pH or mechanical attrition and subsequently rendering active sites useless. Whistler and Danie\textsuperscript{39} reported similar reductions in metal uptake and attributed the reduction in uptake to a loss of amino functional groups on the fungal surface as they are converted to carbonyl groups by the Maillard reaction.\textsuperscript{40} Malik\textsuperscript{4} compared the uptake of biomass samples freeze dried and oven dried and reported little difference in their sorption performance. Our own studies have shown similar results for differently dried samples of \textit{A. filiculoides}. However the consistency of the material was found to differ markedly. The freeze-dried sample was light and fluffy compared to a hard compact oven dried sample. The difference can be explained by the freeze drying technique selectively subliming the frozen water molecules and retaining the physical characteristics of the plant. The oven dried sample allowed cellulosic filaments to stick together, hence reducing accessibility of active sites.

Cadmium biosorption by live fungal biomass was found to be primarily a proton competitive reaction.\textsuperscript{41,42} The charge on the fungal surfaces was predominantly negative in the working pH range (3-10). A direct correlation was found between the fungi with the most negative surface and the highest sorption of cadmium. These findings are in agreement with our own observations of cadmium uptake by the water fern \textit{A. filiculoides}. 


2.3.1 Live Fungal Biosorption

2.3.1.1 Modelling

Modelling of live fungal biosorption has been carried out using several formulae, Langmuir, Freundlich, BET and Scatchard.\textsuperscript{43,44}

The majority of the uptake of metal ions was due to the passive mode, with ion-exchange and complexation reactions can occur on the surface of the cells with functional groups present. Some functional groups believed to be involved include, carboxyl, amine, amide, hydroxyl, phosphate and sulphydryl.\textsuperscript{45,23}

The mechanism of intracellular uptake is not fully understood\textsuperscript{46} but several researchers have shown active uptake of metals such as copper and cadmium.\textsuperscript{47,48}

2.3.2 Kinetics

Kinetics of dead fungal biosorption is reasonably fast. Brady et al\textsuperscript{49} showed that equilibrium was reached in 4-6 hours. pH has also been shown to affect the kinetics of metal uptake\textsuperscript{50} with an increase in pH resulting in a higher rate of sorption.

Tobin et al\textsuperscript{51} linked the sorption capacity of heat treated \textit{R. arrhizus} to the ionic radii of the metal ions. Interestingly the sorption capacity was higher for metals of higher ionic radii, converse to the findings related to bacteria discussed earlier in this chapter.

One of the main reasons for pretreating biomass is to produce sorbents with superior metal removal capacities. A pre-treatment that has been employed on fungi biosorbent material is NaOH washing. In the case of \textit{Aspergillus niger},\textsuperscript{52} NaOH is thought to de-acetylate the chitin present to form chitosan. The metal sorption performance of chitin and chitosan has been tested by several researchers and it is observed that chitosan offers the higher affinity for many metal ions.\textsuperscript{53}
The biosorption of metal ions was observed to be pH dependent with optimum uptake in the range 4-5 and substantial reduction at pH of 2.5. pH changes the speciation and availability of the metal ions present and also the chemical state of the functional groups responsible for biosorption. It has been shown that protons can act as competitive inhibitors. Buffering the wastewater or alternatively preventing the release of hydrogen ions that occur simultaneously with adsorption of the metal ions will result in a greater metal uptake. This was certainly observed by Fourest when using biosorbent in calcium form.

2.3.3 Selectivity

Selectivity is an important parameter. The separation of a specific ion from a solution that may contain a mixture of different competing ions is often the objective of a viable biosorption process. Yakubu and Dudeney discovered a reduced uptake of uranium in the presence of copper, zinc and iron. This is unsurprising since uranium is a large ion and the smaller transition metals can diffuse faster and have higher affinity for the functional groups. However in the selectivity order they published Fe>>U>Cu>Zn it is surprising that uranium is seemingly preferred over copper and zinc. I suggest that the reason for this apparent anomaly is that there are separate mechanisms involved for the sequestration of the transition metal ions and that of the uranium. A precipitation/complexation reaction has been proposed elsewhere. For Penicillium the selectivity series is Fe>Cu>Zn>Ni>Cd, Pb>U. This contrasts with Rhizopus which also showed a preferential position for uranium: U>Pb>Cd>Zn>Cu. Adsorption reactions are generally exothermic. It is therefore possible to increase the extent of the adsorption process by reducing the temperature. Uranium adsorption, however, does not follow this trend and is probably endothermic.

Anions have also been shown to affect the biosorption of metal cations. Biosorption is reduced in the presence of ethylenediamine tetraacetate (EDTA), sulphate, chloride, phosphate, carbonate, glutamate, citrate and pyrophosphate. In these cases it is likely that the anions consequently in solution may bind to the metals prior to contact with the bio-material and produce modified species that are difficult to adsorb. EDTA is known to possess a very high affinity for metal ions. Other anions may also affect
the speciation of the metal in solution. Addition of anions to the solution can easily lead to the formation of neutral complexes with the metal cation. This removes any electrostatic interaction the target metal may have had with the solids negative surface.

Successful regeneration of sorptive materials is an important selection criteria. Copper and cadmium, amongst other metal ions, was successfully removed from biosorbents using a variety of eluants. Zhou and Kiff used 0.1M HCl to regenerate *Rhizopus arrhizus* and it suffered no loss of performance in subsequent operations.

Galun et al. used *Penicillium* biomass and actually recorded an increase in mycelial uptake following NaOH eluting treatment. It is likely that part of this could be due to the NaOH actually washing out the alkali-soluble cell components that are not involved in the biosorption reaction and therefore reducing the non-participating mass.

The technique of immobilising fungal biomass on a rigid structure has attracted substantial research as a way of avoiding the low mechanical strength of the material. The separation of biomass and treated effluent can be difficult with small particles of low density. All manner of materials have been used as substrate to attach the particles of biomass, e.g. sand, paper, polysulphone, alginate and inorganic compounds. *R. arrhizus* was immobilised on several different solids, namely alginate, polyacrylamide, epoxy resin and polyvinyl-formal. In general immobilised biosorbents have a lower capacity than a sample of the pure material, e.g. *R. arrhizus* attached to polyvinyl-formal possesses only 85% of the uptake of the pure material.

### 2.3.4 Mechanism

Tobin et al. reported that uptake of several metal ions was not influenced by the ionic charge or by electrostatic attraction but was only proportional to the ionic radius. The case for *Penicillium* was converse in that it was inversely proportional to ionic radii. Tobin highlighted the active role of functional groups e.g. carboxyl and phosphate.
Chitin and chitosan that are present in many fungi have been isolated as very effective metal binding compounds. During transition metal ion sorption trials on these compounds, Muzzarelli et al. discovered that the free radical was indeed associated with the chitin nitrogen.\textsuperscript{63} This makes perfect sense, since the lone pair of electrons on the chitosan's nitrogen can form co-ordinate bonds. This is the most likely mechanism as the only other functional group present is the hydroxyl which can have mild electrostatic attraction for cations.

Several mechanisms for metal ion uptake by \textit{Rhizopus arrhizus} were suggested by Volesky.\textsuperscript{64}

- Particulate ingestion or entrapment by flagellae or extracellular filaments.
- Active transport of ions.
- Ion exchange.
- Complexation.
- Adsorption.
- Inorganic precipitation.

One characteristic of fungi is the presence of chitin. This and its deacetylated derivative, chitosan, are highly effective at chelating metal ions.\textsuperscript{53,65,66,67,68} Chitin acts as a carbohydrate and nitrogen source and can associate with proteins to form glycoproteins.\textsuperscript{68} It may be possible to artificially enhance biomass surfaces with chitin or chitosan although this would add to the incurred cost. Maruca\textsuperscript{66} suggested that the metal uptake mechanism of chitosan involved nucleation and growth of nodules on the polymer surface. Simple ion adsorption on the chitin/chitosan surface does not appear to be the dominant mechanism since SEM/EDAX analysis shows the presence of metal-containing aggregates on the polymer.
Tsezos\textsuperscript{69} suggested two processes for the uptake of thorium by \textit{R. arrhizus}. In one process, thorium and the nitrogen of the cell wall chitin form a co-ordination complex. The other mechanism involves the adsorption of hydrolysed thorium ions by the outer layers of the \textit{R. arrhizus} cell wall.

Tsezos also analysed the uranium capacity of \textit{R. arrhizus}\textsuperscript{70} and identified three mechanisms:

- IR spectroscopy found uranium associated with nitrogen of chitin monomer. Uranium co-ordinates to amine nitrogen and is retained within the cell wall of the mycelium.
- Hydrolysis of this complex and precipitation of uranyl hydroxide in the cell wall.
- Adsorption of additional uranium by the chitin network close to that complexed by the amine.

### 2.4 Algae as biosorbents

In general, the mechanism of biosorption is based on a number of metal-binding processes taking place with components of the algal cell wall. The algal cell wall can
reversibly biosorb metals and thus functions in a similar way to an ion-exchange resin. Thus, the biosorption mechanism can be considered as being dependent on the composition of the algal cell wall. The cell wall structure and chemical composition of most algal species have not yet been described in detail. There is a wide variation in the chemical composition of eukaryotic algal species (both micro and macroalgae), the only cell wall component that is common to all the eukaryotic algal divisions being cellulose. Algal cell walls can be made up of further polysaccharides; mannan, xylan, alginic acid, chitin etc. These components along with proteins can provide acid binding sites such as amino, amine, hydroxyl, imidazole, phosphate and sulphate groups, see (Fig. 2 and Fig. 3).

\[
\begin{align*}
\text{H-N:} & \quad \text{Amine} \\
\text{C=O} & \quad \text{Carboxyl} \\
\text{N-C=O} & \quad \text{Amide} \\
\text{C-C=N} & \quad \text{Imidazol}
\end{align*}
\]

Fig. 2 Functional groups present in proteins

Recent researchers have shown that judicious tailoring of the surface of carbonaceous materials has been beneficial in the sorption of copper. An important study by Biniak has brought to our attention the features of the adsorbents surface that are related to copper sorption. They did not find any relationship between surface area and uptake of copper ions and hence concluded that the crucial parameters were the equilibrium pH and the nature and quantity of acid functional groups on the solid surface. They have proposed some adsorption schemes for their carbons which could be applied to similar solids with the relevant surface functionality.
One carbon was annealed in a vacuum at high temperature to produce a solid with reduced functionality was found to react to a certain extent with the copper in solution. The authors are proposing carbonium ion reactivity and an oxidation of the carbonyl group.

The oxidised carbon has carboxyl and hydroxyl groups that the researchers propose interact with the copper cation.

The carbon that they have treated with ammonia has gained various amino groups. These are analogous to the amino groups that might be present within protein in biomass samples. These amino groups the authors suggest partake in coordination reactions with the copper ions.

Two comments that may influence their results are that they used the sulphate form of copper and the complex speciation that exists with this ligand is not explained. The pH of the initial solution used in sorption experiments was considerably lower for the oxidised sample than the other two, almost certainly affecting the uptake they reported.

2.4.1 Mechanism

It has been reported that the biosorption mechanism does not involve Van der Waals forces at the cellulose network of the cell wall, thus both ionic charge and covalent bonding are involved in the metal biosorption process. It is thought that proteins and polysaccharides are the major components responsible for biosorption. Covalent bonding is expected with amino and carboxyl groups and ionic charge bonding with carboxyl and sulphate groups. Because of the wide variety in algal morphology and chemical composition the algae can have very different biosorption properties.

Studies by Stary and Kratzer with the microalga Scenedesmus obliquus indicated that the cell wall behaved like a weakly acidic cation exchanger containing various cell wall ligands with different exchange capacities. Further information regarding metal biosorption by algae has been obtained through NMR studies which indicated
that carboxyl groups were responsible for the biosorption of cadmium by the microalga *Stichococcus bacillaris*.

Glooschenco studied mercury sorption by the marine diatom *Chaetoceros costatum* and concluded that the most important process for mercury uptake was surface adsorption. Suggestions that a metallothionein-like substance might be responsible for sequestering heavy metals in the diatom were proposed. The blue-green algae *Chlorella pyrenoidosa* was found to have high mercury sorption capacity at low solution pH. Cadmium uptake by the herbivorous marine crustacean *Pseudodiaptomus coronatus* has been studied by Sick. Evidence of protein-metal interaction has been suggested. The protein which is known to complex with cadmium is rich in cysteine indicating a possible interaction between cadmium and thiol (SH) groups. The thiol group of cysteine can combine via a disulphide bond with another cysteine to make cystine. This can hold proteins together via a crosslink. Dialysed solutions of carageenan were prepared and interaction of cadmium with polysaccharides indicated that binding occurred mainly due to the electrostatic interactions between the sulphate ester groups and the metal cation. In another study, a cadmium binding component with a molecular weight of 12,800 Daltons was detected in lysates of *Chlorella pyrenoidosa*. Evidence that the metal binding component was a metallothionein-like protein was based on the molecular size, heat stability, and the ability to incorporate $^{35}$S along with $^{115}$Cd.
Fig. 3 The 20 common amino acids
Holan\textsuperscript{82} has looked at the biosorption of cadmium by several seaweed biomass types. Both native and crosslinked biomass were investigated and compared with commercial ion exchange resins. The biomass \textit{A. nodosum} demonstrated high equilibrium uptake of cadmium from aqueous solutions. The native biomass was found to be rather soft, with a tendency to disintegrate. In order to improve the stability and mechanical properties of the biomass, crosslinking of \textit{A. nodosum} biomass proved to be promising. The influence of drying procedures on subsequent metal sorption indicated that drying temperatures below 100°C did not affect the biosorbent performance. Solution pH was found to be very important in cadmium biosorption by \textit{A. nodosum}. Cadmium uptake fell from 1.8 mmol g\textsuperscript{-1} at pH 4.9 to less than 0.3 mmol g\textsuperscript{-1} at pH 2. Cadmium sorption was influenced by the type of anionic species in solution. Cadmium uptake from cadmium sulphate solution (0.9 mmol g\textsuperscript{-1}) was less in comparison with cadmium sorption from cadmium acetate solution (1.1 mmol g\textsuperscript{-1}).\textsuperscript{82}

While cadmium uptake by native biomass of \textit{A. nodosum} and \textit{S. natans} was found to be rather encouraging, the dried biomass was found to swell upon rewetting. In order to overcome this problem, an attempt to establish a simple cost effective granulation of \textit{A. nodosum} biomass by crosslinking was investigated by Holan.\textsuperscript{82} The most promising results were obtained using divinylsulfone and formaldehyde under acidic conditions. \textit{A. nodosum} crosslinked with divinylsulfone resulted in slightly decreased metal uptake than the native biomass. This could be attributed to the release of valuable polysaccharides that may have played an important part in metal sequestration. Crosslinking with divinylsulfone or with formaldehyde-urea mixtures may not be considered as pure crosslinking due to the tendency of resin formation that could stabilize the biomass. While the formaldehyde-urea mixture was found to improve the mechanical stability of the material, resin formation blocked or masked the functional groups responsible for the sequestration of the metal.

Aldor\textsuperscript{83} investigated the desorption performance of cadmium laden \textit{Sargassum fluitans}. In addition to various inorganic acids, the complexing agent Na\textsubscript{2}EDTA proved a successful eluant capable of regenerating the cadmium laden biomass. NaCl, CaCl\textsubscript{2} and NH\textsubscript{4}Cl showed good cadmium elution properties. An exchange
equilibrium between protons in solution and cadmium on the biomass was found to exist. Aldor\textsuperscript{83} demonstrated an almost stoichiometric exchange between cadmium and protons during metal desorption.

This worked well but now the copper ions are bound to another substance. Desorption of biomass columns has also been tackled by Aldor et al.\textsuperscript{83} They found that dilute HCl was the best choice overall on grounds of performance, cost and unwanted oxidation.

The shape and structure of the adsorbing material is of great relevance to its performance. Holan\textsuperscript{84} reported that the difference in metal sorption performance of \textit{F. vesiculosus} and \textit{A. nodosum} was linked to the morphologically different architecture of their algal tissues. The thallus of the former consists of parenchymatous cells with apical meristems\textsuperscript{85} instead of predominantly filamentous architecture of the latter. The parenchymatous cells in \textit{F. vesiculosus} were responsible for the increased surface area available for metal sorption.

Seaweeds have been tested with little success in cross flow filtration.\textsuperscript{4} Problems with expansion and lack of mechanical strength have so far prevented further development.

\section*{2.5 Water ferns as biosorbents}

\textit{Azolla} species is an aquatic fern (pteridophyte), floating on the water surface of flooded rice fields, small ponds, and canals as illustrated in Fig. 5. Its size is 1-5 cm, see Fig. 4. \textit{A. filiculoides} has a symbiotic relationship with a blue-green algae that generates nitrogen. It can, therefore, grow on water deficient in nitrogen compounds, and is high in nitrogen and protein. With no limiting factors such as water surface or nutrients it can double its biomass within 2-3 days. Because phosphorus diffusion from soil to water is slow, field population of floating \textit{Azolla} is generally deficient in phosphorus and could well be employed as a successful living phosphorus remover from waste streams. Because of its high nitrogen content \textit{A. filiculoides} has been used as a green manure for wetland rice in northern Vietnam, and central to southern China for centuries.
A. filiculoides has been evaluated as a biosorbent for copper cadmium and lead, untreated and also milled and sieved as non-immobilised and immobilised in three different ways:\(^8^6\)

- Suspension with epichlorohydrin.
- Matrix with tetra ethyl orthosilicate (TEOS).
- Matrix with tetra methyl orthosilicate (TMOS) within a column.
The research showed that the epichlorohydrin immobilised *A. filiculoides* was more efficient per unit weight of biomass at removing copper in column studies. However, using a batch process, mobile biomass was the most efficient. Regeneration of the biomass enables it to be re-used and possibly the metal can also be concentrated and recovered. Tsezos used EDTA disodium dihydrate to desorb copper ions from a column containing 1g of milled and sieved *A. filiculoides*.

A conclusive mechanism for metal ion adsorption has not yet been established. There are probably several mechanisms and these are dependent on the structure of the biomass, the nature of its functional groups and the physical properties of the metal ion(s) in question. Kinetic studies of *A. filiculoides* show that more than one mechanism is involved in the binding process. The first phase is suggested as binding to the biomass surface and the second as an intraparticle sorption.

*A. filiculoides* has been used in a living "bioreactor". The method of metal removal from water using this methodology is different. It was found that metal ions are adsorbed in the plant through the rhizoid and accumulate in the leaves. However, the metals gradually poison the plant and so metal capacity is much lower than with dead material. It was suggested that the metal bound in the biomass can be recovered when the biomass is burnt, where the combustion is probably self-sustaining, thus no external energy would be required.

Zhao obtained adsorption isotherms for chromium and found that the data for *A. filiculoides* obeyed the Langmuir model. For various algae, Aldor et al found a slowly rising linear curve in contrast to the hyperbolic Langmuir curve, proving an advantage for desorption. There are significant benefits of controlling the solution environment for metal uptake. For example, temperature has little effect on the uptake of lead on *A. filiculoides* but change in pH is extremely significant.

Jain et al tested *Azolla pinnata* and compared it with *Lemna minor* for the metal uptake of zinc and lead. They found that *A. pinnata* grew faster than *L. minor* in both metal solutions and reduced the metal ion concentration to a greater extent. They found the growth rate of both plants was higher in zinc solution than in lead solution.
As would be expected, lead at high concentrations was toxic to plant growth. Surprisingly, however, the low concentration lead solutions were actually found to aid plant growth. The lead or zinc concentration within the plant tended to be higher when they were subjected to the metals separately rather than simultaneously. The plants were saturated in 8–10 days and after saturation was reached there was no further adsorption. When the plants were needed for metal ion adsorption analysis they were decomposed to ash for 8 hours in a furnace at 800°C.

Several researchers have used *A. filiculoides* as a living heavy metal remover.\(^{91,92}\) However, metal uptake is greatly enhanced by using the non-viable biomass of the plant.\(^{86}\) Duncan et al.,\(^{88}\) used freshly harvested *A. filiculoides* dried at 60°C and ground to a specific particle size range. This, however, ignores the fact that those metals already sequestered by the plant from its natural environment will not be washed out and heating may denature some of the more sensitive structure of the plant. This methodology also ignores the fact that there are two distinctly different segments of the plant with potentially quite different attributes. We, therefore, performed an elementary experiment to discover the overall ion exchange capacity of the two distinct sections of the plant, i.e., the leaves and the roots (this is discussed in Chapter 5).

### 2.5.1 Mechanism

In a study to identify the physicochemical factors that affect cadmium removal, Huang and Smith\(^{93}\) reported cadmium uptake on 4 activated carbons of differing pH\(_{\text{PZC}}\). An increase in uptake with increase in pH was reported and the samples with the lower PZC were found to be more effective. Is was not mentioned in the paper but we propose that the lower PZC is due to a higher concentration of acidic groups on the surface of the carbon that in turn is responsible for cadmium sorption.

Commentary from Radovic with regard to carbon materials\(^{93}\) discusses the work of Bhattacharyya and co-workers.\(^{94,95}\) They studied the effect of a chelating agent in solution on cadmium uptake by a commercial activated carbon. The characterisation of the material was in no way complete and they postulated the adsorption processes
in the absence of EDTA was due to ion exchange of the carboxylic acid groups with the positive cadmium ions. In the presence of EDTA the cadmium species present was a negative complex that had electrostatic attraction to the positively charged surface groups.

Using a complexing agent might be beneficial if one of three possible scenarios occur:

1. The complexing agent attaches to the surface of the solid and can subsequently interact with the adsorbate.
2. The complexing agent binds with the adsorbate molecule making it more easily adsorbed on the surface of the solid.
3. The complexing agent binds to other competitive adsorbates leaving the surface available for reaction with the target molecule.

2.5.2 Other uses of Azolla

Multiple uses of Azolla have been explored, e.g. it can be employed as a biological herbicide. Covering the water surface reduces light penetration to the soil beneath, resulting in the depression of weed germination. Thus, growth of Azolla reduces occurrence of aquatic weeds in flooded rice fields. Azolla has been used as an animal feed for pig, duck, and fish. It has a high protein content (20-30% on dry weight basis). Its protein is lacking in methionine and cysteine and therefore a combination with cereals is needed.

Many benefits of using Azolla are recognized. As a result, the integrated use of Azolla with rice and fish farming has been developed at Fujian Academy of Agricultural Sciences, China. The integrated approach can increase a farmers income, while reducing the use of pesticides and fertilizers, and, consequently, environmental pollution. Integrated use was also developed by a Filipino farmer. He combined Azolla culture with rice and vegetable culture, and the rearing of pigs and ducks. The excreta from pigs and ducks were introduced to a house scale biogas plant and the effluent from biogas plant was returned to wetland rice fields with Azolla. Rice-duck culture is now common practise in organic rice farming.
### Table 4

A comparison of copper, cadmium and mercury uptake by different biosorbents

<table>
<thead>
<tr>
<th>Organism</th>
<th>Group</th>
<th>Copper uptake [mmol/g]</th>
<th>Cadmium uptake [mmol/g]</th>
<th>Mercury uptake [mmol/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger</td>
<td>Fungi</td>
<td>0.0343</td>
<td>0.0343</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus</td>
<td>Fungi</td>
<td>0.1160</td>
<td></td>
<td>0.2751</td>
</tr>
<tr>
<td>S. cerevisae</td>
<td>Fungi</td>
<td>0.0397</td>
<td>0.1698</td>
<td>0.4749</td>
</tr>
<tr>
<td>S. filipendula</td>
<td>Algae</td>
<td>0.6059</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. nodosum</td>
<td>Algae</td>
<td>1.304</td>
<td>1.004</td>
<td></td>
</tr>
<tr>
<td>L. flavicans</td>
<td>Algae</td>
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<td>1.404</td>
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<td>Algae</td>
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<td></td>
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<tr>
<td>S. fluviatilans</td>
<td>Algae</td>
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<td></td>
<td>0.81100</td>
</tr>
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<td>A. filiculoides</td>
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<td>P. aeruginosa</td>
<td>Bacteria</td>
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<td></td>
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</tr>
<tr>
<td>Carboxymethyl</td>
<td>Icellulose</td>
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</tr>
<tr>
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<td></td>
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</tr>
</tbody>
</table>
\[ \begin{array}{|c|c|c|} \hline
B. subtilis & Bacteria & 0.36^{106} \\
E. coli & Bacteria & 0.10^{106} \\
A. nicotianae & Bacteria & 0.60^{107} \\
B. licheniformis & Bacteria & 0.19^{107} \\
P. aeruginosa & Bacteria & 0.36^{58} \\
\hline
\end{array} \]

2.6 Kinetics

The difference in sorption rate can be directly related to porosity, external mass transfer and intraparticle diffusion. Peel et al\textsuperscript{108} claim that for activated carbon, metal sorption occurs in two stages. The first stage involves rapid sorption in macropores where 70 – 90\% of metal ions are removed. In this first stage the controlling step is film diffusion. The second much slower phase involves sorption in micropores where intraparticle diffusion is the limiting step.

Mohan et al\textsuperscript{109} used an activated carbon derived from fertiliser waste for mercury (II) removal. Maximum uptake was reached after 3 hours. Their experimental range was pH 2 (below which they were concerned with H\textsuperscript{+} ion competition) and 6.5 above which they mention mercury precipitation. They show results that are pH dependent but converse to the findings of this thesis such that sorption increased with decreasing pH.

They also observed linear isotherms for low concentrations of mercury in solution. They have a marked difference in kinetics of uptake by particles of different diameter. They make the logical claim that this indicates pore diffusion is important.
3 Experimental Procedure

A summary of the adsorbent preparation is outlined together with a list of chemicals and equipment used. The methods of physical and chemical characterisation of the adsorbents are detailed. The experiments performed to compare performance and kinetics are explained. Procedures that aim at enhancing the heavy metal uptake of the biomass are described within.

3.1 Chemicals

Aldrich Chemicals, USA, supplied volumetric standard solutions of sodium hydroxide and hydrochloric acid. Potassium iodide and gelatine were supplied by Fisons, UK, and potassium hydrogen phthalate from AnalR, UK. Copper, cadmium and mercury solutions were prepared using laboratory grade reagents: CuCl₂·2H₂O (BDH Chemicals Ltd., Poole, England), CdCl₂·2.5H₂O (May and Baker Ltd., Dagenham, England) and HgCl₂ (Fisher, UK). Chitosan flakes were manufactured by Fluka BioChemika. L-cysteine hydrochloride and sodium alginate were supplied by BDH, Poole, England. Fisher UK also supplied glutaraldehyde solution (50%), casein and calcium chloride salt. Glacial acetic acid was purchased from Sigma. The pectin used was Hercules GENU LM 5CS and the soya protein concentrate was manufactured by RHM.

3.2 Adsorbents

The seaweed varieties used in this study belong to the botanical classification of Phaeophyceae (brown seaweeds). Native seaweed varieties of Ascophyllum nodosum, Lessonia flavigans, Durvillea potatorum and Laminaria hyperborea were supplied by Monsanto plc, Girvan, Ayrshire, Scotland, UK. In addition, algin extracted samples of Ascophyllum nodosum and Lessonia flavigans were provided, which had been prepared in accordance with Monsanto plc standard procedure (included as addendum at the end of the chapter). The dry samples were processed in an electric blender to obtain small particles.
A. filiculoides was received from The University of Liverpool, Department of Biological Sciences. Fresh samples were extensively rinsed with deionised distilled water (DDW) to remove any epiphytes, small animals and soil before being frozen in liquid nitrogen and the leaves separated from the roots. The leaves were then selected as they had been shown to possess a significantly greater total ion exchange capacity than the roots (see Chapter 5 Table 1). The leaves were then ground into particles using a pestle and mortar.

Three types of commercial ion exchange and chelating resins were used in this study. Namely: Purolite S-930 (iminodiacetic), Purolite S-920 (thiouronium) and Rohm and Haas GT-73 (thiol). The beads were ground into particles using a pestle and mortar to obtain particles in the size range 170-210µm.

Following the differing initial steps, the final stages of material preparation were the same. The particles were dry sieved to 170-210µm. The sorbents were then column washed with 100 bed volumes (BV) of 0.1M hydrochloric acid at 10 BVh⁻¹ to remove any salts present (sodium, potassium, magnesium and calcium) or other cations and to convert any acidic groups into hydrogen form. Subsequently the samples were rinsed with DDW until the pH leaving the column was greater than 4. This was to remove remaining unassociated hydrogen ions.

Purolite S-930 and S-920 were air-dried. Rohm and Haas GT-73 was stored under water to prevent any oxidation of the thiol groups. Several samples of the resin were paper dried and then weighed to calculate the dry mass of GT-73 used in experiments. The samples were then placed in an oven at 50°C until constant weight and re-weighed. The dry weight/wet weight ratio of 0.54 has been used in all subsequent calculations. The biological material was freeze-dried using an Edwards Modulyo freeze drier. The freeze drying technique involves freezing the material solid and then subjecting the material to a low pressure and a controlled heat input. Under these conditions the water content, in the form of an ice matrix, is selectively removed via sublimation. This method retains the essential characteristics of the material because the solid constituents are locked into this matrix during the entire dehydration and
cannot interact or compress. An alternative, thermal drying, may cause degradation of the cellular structure of the biological material and may adversely affect metal sorption.

3.3 Sample analysis

Copper and cadmium concentrations were measured using a Varian SpectraAA-200 atomic adsorption spectrophotometer (see Fig. 6), in flame mode. Copper was measured at 324.7nm, cadmium at 228.8nm for concentrations up to 3.0 mg/L and 326.1nm for concentrations of 20 to 200 mg/L. Sensitivity analysis was performed on the spectrophotometer by analysing metal solutions ten times and recording the standard deviation (See Table 5). The solutions were chosen to coincide with the experimental range and encompassed both cadmium calibration ranges.

Table 5 Sensitivity analysis performed on the Varian SpectraAA-200

<table>
<thead>
<tr>
<th>Metal</th>
<th>Calibration</th>
<th>Standard Deviation</th>
<th>Confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>10 ppm</td>
<td>0.058</td>
<td>± 2%</td>
</tr>
<tr>
<td>Cadmium</td>
<td>3 ppm</td>
<td>0.038</td>
<td>± 4%</td>
</tr>
<tr>
<td>Cadmium</td>
<td>200 ppm</td>
<td>4.26</td>
<td>± 6.5%</td>
</tr>
</tbody>
</table>

Mercury analysis was performed using the method reported by Ramakrishna et al. using rhodamine 6G. The coloured solutions were analysed using a UV/VIS spectrophotometer (Perkin Elmer lambda 12) at 575nm using 10mm quartz cells. A sample was analysed three times at the beginning of each analysis to confirm a consistent reading.

In all instances blank samples using the same solutions, under the same conditions, but without adsorbent were prepared for comparison.
3.4 Total Ion Exchange Capacity

A series of experiments were conducted to determine the total sodium exchange capacity of *A. filiculoides* leaves and roots. 50mg of adsorbent was placed into a conical flask containing 25ml of 0.1M volumetric standard sodium hydroxide. This was agitated at 300 min\(^{-1}\) for 48 hours. The solution was filtered and 5ml of the sample was diluted with 5ml of distilled water and back titrated with 0.1M HCl using methyl red as the indicator.
3.5 pH Titrations

15 ml of a 0.1M NaCl solution was added to 50ml Erlenmeyer flasks. The solution pH was varied by adding a total volume of 5 ml, 0.1M NaOH, HCl and/or distilled de-ionised water. Then, 50mg of adsorbent particles, <90μm, were added to the flasks. The samples were stirred for 48 hours to attain equilibrium at room temperature. The initial (before the addition of adsorbent) and final pH were measured. Blank control samples, were run under the same conditions for comparison.

3.6 Zeta Potential

50mg of neutrally bouyant particles, <90μm, were added to 50ml Erlenmeyer flasks. A range of pH across the samples was obtained by the addition of 0.1M NaOH and HCl to a total volume of 20ml. The samples were agitated by an orbital shaker at 300 min⁻¹ for 48 hours to achieve equilibrium. The electrophoretic mobility of the equilibrated samples was measured using a Malvern Instruments Zetasizer 3000HSA.

3.7 Scanning Electron Micrographs (SEM)

Samples of dried biomass were gold sputtered and glued to aluminium platforms to enable electron microscopy pictures of A. filiculoides to be taken using a Cambridge Stereoscan 360 operated at an accelerating voltage of 10 kV. The images presented were provided by the Institute of Polymer Technology and Materials Engineering (IPTME) Loughborough University.

3.8 Energy Dispersive Spectroscopy (EDS)

The stereoscan 360 was equipped with an energy dispersive spectroscopy system. Focusing electrons at the surface of certain areas of the sample will displace electrons in the shells of the elements. A discrete quantity of energy is given out as electrons fall back into the vacant orbitals revealing the elements present. Two areas of the surface of HgCl₂ A. filiculoides were compared, with and without precipitate.
3.9 X-Ray Diffraction (XRD)

The crystallography of the precipitate on the surface of the adsorbent was analysed using a Bruker D8 X-ray diffractometer. A copper X-ray tube was used with a 1° divergence slit, 1° scatter slit and a 0.2 mm receiving slit. Prior to analysis all samples were dried for 24 hours and ground into a fine powder using a mortar and pestle. Samples were specifically analysed in the range 18-25° with a step size of 0.02° at 0.11 steps per minute. As a check, samples were also analysed across the entire range of wave angles.

3.10 X-Ray Photoelectron Spectroscopy (XPS)

The two distinct segments of the A. filiculoides biomass were analysed by XPS. The composition of the sample in terms of the individual atoms, carbon, nitrogen and oxygen were then established. XPS Spectra for the analysis of the biomaterial were obtained with a VG ESCALAB MKI spectrometer under a vacuum of 10^-7 torr. The scan spectra were recorded at a pass energy of 100eV. Samples were dried for 24 hours prior to analysis.

3.11 Batch Sorption Studies

100mg of adsorbent was added to a 500ml polypropylene flask containing 500ml of metal solution of known initial concentration and pH. Samples were agitated and the solution pH was checked and adjusted daily by addition of 0.1M NaOH or HCl until a constant pH was attained. The samples attained equilibrium when no significant change in pH was observed (± 0.1 units) in a 24-hour period. The equilibrated samples were filtered using a 0.2µm PTFE syringe top filter to remove the adsorbent particles, and then analysed. The data produced was fitted with either the Langmuir or Freundlich models, the theory of which is explained in Chapter 4.
3.12 Kinetic Experiments

Initial experiments were performed using the apparatus shown in Fig. 7. This set-up was superseded by that shown in Fig. 8 for all subsequent kinetic experiments. 990ml of distilled water was added to a round-bottomed flask. Then, 0.5g of adsorbent was placed into a rotating basket made of perspex and plastic mesh, opening 50 µm. The basket containing adsorbent was placed in the reactor and connected to a stirrer. The adsorbent was contacted with distilled water for 1 hour prior to the start of the experiment to allow for any trapped air to diffuse out and for any particle swelling to occur. 10ml of metal solution, of known initial concentration, was added to the reactor and the timer and the stirrer motor (set at 250 min⁻¹) started immediately. This was noted as the zero-time of the experiment. Samples were collected at certain time intervals and analysed for metal concentration. The experiments were performed for 3 hours and the temperature was kept at 298 K by a temperature control unit.
1. Temperature controlling unit
2. Plastic syringe
3. Filter
4. Metal solution/adsorbent
5. Water bath
6. Thermometer
7. Glass impeller
8. Variable speed motor

Fig. 7 Initial apparatus for kinetic experiments
1. Temperature controlling unit
2. Plastic syringe
3. Filter
4. Metal solution
5. Water bath
6. Thermometer
7. Glass impeller
8. Variable speed motor
9. Basket with adsorbent

Fig. 8 Basket apparatus used for kinetic experiments

3.13 Mini-column trials

Small polypropylene columns of 7cm³ and 12mm internal diameter were packed with 0.3g of biosorbent in the particle size range of 170-210µm. The packed bed occupied 5cm³. Feed concentrations were standardised as 0.25mM at pH 6 and were passed through the column at 10 bed volumes (BV)h⁻¹. Samples were collected automatically by a fraction collector: Pharmacia Fine Chemicals, FRAC-100, each tube collecting solution for 15 mins. Samples were analysed for metal concentration and pH. The experimental set-up can be seen below in Fig. 9.
Experimental Procedure

1. Feed tank
2. Peristaltic pump
3. Packed column
4. Supports
5. Auto sampler

![Diagram of experimental set-up]

Fig. 9 Experimental set-up to analyse column breakthrough characteristics

3.14 Elution experiments

Regeneration was conducted after a lengthy period of washing where the sorbent bed was exposed to 20 BV’s of distilled de-ionised water. A solution of 0.1M HCl was used to elute any adsorbed species and collected by the fraction collector at an interval of 10 minutes per sample. The apparatus used was the same as for mini column trials, shown in Fig. 9.

3.15 Stirred Cell

The stirred cell apparatus is shown in Fig. 10. 100mg of sorbent particles were placed in the cell with 450ml of distilled de-ionised water. The magnetic stirrer was switched on and 20 minutes was allowed for any swelling of the particles to take place. The peristaltic pump was started and this was noted as the zero time in the experiment. As the solution permeated the membrane it passed the sample valve which was placed as close to the perfectly stirred cell as possible. Down line from the
sample valve the exiting fluid passed a pH meter and a computer recorded this parameter every 5 seconds for the duration of the experiment. The flow rate was calculated during the experiment as the fluid exited into the waste tank.

1. Feed tank
2. Peristaltic pump
3. Stirred cell
4. Membrane
5. Magnetic stirrer
6. Sample valve
7. pH meter
8. Waste tank

Fig. 10 Schematic of stirred cell apparatus

3.16 Surface modification experiments

*A. filiculoides* biomass has a suitable but limited uptake of trace toxic metals. This uptake can be increased with chemical modification of the surface and addition of particular functional groups. Several different techniques have been previously employed in order to enhance biosorbents metal adsorption performance.

3.16.1 Chitosan bonding technique.

Literature had dictated that chitosan is very successful at chelating metal ions from solution (see Chapter 2). Therefore if this substance could be incorporated within or attached to the surface of an adsorbent it might enhance its metal sorption capacity.
A 0.1% Chitosan solution was made up using flakes supplied by Fluka in DDW. The DDW was pH adjusted to 4 using a few drops of concentrated acetic acid. 200ml of the chitosan solution was placed in an Erlenmeyer flask. 1g of the particulate A. filiculoides was added to the flask and stirred for 20 minutes to allow the system to come to equilibrium. 10ml of a crosslinking agent, glutaraldehyde was then added to the mixture to facilitate covalent bonding of the chitosan to the amino groups present on A. filiculoides. The ratio of reactants was decided upon based on previous work immobilising enzymes on chitosan\(^{112}\) and other amino groups.\(^{113}\) The reactants were allowed to interact for 1 hour. The solution was then filtered using a Buchner funnel and Dow membrane type FS61PP. The particles were washed with copious quantities of DDW to remove any excess quantities of the reaction mixture.

### 3.16.2 Cysteine impregnation

The chitosan enhanced material was not expected to result in an increase in mercury sorption due to the differing mechanisms involved in copper, cadmium uptake and that of mercury. Previous researchers have established a high affinity of cysteine for soft metals including mercury, (see Chapter 2.). Volesky et al\(^{114}\) worked on the biosorption of gold. In an effort to increase the capacity of the materials for the soft metal they reacted it with a solution of the amino acid cysteine. They reported an increased in uptake of between 150 and 250%.

The reaction conditions used in this study are equivalent ratios to the method described by Volesky. 2g of A. filiculoides particles was reacted with 100ml of 1mmol/L solution of cysteine. The solution was mixed and allowed to equilibrate for 4 hours. The cysteine uptake was determined from the difference of cysteine concentrations in the initial and final solutions. Cysteine was analysed by a UV/VIS spectrophotometer (Perkin Elmer lambda 12) at 412nm using 10mm quartz cells using the Ellman method.\(^{115}\)
3.17 Algin Extraction Method

Algin extracted samples of *Ascophyllum nodosum* and *Lessonia flavicans* were prepared according to a method used to extract algin by the R&D department, Monsanto plc. In order to extract algin from *Ascophyllum nodosum*, 500 ml of distilled water (70°C) containing 3 ml of CaCl₂ (20% wt/wt) was put into a 1l nominal capacity stainless steel beaker. The beaker was placed in a water bath maintained at 70°C (±1°C). 30g of sun dried algal particles were added into this solution. The slurry was hand-stirred occasionally over a 15 minute period using a polypropylene rod. The suspension was then allowed to stand for 15 minutes in order to settle the algal particles. 350 ml of the supernatant solution was decanted. 20 ml of 1.8%(v/v) formaldehyde solution was added into the remaining seaweed slurry. The slurry was hand-stirred for 1 minute and then left in the water bath for a further 30 minutes. The beaker was then removed from the water bath and made up to a final reactant mass of 600g by addition of hot DDW water (70°C). 5.5g of sodium carbonate and 5.5 ml of sodium hydroxide (10% w/v) was thoroughly mixed with the seaweed slurry, and the beaker replaced in the water bath. The slurry was stirred occasionally (approximately every half hour) over a period of 2.5h.

Algin extraction from *Lessonia flavicans* was carried out under slightly different conditions. 23.5g of dry seaweed meal was added to 200 ml of tap water (80°C) in a 1l nominal capacity stainless steel beaker. The beaker was placed in a temperature controlled water bath (80°C ± 1°C). The algal slurry was hand-stirred for 15 minutes using a polypropylene rod. 10 ml of 1.8% (v/v) formaldehyde solution was added to the mixture which was hand-stirred for a further 1 minute. The mixture was allowed to react for a further 30 minutes after which time the beaker was removed from the water bath. Hot tap water (80°C) was added to the algal mixture to obtain a final reactant mass of 400g. 4.7g of sodium carbonate and 4.8 ml of 10% sodium hydroxide solution were added to the algal mixture, hand-stirred thoroughly and then the beaker was replaced into the water bath. The slurry was stirred occasionally (every 30 minutes) over a period of 4h.
3.17.1 Dilution and separation step

Following extraction of algin from the seaweeds, cold tap water was added to the extract mix (4 parts water for each part of algal slurry) in order to aid dissolution of the alginate. Phase separation was achieved via solids sedimentation over an 18h period. The beaker was removed from the water bath and the extract slurry transferred to a 4l container containing a nominal amount of cold water. A final mass of 3 kg was attained by addition of cold tap water. The diluted extract was mechanically stirred for 15 minutes after which time, 8 ml of formaldehyde (40% v/v) solution was added to the system. After 2h, 30 ml of bentonite bulking solution was added to the weed extract slurry. The mixture was stirred for a further 2h 25 min and then 60 ml of Magnafloc R140 polyacrylamide settling agent was added. The stirrer speed was reduced to a minimum rate such that the solids were kept 'just suspended' in solution. The stirring was continued for a further 2 min. The stirrer was then removed and the dilute (algin extract/seaweed) slurry was allowed to settle.

3.17.2 Recovery of dealginate seaweed solids

A distinct solid/liquid interface was evident after 18h (approx.) settling time. The supernatant solution (containing algin) was carefully siphoned off. The remaining seaweed solids were washed by adding cold tap water to make up a 3 kg mass. The slurry was stirred for 15 min and then allowed to settle until a good phase separation had been achieved. The supernatant liquid was again siphoned off. The remaining solids were termed "dealginate seaweed" and represented the residue waste material produced by algin extraction from marine algae.

3.18 Amino acid analysis

Samples of biosorbent have been analysed for amino acid content. Samples were analysed by first hydrolysing the sample and making the amino acids available. This was achieved by adding 0.5 ml of 0.1M phenol (to reduce oxidation) and 4.5 ml of 6.6M HCl to 100mg of sample. 10 mg of tryptamine was added to help reduce acid hydrolysis of amino acids. The samples were heated at 110°C for 24 hours and then
allowed to desiccate until dryness. Distilled water was twice added and evaporated to ensure removal of all HCl. The amino acids were then obtained as the hydrochlorides. The sample was then analysed by high performance liquid chromatography (HPLC). The results are compared against standards and hence quantities of each amino acid present can be ascertained.
4 Theory

This chapter summarises the fundamental theory that lies behind the experimental data presented within this thesis. An introduction to adsorption is presented along with an explanation of the assumptions behind the more popular adsorption models. Background theory regarding zeta potential and column experiments are discussed.

4.1 Ion-exchange and adsorption

Adsorption is the process by which liquid or gaseous molecules are concentrated on the surface of a solid. This differs from absorption where molecules are encapsulated by a liquid or a gas. Several mechanisms can be involved in the adsorption process and these can be classified into two general groups, i.e. physical adsorption, involving Van der Waals forces, and chemical adsorption or electrostatic attraction.

Physical forces develop from temporary dipoles that are formed by irregular electron movements in the bonding of atoms. The negative end of the temporary dipole will repel electrons in the second proximal molecule thus inducing charge separation in the neighbouring molecule. When oppositely charged molecules are in close proximity an attraction occurs, known as London forces or hydrophobic bonding. In polar substances, atoms with strong electron affinity result in permanent charge separation on the molecule. This is termed dipole-dipole attraction. There is a special kind of dipole-dipole attraction that is called hydrogen bonding. The force of attraction between two opposite charges is proportional to the magnitude of their charges divided by the square of the distance between them. If hydrogen is close to an atom with a high affinity for electrons (e.g. O, N or Cl), the electronegative atom becomes negatively charged and conversely hydrogen becomes positively charged. Hydrogen is a very small atom and so the bond length between the two molecules becomes very small. This combination of high polarity and short distance results in a very strong interaction.
Electrostatic bonding occurs through the process of ion-exchange or co-ordination. In ion-exchange, ions of a given charge (either cations or anions) in solution are sorbed onto a solid material (the ion-exchanger) and are replaced by equivalent quantities of other ions of the same charge released by the solid. Ion-exchange can only occur when the functional groups on the adsorbent and the sorbate species are dissociated. For example, dissociation of the carboxylic acid group found in amino acids, fatty acids and several other cellular components is shown below:

\[
\begin{align*}
\text{R-C-CH}_2\text{OH} & \quad \rightleftharpoons \quad \text{R-C-CHO} + \text{H}^+ \\
\text{Fig. 11 Simple hydrogen dissociation of a carboxyl group}
\end{align*}
\]

This means that there are several criteria for ion exchange to be successful. The aqueous solution conditions must be such that the metal is in an ionic form. Also the solution conditions should result in a negatively charged adsorbent surface, through dissociation of functional groups.

The pK (log of a dissociation constant value) of functional groups is often used to describe the extent to which a particular group will have dissociated under specific conditions. The Henderson-Hasselbach equation describes the relationship between the pH of solution and the ratio of dissociated to complexed groups on the solid. For example, the dissociation of carboxylic acid shown in Fig. 11 can be expressed as follows:117

\[
pH = pK + \log \frac{[\text{COO}^-]}{[\text{COOH}]} \quad (1)
\]

Equation 1 shows that 50% of the specific group has dissociated when the pH of the solution is equal to the pK value (see Table 6).
Table 6 Relationship between pH and pK showing the distribution of species for a carboxylic acid group with a pK of 3

<table>
<thead>
<tr>
<th>pH</th>
<th>Percentage of Species as:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-COOH</td>
<td>R-COO⁻ + H⁺</td>
</tr>
<tr>
<td>1</td>
<td>99</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>99</td>
</tr>
</tbody>
</table>

Having considered the state of the functional groups on the solid it is now important to predict the metal species present in solution. Speciation diagrams were calculated for the chloride salts; CuCl₂, CdCl₂ and HgCl₂ in distilled water. A program Hydragl¹¹⁸ was used, that relies on pK data concerning the species present in the system (see Fig. 12, Fig. 13 and Fig. 14). Manual calculations were also performed to verify the results, an example follows below.

Fig. 12 Speciation diagram for 1mM solution of CuCl₂
Fig. 13 Speciation diagram for 1mM solution of CdCl₂

For the usual solutions used the species present were calculated and plotted on a speciation diagram. An example of the speciation used is shown below for an 1mM solution of cadmium chloride.

Fig. 14 Speciation diagram for 1mM solution of HgCl₂
Fig. 15 Speciation diagram for 1mM HgCl₂ solution produced on a log scale

For the metal solutions used the species present were calculated and plotted on speciation diagrams. An example of the mathematics used is shown below for an aqueous solution of cadmium.

\[
\begin{align*}
Cd^{2+} + H_2O & \leftrightarrow Cd(OH)^+ + H^+ \quad pK_{119}^{119} = 10.1 \quad K_1 \\
Cd^{2+} + 2H_2O & \leftrightarrow Cd(OH)_2(aq) + 2H^+ \quad pK_{22}^{22} = 20.4 \quad K_2 \\
Cd^{2+} + 3H_2O & \leftrightarrow Cd(OH)_3^- + 3H^+ \quad pK_{33}^{33} = 33.3 \quad K_3 \\
Cd^{2+} + 4H_2O & \leftrightarrow Cd(OH)_4^{2-} + 4H^+ \quad pK_{44}^{44} = 47.4 \quad K_4
\end{align*}
\]

The total concentration of cadmium (CM) is the sum of the concentrations of all cadmium containing species:

\[
CM = [Cd^{2+}] + [Cd(OH)^+] + [Cd(OH)_2(aq)] + [Cd(OH)_3^-] + [Cd(OH)_4^{2-}] \quad (2)
\]
The equations for the dissociation constants have the form:

\[ K_1 = \frac{[\text{Cd} \ (\text{OH})^+] [H^+]}{[\text{Cd}^{2+}]} \]  

(3)

Inserting equation (3) and the other \( K \) equations into (2) gives:

\[ CM = [\text{Cd}^{2+}] + K_1 \frac{[\text{Cd}^{2+}]}{[H^+]^1} + K_2 \frac{[\text{Cd}^{2+}]}{[H^+]^2} + K_3 \frac{[\text{Cd}^{2+}]}{[H^+]^3} + K_4 \frac{[\text{Cd}^{2+}]}{[H^+]^4} \]  

(4)

and simplification leads to equation (5):

\[ \alpha = \frac{[\text{Cd}^{2+}]}{CM} = \frac{1}{1 + \frac{K_1}{[H^+]^1} + \frac{K_2}{[H^+]^2} + \frac{K_3}{[H^+]^3} + \frac{K_4}{[H^+]^4}} \]  

(5)

The other alpha values for the remaining cadmium species were calculated in the same way. Then using equation (6):

\[ pH = -\log[H^+] \]  

(6)

a chart can be produced showing the fraction of each species over a range of pH values. The series can be easily extended to include the chlorine ligand.

### 4.1.2 Lewis Acids

The electron configuration of the metal sorbate ions gives them characteristic bonding properties that affect the sorption potential. The electron configuration of the target metals used in this study (Cu, Cd and Hg) are as follows:
Ground state electron configurations of the d-block elements: e.g. (Ar) represents 1s² 2s² 2p⁶ 3s² 3p⁶

Cu⁺ (Ar) 3d¹⁰ 4s¹  Cu²⁺ (Ar) 3d⁹
Cd (Kr) 4d¹⁰ 5s²  Cd²⁺ (Kr) 4d¹⁰
Hg (Xe) 4f¹⁴ 5d¹⁰ 6s²  Hg²⁺ (Xe) 4f¹⁴ 5d¹⁰

Since metal ions have empty valence orbitals, they can act as Lewis acids (electron pair acceptors). Ligands that can bond with the metals have unshared pairs of electrons, and so function as Lewis bases (electron pair donors). The bond between the metal ions exists as a result of sharing a pair of electrons donated by the ligand. This is a coordinate bond.

Metal complexes can be formed when a group of surrounding molecules or ions are bonded to a central metal ion. The molecules or ions that surround the metal are ligands that are normally either anions or polar molecules because each coordinate bond requires an available pair of electrons. In aqueous solution copper exists as the hexa-aqua ion (see Fig. 16).

\[ \text{H}_2\text{O} - \text{Cu}^{2+} - \text{OH}_2 \]

\[ \text{H}_2\text{O} \quad \text{H}_2\text{O} \]

**Fig. 16 Copper ion in aqueous solution takes the form of the hexa-aqua ion**

### 4.2 Adsorption Modelling

Adsorption is commonly described by an adsorption isotherm that reveals the distribution of the solute between the solid and liquid phases at a constant

---

*The copper atom fills the 3d shell before the 4s one as this is the more stable arrangement.*
temperature. The adsorption isotherm is dependent on several parameters including solution concentration, the sorbed species, any competing ions and characteristics of the adsorbent used.

4.2.1 Langmuir type isotherm

The Langmuir type isotherm is the most common and characterises the interactions where a high affinity of the adsorbent for the adsorbate is observed. It is normally applied to non ion-exchange reactions. The main assumptions of the model are:

- The adsorption occurs in a monolayer.
- There are no adsorbate-adsorbate interactions.
- The surface of the adsorbent is homogeneous.

As the initial sites are filled it becomes increasingly difficult for the adsorbate molecules to find a vacant site resulting in the formation of the monolayer.

The Langmuir equation is written as below:

\[
q = \frac{q_m b C_{eq}}{1 + b C_{eq}}
\]  

To use this equation for modelling experimental data we can take reciprocals and rearrange to give the following:

\[
\frac{1}{q} = \frac{1}{q_m b C_{eq}} + \frac{1}{q_m}
\]

A Lineweaver-Burk plot of \(1/q\) vs \(1/C_{eq}\) should give a straight line of gradient \(1/b q_m\) and an intercept of \(1/q_m\). The constant \(b\) is related to the energy of adsorption and increases as the strength of the adsorbate/adsorbent bond increases. Deviations often occur from the Langmuir correlation due to multi-layer formation at higher adsorbate
surface concentrations. Freundlich developed a model that can overcome this limitation by including heterogeneous sorption that allows for multi-layer formation.

**4.2.2 Freundlich type isotherm**

The Freundlich isotherm is often used to describe adsorption to multi-site or heterogeneous solids across a wide range of adsorbate concentrations. It simulates the layering of adsorbate on the surface with decreasing adsorption energy. It is relatively insensitive, as logarithmic plots do not give all data points equal weighting and hence tend to fit most data well. The constant $K$ is primarily related to the capacity of the adsorbent for the adsorbate and $1/n$ is a function of the strength of adsorption. Low values of $1/n$ suggest a strong interaction between the adsorbent surface and the adsorbate, often resulting in irreversible adsorption.

$$q = kC_{eq}^{1/n}$$

(9)

After linearisation equation 5 can be expressed as follows:

$$\ln q = \ln k + \frac{1}{n} \ln C_{eq}$$

(10)

Many other adsorption isotherm correlations exist in the literature, however these are often only used when application of the Langmuir and Freundlich models fail. There is a very good explanation of the theoretical basis of the models in the book by Ruthven.\(^{121}\)
4.3 Kinetics

4.3.1 Sorbate distribution

For an adsorption reaction to occur the sorbate molecules must come into close proximity with the active sites on the adsorbent. The distribution of the sorbate molecules within the carrier fluid is governed by dispersion and diffusion. The dissolved metal ions are dispersed within the carrier fluid by its motion and turbulence. This advection is the fastest form of chemical transport in porous media. The concentration of the molecule decreases in the direction of flow.

Diffusion is the movement of a moiety from a region of high concentration to a region of low concentration until the molecules reach a state of dynamic equilibrium. Fick's Law (see Equation 11) states that the rate of diffusion of a substance is proportional to the distance moved and the concentration gradient. For biological cells this means that the thickness of the cell membrane has a critical effect on the rate of diffusion and is one reason why the membrane is extremely thin (generally less than 10 nm).

\[ q_x = -D \frac{\partial C}{\partial x} \]  

(11)

In order to design an adsorption process knowledge of adsorbent capacity and the kinetics of adsorption is required. The process of metal removal from the aqueous phase can be described as a heterogeneous reaction between solid and liquid. A sequence of reactions that can result in the sorption of an adsorbate molecule are as follows: (See Fig. 17).

1. Diffusion of ions through the liquid film surrounding the particle.
2. Diffusion of ions through the porous structure of the solid.
3. Chemical reaction with functional groups present on the adsorbent.

The slowest of the three stages will become the rate determining step.
In surface diffusion, molecules migrate by a surface hopping mechanism; i.e. when an adjacent adsorption site is available, and the molecule has enough energy to leave the site it is presently occupying, it will hop from one to the other. Pore diffusion occurs when the adsorbate molecule diffuses in the liquid filling the pore. The surface diffusion flux is many times greater than the pore diffusion flux for strongly adsorbed species. This enables the contribution of pore diffusion to the adsorbate transport to be neglected.\textsuperscript{122}

If ion-exchange is believed to be a mechanism then it is unlikely to be the rate determining step as it is generally considered to be a spontaneous reaction. However, there are instances when changes in the ionic species present are required to enable an ion-exchange reaction and these are responsible for the overall ion-exchange reaction proceeding at a slower rate.
In most sorptive reactions the particle diffusion step is believed to be the rate-determining step. This is particularly true for some commercially produced resins with high degrees of cross-linking and tortuous porosity. If there is little resistance to diffusion within the solid then the liquid diffusion can be the limiting factor, particularly in cases of low convective mixing.

### 4.4 Electrophoretic mobility

Colloidal suspensions and dispersions of fine particles in the liquid phase possess an electric charge that is dependent on the nature of the surrounding medium and the solids themselves. Charged particles will exhibit electrokinetic effects when exposed to an electric field. One of these effects is called electrophoresis. This is the movement of a charged particle suspended in an electrolyte under the influence of an applied electric field. Zeta potential is related to electrophoretic mobility and quantifies the magnitude of interaction between particles. The higher the zeta potential (positive or negative), the greater the dispersal of colloidal particles.

The charge that develops at the interface between a colloidal particle and the liquid medium in which it is suspended may arise by one of several mechanisms. Among these are the dissociation of ionogenic groups on the particle surface and the adsorption from solution of charged ions onto the surface region. Ion-exchange mechanisms may also be important.

The development of a net charge at the particle surface affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter-ions close to the surface. Thus, an electrical double layer is formed in the region of the particle-liquid interface.
The inner region is called the Stern layer and counter ions present within this area are strongly bound to the surface of the particle. The second layer is described as the diffuse region containing a lower density of particles with a greater degree of mobility. The potential at the boundary of this region is the zeta potential as shown in Fig. 18. Zeta potential is a function of pH and as such can be plotted against the physical parameter to elucidate an important characteristic of the solid the iso-electric point or IEP. The IEP describes the pH at which there is no net charge on the particle.
4.5 Packed bed columns

The steepness of the breakthrough curve determines the extent to which the full capacity of an adsorbent bed can be utilised. Thus, the shape of the curve is very important in determining the length of the adsorption bed. In actual practice, the steepness of the concentration profiles can increase or decrease, depending on the type of adsorption isotherm involved.

Fig. 19 Idealised breakthrough curve detailing the normalised sorbate exit concentration from the column.
Fig. 20 shows that for the favourable isotherm of the Langmuir or Freundlich type, the high concentration regions move faster than the low concentration regions, and the wave front becomes steeper with time until a constant pattern front is developed. (self-sharpening).

Fig. 20 Shows a favourable isotherm and a self-sharpening wave front as it travels through a column.
4.6 X-Ray Photoelectron Spectroscopy (XPS)

The surface chemical composition can be studied using different surface spectroscopy techniques. The most commonly used are X-ray Photoelectron Spectroscopy (XPS), Auger Electron Spectroscopy (AES), Secondary-Ion Mass Spectroscopy (SIMS) and Low-Energy Ion Scattering Spectroscopy (ISS).

In XPS a sample is irradiated with non-monochromatic X-rays. The X-rays excite photoemission from the core levels of the atoms present in the sample and the resultant photoelectrons emerging from the sample surface are collected and energy analysed to yield the photoelectron spectrum. Since the kinetic energy of the photoelectron depends on the binding energy of electrons in the core levels from which the photoemission is excited, each element gives rise to a set of peaks at characteristic energies in the photoelectron spectrum. Measurement of these energies therefore allows the elements present in the sample surface to be determined. Quantitative analysis is then obtained from the measurement of the relative intensities of the photoelectron peaks.

The inelastic mean free path of photoelectrons generated in a solid state is typically 2-5nm. This means that only those photoelectrons generated in the outermost atomic layers of the sample are likely to escape the surface, and be detected, with their initial kinetic energy intact. XPS is thus an analysis technique specific to the surface of solid materials, allowing routine analysis of the species present in amounts as small as 0.1 of a monolayer.

In addition to providing quantitative analysis of the outermost atomic layers of the sample, XPS may also be used to probe the chemistry of the surface. The binding energy of a core level depends principally on the charge on the nucleus of the atom concerned and to a lesser extent on the bonding between the atom and its neighbours. The change in binding energy due to chemical bonding is often referred to as the 'chemical shift'. Comparisons between measured chemical shifts and published values for a wide variety of chemical compounds may allow the chemistry of the surface to be inferred and the functional groups to be identified.
4.7 Electron Dispersive Spectroscopy (EDS)

Several different phenomena result from the bombardment of the sample with electrons: (see Fig. 21)

(i) Backscattered electrons - reflected back from sample
(ii) Secondary electrons removed from atoms

X-rays are emitted resulting from relaxation of excited electrons

X-ray techniques work well for elements with atomic numbers equal to sodium or higher

1. The electrons involved are independent of molecular interactions (non valence)

2. The wavelength of the emitted X-ray is characteristic of the atom
   (i.) Energy required to excite an electron increases with atomic number
   (ii.) This increase in energy required to excite electrons is due to the increased (+) charge on the nucleus
Fig. 22 shows the sequence of events during the EDS analysis.

**Fig. 22** Diagram showing the electron sequence during EDS analysis

### 4.8 X-Ray Diffraction (XRD)

The crystal structure of each material is unique and depends on the order of its atoms. The atoms affect the X-rays that are fired at the sample surface causing them to scatter. The majority of the waves are cancelled out through destructive interference; however, some can be enlarged by synergistic waves. The direction of the enhanced waves is related to the distance between the atomic planes and is measured by the diffractometer to predict the composition of the sample, see Fig. 23.

**Fig. 23** Schematic of an X-ray powder diffractometer
Chapter Nomenclature

\[ pH = \text{The negative log of the hydrogen ion concentration} \]
\[ pK = \text{The negative log of the dissociation constant} \]
\[ A' = \text{The basic form of the surface group [Moles/l]} \]
\[ HA = \text{The acidic form of the group [Moles/l]} \]
\[ q_f = \text{Rate of mass transport in the x direction [M/l^2T]} \]
\[ D = \text{Diffusion coefficient [l^2/T]} \]
\[ C = \text{Concentration [M/l^3]} \]
\[ x = \text{Distance [m]} \]
\[ q_m = \text{Complete monolayer capacity [mmol/g]} \]
\[ b = \text{Langmuir constant [l/mmol]} \]
\[ C_{eq} = \text{Equilibrium sorbate concentration [mmol/l]} \]
\[ q = \text{Equilibrium concentration in solid phase [mmol/g]} \]
\[ k = \text{Freundlich adsorption constant} \]
\[ n = \text{Freundlich exponent} \]
5 Azolla filiculoides, Characterisation and Evaluation

It has been shown earlier that a key parameter in the adsorption process is the functionality of the adsorbent surface. It is known that surface groups in biomass may contain heteroatoms such as nitrogen and sulphur. Functional groups can directly interact with metal species by ion-exchange and/or coordinate bonding or chemical reaction. Further to this the acidic or basic nature of the functional groups has a strong influence on the surface electrostatic charge of the sorptive materials allowing for attraction or repulsion of charged metal species. This chapter describes the sorbent material that was produced by characterising it both chemically and physically. The biosorbent has been challenged with the target metal ion solutions and its performance measured. Sorption mini column runs have been performed and breakthrough curves plotted. The kinetics of the sorption processes have been quantified and compared. The study of proton – metal interactions with the biosorbent surface and XRD data has yielded information regarding the mechanisms of metal biosorption. The material has also been chemically enhanced to yield a higher uptake of the targeted metal ions.

5.1 Chemical Characterisation

5.1.1 Total Ion Exchange Capacity

Functional groups present on the surface of adsorbents e.g. carbonyl, carboxyl, phenolic, lactonic and amino dissociate at increasing pH values. The total ion exchange capacity is determined in alkali solutions, this ensures that groups are dissociated to a high degree and available for ion exchange. The common salt available for this purpose is sodium hydroxide. Under these conditions therefore the total ion exchange capacity can be determined.

The ion-exchange capacity has been determined for the two distinct components of the water fern A. filiculoides. This has been compared with previously reported data for granular activated carbon see Table 7.
Table 7 shows the ion-exchange capacity of the adsorbents. The sodium capacity is greatest for the acid oxidised carbon, however the capacity of the biosorbent is significant and much greater than that of the as-received activated carbon.

<table>
<thead>
<tr>
<th>Material</th>
<th>Ion-Exchange Capacity [mmol/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. filiculoides</em> roots</td>
<td>2.6</td>
</tr>
<tr>
<td><em>A. filiculoides</em> leaves</td>
<td>3.8</td>
</tr>
<tr>
<td>Commercial GAC AUG WHK\textsuperscript{123}</td>
<td>1.6</td>
</tr>
<tr>
<td>Acid oxidized WHK\textsuperscript{123}</td>
<td>4.6</td>
</tr>
</tbody>
</table>

In the subsequent characterisation and evaluation of the biosorbent it was important to perform experiments using an homogeneous adsorbent. With that in mind it was decided to conduct all future experiments with the leaves of *A. filiculoides* based on their superior ion exchange capacity.

### 5.1.2 pH titrations

Ion exchange and adsorptive materials can possess acidic and basic properties. The quantity and type of these functional groups can be easily assessed through a technique know as pH titration. Information can be obtained regarding the nature of the adsorbent (mono or polyfunctional) and the appropriate working range based on its dissociation contants.

When the adsorbent is contacted with the electrolyte solution then the electrolyte that is in equilibrium with the solid will react with surface functional groups and alter the pH of the solution.

pH titrations were performed on *A. filiculoides* particles. Typical pH titration curves are shown below (see Fig. 24).
Fig. 24 pH titration curves for *A. filiculoides* and blank experiments

It can be seen from Fig. 24 that when comparing the titration of the adsorbent with that of a blank experiment there is a drop in pH in alkali solutions. It is suggested that this is due to dissociation of acidic groups and a release of H\(^+\) ions into solution. The H\(^+\) ions released were quantified by calculating the difference between the two curves at any given pH and using the values in the following equation:

\[
A = \frac{C(V_b - V_s)}{m}
\]  
(1)

- **A** = Ions released [mmol/g]
- **C** = Concentration of titrant [Moles]
- **V\(_b\)** = Volume of blank experiment [ml]
- **V\(_s\)** = Volume of experiment in presence of solid [ml]
- **m** = Mass of solid added [g]
Plotting the values obtained from equation (1) gives the proton binding curves (see Fig. 25). The shapes of proton-binding curves reveal important information as to the changing state of the solids surface. The point of zero charge (PZC) is a useful parameter and it can be determined by pH titration. PZC is the pH at which the net surface charge, internal and external, is zero. Biomaterial has the potential (depending on surface functionality) to be an amphoteric sorbent i.e. adsorb acids or bases depending on pH. It follows that if it displays basic properties it can perform anion exchange and where it is acidic in nature cation exchange will be possible. Therefore it is necessary to titrate the material in both acid and alkali solution in order to fully describe its ion-exchange nature. *Azolla filiculoides* shows a curve that is characteristic of weak acid cation exchangers with slow dissociation of groups giving a flat curve. The shape of the curve for *A. filiculoides* is very interesting as it displays a significant step in hydrogen ions released between pH 4 and 5. This ties in with the sorption data given later in this chapter that shows a large increase in metal ion uptake between these two pH values compared to the increase displayed between pH 5 and 6. This can be attributed to the dissociation of a functional group present within the biosorbent and is discussed in more detail in subsequent sections of this chapter. At
high pH the biosorbent differs from the carbon in that it does not have such a dramatic release of hydrogen ions. The carbon has a higher concentration of oxygen containing groups and at pH > 10 hydroxyl groups can dissociate.

5.1.3 Electrophoretic Mobility Measurements

![Electrophoretic mobility measurements of A. filiculoides and as received carbon WHK.](image)

Fig. 26 Electrophoretic mobility measurements of *A. filiculoides* and as received carbon WHK.

The electrokinetic behaviour of a solid is relevant when charge related processes are taking place. The important condition to consider is when the zeta potential is zero. This is termed the isoelectric point (IEP) of the interface, as described in Chapter 3. At pH greater than pH\(_{IEP}\) the sorbents surface will attract cations from solution. At pH < pH\(_{IEP}\) the sorbent will attract anions.

In Fig. 24 *A. filiculoides* particles exhibit slow dissociation of acidic groups with increase in solution pH. It is observed in Table 8 that the IEP of *A. filiculoides* follows the same trend as the PZC, however the PZC is displaced slightly to the right. This is most likely due to the inclusion of internal surface area, increasing the total number of functional groups available for the reaction. The surface charge below and
above the IEP can be explained in relation to the protonation and dissociation of acidic groups.

As mentioned above, The PZC takes into account the internal and external surface whereas the IEP, due to the distance from the particle at which it is measured, refers only to the external surface of the adsorbent. Table 8 reports the difference PZC-IEP that shows the homogeneity of a sample in terms of weak acidic surface groups. It can be deduced that the distribution of acidic groups appears to be reasonably homogeneous in *Azolla filiculoides*. Possible explanations for this are that the material contains either very large pores or that there is very little porosity.

Table 8. A comparison of the charge related properties of the adsorbents (pH units)

<table>
<thead>
<tr>
<th>Sample</th>
<th>PZC</th>
<th>IEP</th>
<th>PZC-IEP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. filiculoides</em></td>
<td>2.8</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Commercial GAC</td>
<td>4.5</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>AUG WHK\textsuperscript{123}</td>
<td>3.5</td>
<td>0.8</td>
<td>2.7</td>
</tr>
</tbody>
</table>

It can be seen in Table 8 that the carbon materials which possess a defined pore structure and large surface area have a difference in surface to total surface charge that is an order of magnitude greater than the biomaterial. The difference in homogeneity of the two carbon samples is skewed by the oxidation reactions which are diffusion limited and hence primarily occur at the surface of the sample.

5.1.4 Nitrogen content

Previous work on metal uptake by biosorbents generally focussed on the ion-exchange mechanism between the cationic metal ions and ionisable groups such as carboxylic acid present on the adsorbent.\textsuperscript{125} However during a study on different classes of seaweed\textsuperscript{4} such as *Ascophyllum nodosum* and *Lessonia flavicans* it was found that after the main source of carboxyl groups (alginate) was extracted there was still a significant residual sorption capacity. It was suggested that this may arise from the presence of
amino acids present within the biosorbent structure. The following experiments were performed with the aim of characterising these materials with respect to their nitrogen content and amino acid profile.

Table 9 Kjeldahl nitrogen determination for assorted biological materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Percent Nitrogen [w/w]</th>
<th>Estimated Percent Protein* [w/w]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. filiculoides</td>
<td>3.80</td>
<td>23.76</td>
</tr>
<tr>
<td>De-alginate L. flavicans</td>
<td>3.20</td>
<td>20.02</td>
</tr>
<tr>
<td>L. flavicans</td>
<td>1.90</td>
<td>11.86</td>
</tr>
<tr>
<td>De-alginate A. nodosum</td>
<td>1.66</td>
<td>10.38</td>
</tr>
<tr>
<td>Laminarea hypoborea</td>
<td>1.58</td>
<td>9.90</td>
</tr>
<tr>
<td>Ascophyllum nodosum</td>
<td>0.79</td>
<td>4.94</td>
</tr>
<tr>
<td>D. potatorum</td>
<td>0.69</td>
<td>4.33</td>
</tr>
<tr>
<td>L. flavicans alginic acid</td>
<td>0.20</td>
<td>1.23</td>
</tr>
<tr>
<td>A. nodosom alginic acid</td>
<td>0.14</td>
<td>0.88</td>
</tr>
</tbody>
</table>

* calculated from ASOC protein factor of 6.25

Table 9 shows that there is indeed a substantial quantity of nitrogen associated with these materials. This result was not wholly unsurprising for A. filiculoides which, as described in Chapter 1, is used in animal feed as a source of amino acids and the protein concentration calculated as part of these experiments closely matches that reported in the literature.126 A. filiculoides contained a much larger quantity of nitrogen than the native seaweeds studied and hence was an ideal candidate for studying metal ion – protein interactions.
5.1.5 Amino acid analysis

Following the successful nitrogen analysis it was considered crucial in the understanding of any metal binding potential of protein residues to further characterise them by an amino acid analysis. The method described in Chapter 3 was employed and the results are shown in Fig. 27. It is assumed that the amino acids are still present within the primary protein structure where peptide bonds nullify the reactive carboxy and amino groups inherent on every amino acid. The active groups are present on the side chains of the amino acids and are considered available for reaction. Fig. 27 shows only the amino acids with reactive side chains. The concentration of amino acids with un-reactive side chains was 1.1 mmol/g. Ideally, the mixture of amino acids will accurately reflect the composition of the original protein. However a number of factors can cause the amino acid composition of the acid hydrolysate to differ from that of the protein from which it is derived. The principle reasons are artefact formation, incomplete liberation of amino acids, racemization of amino acids, and destruction of the amino acids during hydrolysis.

![Fig. 27 HPLC amino acid profile for A. filiculoides](chart.png)

- GLU - Glutamic Acid
- ASP - Aspartic Acid
- SER - Serine
- PRO - Proline
- THR - Threonine
- ARG - Arginine
- LYS - Lysine
- PHE - Phenylalanine
- TYR - Tyrosine
- HIS - Histidine
- MET - Methionine
- CYS - Cysteine
- TRY - Tryptophan
Artefact formation can be minimised by rapid removal of HCl after hydrolysis, at slightly elevated temperatures and under reduced pressure. Incomplete liberation of amino acids can occur if the acid hydrolysis stage is not long enough. However, a prolonged hydrolysis step can destroy some amino acids e.g. serine and threonine. These amino acids break down and give rise to ammonia and hence ammonium chloride was analysed to reveal degradation levels and accounted for 0.2mmol/g.

From Fig. 27 showing the amino acid profile for *A. filiculoides* and including the 1.1 mmol/g of unreactive amino acids it is possible to calculate that the total concentration within the adsorbent is 2.41 mmol/g. With reference to Table 10 of the amino acids with functional R chains it is possible to deduce that there are 1.33 mmol/g of groups present within these amino acids that are capable of ion-exchange, physisorption or co-ordination reactions with cations. Of course this is a theoretical maximum, some of the groups involved have very weak interactions with cations such as carbonyl and hydroxyl. These two groups may have intermittent dipole attractions for cations emanating from the slightly negatively charged oxygen atom but will not manage dissociation reactions below a pH of approximately 10. The other groups considered are: carboxyl, amino, imine, thiol and thioester. Purely from an ion-exchange point of view there are 0.49mmol/g of carboxyl groups. This can account for 0.25mmol/g of divalent cation sorption. These can only be suggested as part of the mechanism for cation sorption by *A. filiculoides* as there are several additional parameters that are crucial to the sorption process including spatial distribution of these groups and the surface/pore topography and geometry for adsorbate access to the binding sites.
Table 10 Amino acid functionality and concentration in *A. filiculoides*

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Functional ‘R’ Chain Group(s)</th>
<th>pK</th>
<th>Cation Sorption mechanism</th>
<th>Concentration in <em>A. filiculoides</em> [mmol/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine (Guanidino gp.)</td>
<td>Imine</td>
<td>12.5</td>
<td>Co-ordinate</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>1(^y) amino</td>
<td>N/A</td>
<td>Co-ordinate</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>2(^y) amino</td>
<td>N/A</td>
<td>Co-ordinate</td>
<td>0.10</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Carbonyl</td>
<td>N/A</td>
<td>H-bonding</td>
<td>Not analysed for</td>
</tr>
<tr>
<td></td>
<td>Amino</td>
<td>N/A</td>
<td>Co-ordinate</td>
<td></td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>Carboxyl</td>
<td>3.9</td>
<td>Ion exchange</td>
<td>0.22</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Thiol</td>
<td>8.3</td>
<td>Ion ex. / Co-ordinate</td>
<td>0.01</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Carbonyl</td>
<td>N/A</td>
<td>H-bonding</td>
<td>Not analysed for</td>
</tr>
<tr>
<td></td>
<td>Amino</td>
<td>N/A</td>
<td>Coordinate</td>
<td></td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>Carboxyl</td>
<td>4.3</td>
<td>Ion exchange</td>
<td>0.27</td>
</tr>
<tr>
<td>Histidine (Imidazole Ring)</td>
<td>2(^y) amino</td>
<td>N/A</td>
<td>Co-ordinate</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Imine</td>
<td>6.0</td>
<td>Ion ex. / Co-ordinate</td>
<td>0.04</td>
</tr>
<tr>
<td>Lysine</td>
<td>1(^y) Amino</td>
<td>10.8</td>
<td>Co-ordinate</td>
<td>0.10</td>
</tr>
<tr>
<td>Methionine</td>
<td>Thioester</td>
<td>N/A</td>
<td>Co-ordinate</td>
<td>0.03</td>
</tr>
<tr>
<td>Proline</td>
<td>2(^y) Amino</td>
<td>N/A</td>
<td>Co-ordinate</td>
<td>0.14</td>
</tr>
<tr>
<td>Serine</td>
<td>Hydroxyl</td>
<td>13.0</td>
<td>H-bonding</td>
<td>0.14</td>
</tr>
<tr>
<td>Threonine</td>
<td>Hydroxyl</td>
<td>13.0</td>
<td>H-bonding</td>
<td>0.12</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>2(^y) Amino</td>
<td>N/A</td>
<td>Co-ordinate</td>
<td>0.00</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Hydroxyl (Phenol)</td>
<td>10.1</td>
<td>H-bonding</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>1.33</strong></td>
</tr>
</tbody>
</table>

A comparison of the total nitrogen obtained by the Kjeldahl method and the sum of the nitrogen atoms present in the form of amino acids shows consistency. The Kjeldahl method gave a value of 2.7 mmol/g of nitrogen. The total nitrogen found from the amino acid analysis was 3.1 mmol/g.
5.2 Physical Characterisation

5.2.1 Scanning Electron Micrographs (SEM)

SEM photographs were performed to gain information regarding the surface topography of the samples and elucidate any indication of pore structure. The SEM images clearly show differences of physical structure between the leaves (Figs. 5,6) and roots (Figs 7,8) of *Azolla filiculoides*. *A. filiculoides* leaves do not seem to possess porosity of a regular nature. There are, however, occasion breaks in the surface due to mechanical processing and stomatal pores.

![Fig. 28 SEM of *A. filiculoides* leaf particles (protonated)](image)

![Fig. 29 SEM of *A. filiculoides* leaf particles (protonated)](image)
5.2.2 Swelling tests

The swelling properties of the material were investigated after processing the biomaterial into a particulate hydrogen form adsorbent. The particles were examined under a light microscope and allowed to equilibrate with de-ionised distilled water (DDW). Swelling characteristics are important since particles can exert a force on any mechanical containment. For example ion-exchange resins shrink and swell when changing ionic form.
Fig. 32 *A. filiculoides* particles contacted with DDW at $t = 0$ observed under a stereo light microscope

Fig. 33 *A. filiculoides* particles in contact with DDW at $t = 20$ minutes observed under a stereo light microscope

Particles of *A. filiculoides* were ground and sieved to 499-553µm and studied under a stereo microscope (Leitz Orthoplan) with images captured by a video camera (JVC TK-1280E). The particles were contacted with DDW as they were in position on the microscope slide and the lack of swelling can be seen by a comparison of Fig. 32 and Fig. 33. The extra shadows visible on the sample at $t = 0$ are due to the refraction of water that has pooled around the particle.
Fig. 34 Particles of *A. filiculoides* sieved to 499-553 µm observed under a light microscope

Fig. 34 shows the variety of particle shapes generated by the processing of *A. filiculoides* plants into a particulate adsorbent.

### 5.2.3 XPS data

The material generated was subjected to XPS analysis in order to obtain information regarding its atomic make-up.
Fig. 35 X-Ray photoelectron spectrum of *A. filiculoides*

The peaks presented in each diagram correspond to an element, which can be identified with its corresponding binding energy. The wide spectrum for *A. filiculoides*, illustrated in Fig. 35, clearly shows the presence of carbon, nitrogen and oxygen at the binding energy of 285, 400 and 533 eV respectively.

Fig. 36 XPS focussed on the main hydrocarbon peak of 285 eV

The C1’s spectra (Fig. 36) show asymmetry on the higher binding energy side of the main hydrocarbon peak at 285 eV but no resolvable additional peaks of shoulders.
Fig. 37 XPS focussed on the oxygen peak at 533 eV

There are also no resolvable additional peaks or shoulders shown for the oxygen peak in Fig. 37. This information suggests a mixture of carbon-oxygen functionalities which could only be quantified through specific derivitisation.

The data presented in the graphs above can be used to quantify the elements present on the surface of the biosorbent, see Table 11 below. The surface composition shows a small percentage of nitrogen, assumed to be present as part of the amino acids that make up the plant protein. Also in evidence is a large proportion of oxygen atoms. If we compare the oxygen content of the biosorbent with that of the carbon samples we can see that it has a much greater concentration than the as received sample. It also has a larger quantity than the acid oxidised carbon. The oxygen content reported here is directly related to the amount of oxygen containing groups on the carbon surface. It should therefore be possible to predict the total ion-exchange capacity in relation to this comparison.
Table 11 Surface composition of *A. filiculoides* and samples of GAC WHK by XPS

<table>
<thead>
<tr>
<th>Sample</th>
<th>C</th>
<th>N</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. filiculoides</em></td>
<td>73.2</td>
<td>3.3</td>
<td>23.4</td>
</tr>
<tr>
<td>As received WHK\textsuperscript{123}</td>
<td>91.5</td>
<td>0.0</td>
<td>8.1</td>
</tr>
<tr>
<td>Acid oxidised WHK\textsuperscript{123}</td>
<td>79.9</td>
<td>3.0</td>
<td>17.1</td>
</tr>
</tbody>
</table>

5.3 Heavy Metal Sorption

5.3.1 Mercury Sorption

*A. filiculoides* was challenged with the removal of mercury from aqueous solutions. There is little literature on the removal of mercury from solution, particularly using biological adsorbents.\textsuperscript{127,125} It has been attempted with *E. coli* genetically modified to produce larger than normal concentrations of metallothionein\textsuperscript{128}, a sulphur containing amino acid with high mercury affinity.

The mercury sorption isotherms (Fig. 38) show an increase in uptake for the metal with increase in pH. This follows the same trend observed for copper and cadmium sorption. There is however a marked difference in the modelling of the experimental data.
 Whereas copper and cadmium sorption had been accurately described by the Freundlich model, in mercury trials it is the Langmuir expression that better defines the uptake. This suggests that a different mechanism is responsible for the biosorbent removal of mercury from solution. One of the main differences in the two models mentioned is that Langmuir assumes a mono-layer coverage of sorbent moeties compared to the multi-layer sorption accommodated by Freundlich’s equation.

Table 12 Model parameters for mercury sorption by *A. filiculoides*

<table>
<thead>
<tr>
<th>pH</th>
<th>Langmuir/Freundlich Fit [R^2]</th>
<th>Langmuir parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.902 0.842</td>
<td>(q_m) 0.23 (b) 116.3</td>
</tr>
<tr>
<td>5</td>
<td>0.989 0.912</td>
<td>(q_m) 0.44 (b) 50.8</td>
</tr>
<tr>
<td>6</td>
<td>0.998 0.919</td>
<td>(q_m) 0.56 (b) 37.2</td>
</tr>
</tbody>
</table>

During sorption trials, the biomaterial was noticed to have a lighter appearance following contact with the mercury solution. This observation confirms an indication in the literature\textsuperscript{129} that showed backscatter SEM images that are thought to be a mercury based precipitate. The SEM study was repeated as part of this work in order...
to compare the mercury sorption mechanism of *A. filiculoides* with an activated carbon.

### 5.3.1.1 Scanning Electron Microscopy (SEM)

Backscatter images (Fig. 39) of mercury contacted *A. filiculoides* samples show the presence of many light areas on the surface, of an apparent crystalline nature. Due to the technique of backscatter SEM molecules of high atomic number show up as lighter areas than those with lower atomic number.
Fig. 39 SEM's of *Azolla filiculoides* equilibrated with HgCl$_2$ at pH 6. (a) Large Scale coverage, (b) Crystal forming in crevice, (c) Large scale crystal.

5.3.1.2 Energy Dispersive Spectroscopy (EDS)

The equipment utilised for the scanning electron micrographs possessed an energy dispersive attachment. In order to know the chemical composition of the light areas present on the sorbents surface, Electron Dispersive Spectroscopy (EDS) was conducted. EDS profiles of each sample were generated using the detector associated with the microscope. Fine cross-hairs could be orientated on the sample and an elemental analysis of a specific area made. The EDS spectra for the sorbent at different locations is presented in Fig. 40. These spectra show that both mercury and
chlorine are present, which is due to the removal of HgCl₂ from solution. The presence of silicon and gold is because samples were glued to aluminium platforms and sputter coated in gold, to enable them to conduct electricity, prior to analysis. Mercury peaks from other displaced electrons falling back into their orbitals can be seen clearly at higher eV values.

Fig. 40 EDS spectra of A. filiculoides samples (a) equilibrated with HgCl₂ at pH 6, (b) sample blank

5.3.1.3 X-ray Diffraction (XRD)

The results of SEM's of the adsorbents surface and the EDS analysis of the surface composition prove that there is mercury present on the adsorbent and show what appears to be a precipitate. There is as yet, however, no indication as to what mercury complex is responsible. XRD is a method by which powder samples can be evaluated for their crystallogical form and compared against a standard database to reveal the elements present and their complexes.
Experiments using XRD were carried out in order to elucidate the crystallographic form of the precipitate observed by SEM studies. The spectra shown in Fig. 41 for *Azolla filiculoides* reveals two peaks at 2θ angles of 21.4 and 28.2, which correspond to mercurous chloride (Hg₂Cl₂). These spectra show no evidence of a peak at 20.5 that would indicate that mercury is present as mercuric chloride (HgCl₂). These results confirm that mercury is, at least in part, removed by the reduction of Hg(II) (uncharged aqueous species HgCl₂ that predominates at pH lower than 6, (see Fig. 4 Chapter 4) to Hg(I) followed by Hg₂Cl₂ precipitation. The suggested reduction reaction of HgCl₂ with surface hydroxyl groups is as follows:\(^\text{130}\)

\[
2(-\text{OH}) + 2\text{HgCl}_2 (\text{aq}) \rightarrow 2(=\text{O}) + \text{Hg}_2\text{Cl}_2 (\text{s}) + 2\text{HCl}
\]

![Normalized intensity graph](image)

**Fig. 41 Combined XRD spectra of *Azolla filiculoides* contacted with HgCl₂ at pH 6**

**Table 13 Main X-Ray Diffraction lines for Mercury salts\(^\text{131}\)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Main Lines (2θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg₂Cl₂</td>
<td>21.45, 28.15</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>20.45</td>
</tr>
</tbody>
</table>
5.3.2 Cadmium sorption

This section reports on the removal of cadmium by *A. filiculoides* from aqueous solution. *A. filiculoides* shows a perfectly acceptable uptake with the maximum sorption observed at pH 6 of 0.35 mmol/g.

![Cadmium sorption isotherms for A. filiculoides](image)

**Fig. 42 Cadmium sorption isotherms for A. filiculoides**

pH can be considered a crucial parameter in the adsorption and ion exchange process. It governs the distribution and nature of the species present in solution as shown in Fig. 3 Chapter 4. Also the state of the functional groups present on the adsorbent is dependent on the solution pH. The sorption of cadmium by *A. filiculoides* increases with increase in pH. The lowest uptake is observed at pH 4, however if the pH were reduced further there would be a point where almost none would be removed. The cadmium would be competing for sites with very large quantities of hydrogen ions. The low pH would mean that very few of the ionisable groups would dissociate making it impossible for ion exchange to occur.

The copper, cadmium and mercury sorption isotherms show a greater increase in capacity from pH 4 - 5 than from 5 - 6 (see Figs. 15, 19 & 20). This may be explained in respect to the dissociation of carboxyl groups on pectin. Its pKa is approximately 3.5, this means that at pH 4 76% of the groups are dissociated, at pH 5
this value is 97% and at 6 it will be >99%. Freundlich’s model best described the experimental isotherm (see Table 14).

Table 14 Model parameters for cadmium sorption by *A. filiculoides*

<table>
<thead>
<tr>
<th>pH</th>
<th>Langmuir/Freundlich Fit [R^2]</th>
<th>Freundlich parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.891 0.989</td>
<td>k 0.166</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n 2.717</td>
</tr>
<tr>
<td>5</td>
<td>0.774 0.902</td>
<td>k 0.300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n 3.490</td>
</tr>
<tr>
<td>6</td>
<td>0.946 0.972</td>
<td>k 0.370</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n 4.685</td>
</tr>
</tbody>
</table>

5.3.3 Copper sorption

The uptake of copper from solution was evaluated for the particles of *A. filiculoides*. The metal ion is prevalent in the environment and in previous adsorption research and thus can easily be used as a comparison between this biosorbent and other sorbents. The maximum uptake observed was of 0.73mmol/g at pH 6 and 1mmol/l equilibrium concentration. This result shows the material performs better than the majority of biosorbent with only the algae class showing greater uptake (See Table 1 Chapter 2).

![Fig. 43 Copper sorption isotherms for *A. filiculoides*](image-url)
Similarly to cadmium a pH effect is evident for the sorption of copper from aqueous solution by *Azolla filiculoides*. Also a greater increase in uptake is evident between pH 4 and 5, than between 5 and 6. As before the dissociation of carboxyl groups is suggested as responsible for this observation. Similarly to cadmium sorption the isotherms were best described by the Freundlich equation (see Table 15).

**Table 15 Model parameters for copper sorption by *Azolla filiculoides***

<table>
<thead>
<tr>
<th>pH</th>
<th>Langmuir/Freundlich Fit [R²]</th>
<th>Freundlich parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>k, n</td>
</tr>
<tr>
<td>4</td>
<td>0.954 0.987</td>
<td>0.226 1.590</td>
</tr>
<tr>
<td>5</td>
<td>0.938 0.991</td>
<td>0.513 3.318</td>
</tr>
<tr>
<td>6</td>
<td>0.814 0.949</td>
<td>0.721 5.162</td>
</tr>
</tbody>
</table>

Fig. 44 A comparison between copper and cadmium adsorption by *Azolla filiculoides* at pH 6
Fig. 44 Shows the greater affinity of copper than cadmium for *A. filiculoides* at pH 6. *A. filiculoides* has a higher uptake of copper than cadmium. Comparably higher copper uptake has been observed by other researchers\textsuperscript{132,133} and explained as follows. The unusual electron configuration (shown in Chapter 3) enables copper to form distorted octahedral complexes. In these complexes some of the bonds are shorter than others and hence of higher strength.

### 5.4 Pectin’s role in the sorption process

One of the main molecular components of *A. filiculoides* biomass is the biopolymer pectin. Pectin is an acidic structural polysaccharide, found in fruit and vegetables and mainly prepared from waste citrus peel and apple pomace. The majority of the structure consists of homopolymeric partially methylated D-galacturonic acid residues, which contain hydroxyl, carbonyl and carboxyl functionality. To evaluate pectins role in the sorption of heavy metals pure samples of the solid were challenged with the three target metal solutions. The type of pectin chosen for this study was a low methyl grade. This was considered suitable since the acid wash stage of the material processing of *A. filiculoides* would result in a low methyl content pectin. Tel-Or et al examined the involvement of Pectin in strontium sorption by *A. filiculoides*.\textsuperscript{134} Pretreating the sorbent material with the enzyme pectinase reduced the binding capacity of the plant to Sr\textsuperscript{2+}. They also suggested that the higher sorption for Sr at elevated pH may in part at least be due to the de-esterification of pectin and exposure of additional carboxyl groups.
Fig. 45 Mercury sorption isotherm for Pectin, pH 4

Fig. 46 Cadmium sorption isotherm for Pectin, pH 4
The sorption isotherms for pectin show that this solid is proficient at removing trace toxic metals from aqueous solution. There are several salient points that can be elucidated from the graphs. In the case of all three metals tested there is very little or no uptake at low metal concentration. Initially this looks odd particularly when you compare it to the Langmuir (mercury) and Freundlich (copper/cadmium) models for *A. filiculoides*. This phenomena can be explained, however, by reference to the food industry where they use low-methyl pectin as a thickener in reduced sugar products. When used as a thickener it requires divalent cations of calcium to form a network of pectin molecules crosslinked by calcium as described in Fig. 48 below. It is suggested that at low divalent ion concentration there are not enough crosslinking atoms to produce the network and the pectins solubility reveals the limits of the experimental technique.

Generally, pectins do not possess exact structures. D-galacturonic acid residues form most of the molecules, in blocks of 'smooth' and 'hairy' regions. The molecule does not adopt a straight conformation in solution, but is extended and curved ('worm like') with a large amount of flexibility. The carboxylate groups tend to expand the
structure of pectins as a result of their charge, unless they interact through divalent cationic bridging.

![Diagram of pectin molecules](image)

**Fig. 48** Pectin molecules "gelling" by network formation with divalent calcium atoms.

The differences in shape of the curves suggest that the mechanism of mercury removal is different to that of the copper and cadmium. There is almost identical uptake for both copper and cadmium, which suggests that they are behaving similarly to calcium in the manufactured network and the difference in hydrated ionic radii has little or no effect.

Surprisingly there was no change in copper sorption capacity with increase in pH (see Fig. 49). This observation indicates that the mechanism of metal removal for the pure substance where it has freedom to orientate itself around the target molecule differs from that of, for example, *A. filiculoides* where any pectin present is contained within a rigid structure and interactions with neighbouring molecules may occur.

From the results shown above an equal uptake is displayed for both cadmium and copper ions. This was not the case for a recent study of heavy metal binding by pectin
performed by Kartel et al.\textsuperscript{137} In this study they tested pectin samples from a range of different sources and discovered the selectivity changed between samples with the common factor being poor adsorption performance with zinc and cadmium. Unfortunately during their experiments they did not use metals of a common salt and hence their sorption experiments cannot be compared as like for like. The maximum copper uptake that they observed (0.45 mmol/g) is approximately half of the uptake observed in this study. This can be accounted for by the fact that the pectin source used in this study was one of low methyl content and has approximately twice the proportion of carboxyl groups.

![Graph showing copper sorption by pectin at pH 4 and 6](image)

\textbf{Fig. 49 Comparison between copper sorption by pectin at pH 4 and 6}

It is interesting to note that the mercury sorption isotherm shows a lower capacity than that for copper and cadmium. This observation elucidates at least part of the mechanism responsible for metal uptake by \textit{A. filiculoides}. For copper and cadmium, the sorption capacity realised in the pectin trials reveals a mechanism that could be responsible for a large proportion of the biosorbents ability to sequester those metals. However the same cannot be said for mercury which gives a much lower uptake. This
leads to the conclusions that although pectin certainly plays a role in the uptake of the metals by *A. filiculoides* it is not the only cellular component responsible.

### 5.5 Proteins role in the sorption process

Following the observations of previous researchers that carboxyl bearing cellular residues such as pectin, and/or alginic acid were not the only biological components with the ability to adsorb metal ions from solution, insoluble proteins were also evaluated. Two proteins were chosen to trial due to their different sources and their readily available amino acid profile. Casein a protein from milk was sourced from Fisher, UK and has the amino acid profile shown in Table 16. Secondly a soya protein from RHM called Protena 1750 that is actually 50% soya protein isolate and 50% defatted soya flour, its amino acid profile is shown in Table 16.
If we compare the proline concentrations in the two protein sources it can be seen that casein contains 2.5 times as much. The conformation of caseins due to the high number of proline residues inhibits the formation of close-packed, ordered secondary structures. Also caseins contain no disulfide bonds. As well, the lack of tertiary structure accounts for the stability of caseins against heat denaturation because there is very little structure to unfold. Without a tertiary structure there is considerable exposure of hydrophobic residues. This results in strong association reactions of the caseins and renders them insoluble in water. Due to the minimal amount of secondary structure in casein proteins the groups which display charge separation are available

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Concentration [mmol/g]</th>
<th>Casein</th>
<th>Protene 1750</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic Acid</td>
<td>0.15</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>0.08</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>0.07</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>0.07</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>0.02</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>0.09</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Iso-Leucine</td>
<td>0.02</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>0.03</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>0.05</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.03</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.02</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.01</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>
to hydrogen bond with other molecules. In milk, casein forms a micelle with Ca\(^{2+}\) and phosphate groups, its biological function is creating a large network of casein molecules in order to transport CaP to mammalian young.

The heat treatment to deactivate the defatted soya flour could also cause glycolysation which would result in a loss of amino and hydroxyl groups which may not be accounted for in the amino acid mass balance. Heat treatment can also affect the secondary and tertiary structure of the protein chain. Without modelling the protein and metal ion interaction it would be impossible to say how this would affect the sorption uptake.

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Concentration [mmol/g]</th>
<th>Ratio Casein/Protena</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protena 1750</td>
<td>Casein</td>
</tr>
<tr>
<td>Carboxyl</td>
<td>0.153</td>
<td>0.228</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>0.153</td>
<td>0.228</td>
</tr>
<tr>
<td>Hydroxyl</td>
<td>0.063</td>
<td>0.044</td>
</tr>
<tr>
<td>Amino</td>
<td>0.129</td>
<td>0.211</td>
</tr>
<tr>
<td>Imine</td>
<td>0.045</td>
<td>0.044</td>
</tr>
<tr>
<td>Thiol</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>Thioester</td>
<td>0.005</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Table 17 Concentration of functional groups on proteins Protena 1750 and Casein
Table 18 Concentration of functional groups present as part of the amino acid content of *A. filiculoides*

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Concentration [mmol/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxyl</td>
<td>0.49</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>0.49</td>
</tr>
<tr>
<td>Hydroxyl</td>
<td>0.20</td>
</tr>
<tr>
<td>Amino</td>
<td>0.49</td>
</tr>
<tr>
<td>Imine</td>
<td>0.14</td>
</tr>
<tr>
<td>Thiol</td>
<td>0.01</td>
</tr>
<tr>
<td>Thioester</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Fig. 50 Cadmium sorption isotherms for proteins casein and soya protein at pH 4
Sorption trials on the protein samples with copper (Fig. 52) cadmium (Fig. 50) and mercury (Fig. 51) show that proteins do indeed have an affinity for the metal ions. A greater difference in mercury and cadmium uptake between the two sources of protein
is observed on Fig. 50 and Fig. 51 compared with copper sorption Fig. 52. It may be possible to attribute some of this difference to the greater proportion of thioester groups present on the casein sample. As discussed earlier the sulphur atom in the thioester group has a great affinity for soft metals such as cadmium and especially mercury. The uptake of mercury by casein is shown as 0.3 mmol/g at an equilibrium concentration of 0.5 mmol/l and a solution pH of 4. This is a superior uptake to that reported by Mishra et al.\textsuperscript{103}

The general trend is for approximately double the uptake to be realised in the casein sample compared with the soya protein. Table 17 compares the quantity of functional groups available on both samples and casein has approximately 1.5 times as many of the crucial carboxyl and carbonyl groups. Also due to the lower quantity of functional groups as a whole, the probability of metal ions forming a stable complex with suitably positioned active sites are greatly reduced.

\section*{5.6 Enhancement experiments}

\subsection*{5.6.1 Chitosan Bonding Technique}

Initial sorption experiments have shown that the biomaterial can adsorb the three metal ions of choice. The uptake observed was acceptable but significantly less than the specially tailored commercial resins, e.g. Purolite S-920, S-930 and Rohm and Haas GT-73. If the biomaterial could be modified in a simple and cost-effective manner then it may be possible to enhance the uptake for the metals in question. Several attempts were made and the more successful are outlined below with suggested future development.
Fig. 53 Comparison of cadmium uptake by *A. filiculoides* at pH 4 following different pre-treatment steps

An attempt was made to increase the uptake of *A. filiculoides* for cationic metal ions by applying different pre-treatment stages. As previously discussed, the literature states that chitosan is an effective heavy metal chelater. Elsewhere researchers have been immobilising enzymes on chitosan for certain specific applications. Using these two pieces of information it was attempted to attach chitosan molecules to the surface of *A. filiculoides* and increase its uptake of cationic metals.

Fig. 53 shows cadmium uptake by *A. filiculoides* following several different pre-treatments. Contacting the particles with a chitosan solution and then rinsing with DDW increases the capacity by 100%. The reason for the increase is possibly explained by the fact that chitosan solution becomes trapped within cracks on the sorbents surface or precipitates at the surface. It could also be held by weak electrostatic forces between functional groups on the solid surface and groups on the chitosan in solution. This method of enhancement is far from ideal as the chitosan molecules can detach from the sorbent surface easily and re-mobilise any complexed metal ions.
Secondly the material was contacted with glutaraldehyde solution which has the ability to form Schiff bases with amino groups. It is suggested that the following reaction takes place:

![Chemical structure of Schiff base formation](image)

**Fig. 54 Schiff base formation on reaction of an aldehyde with surface amino group**

Adsorption through coordination with the nitrogen atom as part of an amine or amide group is a well known phenomenon. A Schiff base is an amine–aldehyde complex as shown in Fig. 54, and are well known transition metal ion complexers. They are used, for example, in the production of catalysts for a wide range of oxidation reactions. The glutaraldehyde contacted material showed an increase in cadmium sorption capacity over the original material of 130%.
Fig. 55 Following the reaction of glutaraldehyde with the surface amino groups of *A. filiculoides* (Fig. 54) this is the subsequent reaction with chitosan. Dotted lines indicate that the chitosan polymer can continue with the repeating unit.

Finally the material was contacted with glutaraldehyde and chitosan simultaneously as shown above in Fig. 55. The greater quantity of Schiff bases now available on the adsorbent together with an increase in concentration of hydroxyl groups enhances the cadmium uptake of *A. filiculoides* by approximately 200%.
Fig. 56 Ions released by *A. filiculoides* and chitosan enhanced *A. filiculoides*

The pH titration curves of the chitosan attached Azolla and the original material is shown in Fig. 56. They follow almost the same pattern. At elevated pH the chitosan enhanced material deviates from the original material curve. This is attributed to the increase in hydroxyl groups and amine groups following the enhancement process. Both these groups have pK dissociation values of between pH 9 and 11.
5.6.2 Cysteine Impregnation

Fig. 57 A comparison of Hg uptake by *A. filiculoides* at pH 4 following different pre-treatments

The cysteine contact process is described in Chapter 3 and is a method extracted from a paper by Volesky in which he aimed to improve gold biosorption. The reactive groups on the cysteine molecule are amino, sulfhydryl and carboxyl groups, shown in Fig. 58. The dissociation constants (pKa) for these groups are 10.36, 8.12 and 1.90 respectively. The cysteine in solution can theoretically bind to positive groups on the biosorbent through its carboxyl group and any negative groups through its amino group. Volesky found that the main atoms responsible for gold binding were O, N and S. This is primarily due to the gold being in anionic form when complexed with cyanide. The atom considered important for mercury uptake is the sulphur that is present within cysteine. Sulphur and mercury form very stable complexes and therefore are extremely attracted to one another. An example of this is in mercury poisoning the method by which the mercury exhibits its toxicity is through binding with sulphur containing amino acids and reduces the molecule's availability for normal metabolic functions.
Fig. 58 Individual cysteine molecule in solution at pH 4

Fig. 59 Ions released curves for *A. filiculoides* and cysteine contacted *A. filiculoides*

The PZC on the modified Azolla adsorbent is slightly lower than the original material. This can be explained by the form of the cysteine that is used in the contact process. It is a salt of the hydrochloride, meaning it is bound to several molecules of HCl acid. If these remain with the cysteine molecule when it is adsorbed on the surface of the Azolla then they may dissociate when placed in solution under different conditions. The curve follows the trend of the original material and there is reasonable dissociation between pH 4 and 5 suggesting the carboxyl groups present due to pectin are not all involved in the cysteine adsorption process. It may be possible to
distinguish an additional inflexion at approximately pH 7. This can be explained by the dissociation of the thiol group on cysteine. It is slightly lower than the quoted dissociation constant for the cysteine thiol group at 8.12. This is not unsurprising as electron attracting or repelling atoms in close proximity to the thiol group can shift this value up or down. For example the pH titration of Rohm and Haas GT-73 (a thiol containing ion-exchange resin) shows a pK at approximately 6.5 (See Chapter 6).

It would be useful to confirm the presence of thiol groups on the surface as a positive way of characterising the material. This could back up the mass balance technique applied in this study. A possible approach would be attempt to observe an SH stretching band at 2560 cm\(^{-1}\) on IR analysis.\(^{142}\)

The model fitting also changes following the modification of the adsorbents surface. The Freundlich model now has the better fit.

5.7 Kinetics

There are three main factors when choosing an adsorbent for a particular process. The first is affinity of the adsorbate for the adsorbent. The second is the capacity of the adsorbent for the adsorbate and the third is the speed at which the adsorbent can immobilise the adsorbate. This third factor is studied in the following kinetics experiments.

The process of metal removal from the aqueous phase can be described as a heterogeneous reaction between solid and liquid. There is a sequence of reactions that can result in the sorption of a sorbate molecule.

- Diffusion of ions through the liquid film surrounding the particle.
- Diffusion of ions through any pore structure the solid possesses.
- Chemical reaction with the functional groups present on the adsorbent.

The slowest of the three stages will become the rate determining reaction.
If ion exchange is believed to be a mechanism then it is unlikely to be the rate determining step as it is generally considered to be a spontaneous reaction. However there are instances when changes in the ionic species present are required to enable an ion exchange reaction and these are responsible for the overall ion exchange reaction proceeding at a slower rate.

In most sorptive reactions the particle diffusion reaction is believed to be the rate-determining step. This is particularly true for some commercially produced resins with high degrees of cross linking and tortuous porosity. If there is little resistance to diffusion within the solid then the liquid diffusion can be the limiting factor, particularly in cases of low convective mixing.

![Graph](image.png)

**Fig. 60** Comparison of copper sorption by identical adsorbents of particle size range 1000 - 1200µm at 25°C and 6ppm initial concentration

Kinetic studies on the sorption of moieties can and has been studied in many different ways. One of the most popular appears to be having free particles in a reactor and the solution stirred by an impeller, see Chapter 3. The technique adopted by the author was one of containing the adsorbent particles within a pervious basket that is rotated within a solution of adsorbate (see Chapter 3).
A significant difference in sorption rates can be observed in the two different techniques used to show the rate of uptake of sorbate. There are several reasons for this. The impeller method creates a swirling bulk solution. The particles sit in this swirling bulk with little or no relative velocity. This means there is a much greater resistance to film transfer and subsequently slower sorption. In the case of the rotating basket the particles are essentially static and the liquid is forced past them resulting in a greater mass transfer rate due to a shallower liquid film. Other difficulties associated with the impeller method include particle break up as they get smashed on the fast moving blades. This is avoided in the rotating basket.

Fig. 61 Copper sorption rate for *A. filiculoides* at varying stirrer rates, 25°C and 6ppm initial concentration

The results presented in Fig. 61 show that sorption rate increases as the flow rate passing the adsorbent particle increases. The reason for the increase in sorption rate is that the liquid film that surrounds these particles decreases in size as the fluid velocity past the surface of the particles increases. This reduces the time it will take for any adsorbate to diffuse to the particles surface.
A kinetic study on the rate of copper uptake by the brown seaweed *Ascophyllum nodosum* revealed very little difference with increasing the stirrer speed. It was concluded that film diffusional resistance was insignificant in the sorption process. The method used was the simple impeller and this may not have been sensitive enough to show differences in sorption rate at different stirring rates.

![Graph showing metal uptake rates](image)

**Fig. 62** A comparison of metal uptake rates by *A. filiculoides* particles of 170-210µm at 25°C and 0.1mM initial concentration at 250 rpm

**Fig. 62** compares the sorption rate of different metals as they are removed from solution by the adsorbent *A. filiculoides*. Copper and cadmium are removed at nearly the same rate, with copper being slightly faster. This is because they are both largely in the 2+ ionic form with a strong attraction for negative surfaces. The copper ion is slightly smaller (see Table 19) and therefore will possess a faster diffusivity. The mercury sorption rate is considerably slower. This can be explained by the larger size of the mercury molecule compared to copper and cadmium. It is also in an uncharged state and as such does not benefit from electrostatic attraction to the solids surface. Furthermore, the mechanism is more complicated, and slower, involving reduction-precipitation compared to the ion exchange reaction attributed to copper and cadmium removal.
Fig. 63 Diagram showing the fluid streamlines around particles at (i) slow liquid velocity (ii) fast liquid velocity

Fig. 63 shows that the fluid streamlines move closer to the particle and reduces the liquid film boundary as the fluid velocity increases. In this way, it is possible to determine the effect of film diffusion by measuring the rate of adsorption under the same conditions and changing the fluid velocity.
Table 19 A comparison of the radii of the metal ions

<table>
<thead>
<tr>
<th>Metal</th>
<th>Complex Radii* [nm]</th>
<th>Hydrated Ionic Radii [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>0.419</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.426</td>
<td></td>
</tr>
<tr>
<td>Mercury</td>
<td>0.498</td>
<td>&gt;0.498</td>
</tr>
</tbody>
</table>

* estimated from addition of atom radii and bond lengths

These experiments revealed a difference in metal sorption rate with change in particle size. Fig. 64 shows that smaller particles reach their equilibrium uptake faster. This might indicate that there is an intra-particle diffusion resistance to overcome. It could also, however, be easily explained by the increase in surface area that smaller particles of the same mass would possess.

Kinetic properties of the different sized particles, different stirrer speeds and different sorbate molecules were compared by applying the Adam-Bohart-Thomas relation as
Azolla filiculoides, Characterisation and Evaluation

This mathematical expression was previously used for the sorption of organic compounds onto adsorbents and is represented as follows:

\[
\frac{dq}{dt} = K_1 C (q_m - q) - K_2 q
\]  

(2)

Where:

- \( q \) = Adsorption capacity [mg/g]
- \( C \) = Solution concentration [mg/l]
- \( K_1 \) = Adsorption kinetic constant [L/mg.s]
- \( K_2 \) = Desorption kinetic constant [1/s]
- \( q_m \) = Maximum surface concentration [mg/g]
- \( t \) = Time [s]

When \( t \to 0 \), \( q \to 0 \) and \( C \to C_0 \). Then equation (2) can be written as:

\[
\left( \frac{dq}{dt} \right)_{t \to 0} = \left( \frac{d(C - C_0)}{dt} \right)_{t \to 0} \frac{V}{m} = K_1 C_0 q_m
\]  

(3)

Where \( C_0 \) represents the initial concentration [mg/l], \( V \) the solution volume [l] and \( m \) the adsorbent weight [g]. It is then possible to calculate the initial adsorption kinetic coefficient “\( \gamma \)” represented as:

\[
\gamma = -K_1 q_m = -\frac{V}{C_0 m} \left( \frac{dC}{dt} \right)_{t \to 0}
\]  

(4)

The differential term \( (dC/dt)_{t \to 0} \) was calculated using the concentration decay after one minute of the start of the experiment. The values of “\( \gamma \)” are calculated by the model described above and shown in Tables 20 - 22.
Table 20 Initial adsorption kinetic co-efficient of copper using *Azolla filiculoides* of different particle sizes at 250 RPM

<table>
<thead>
<tr>
<th>Particle size µm</th>
<th>γ (L/mg min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>170-210</td>
<td>0.16</td>
</tr>
<tr>
<td>499-553</td>
<td>0.33</td>
</tr>
<tr>
<td>710-850</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Table 20 presents a faster kinetic coefficient for the smaller particles of *Azolla filiculoides* with adsorbing copper from solution. This result indicates a small resistance to intra-particle diffusion.

Table 21 Initial adsorption kinetic coefficient of copper using 499-553 µm *Azolla filiculoides* at different RPM

<table>
<thead>
<tr>
<th>RPM</th>
<th>γ (L/mg min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>0.70</td>
</tr>
<tr>
<td>200</td>
<td>0.36</td>
</tr>
<tr>
<td>250</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Table 21 reveals a faster sorption rate at higher liquid flow rate. Fig. 63 shows how the insulating film layer becomes smaller as the fluid velocity past the particle increases. This result suggests that there is also a film diffusion effect with the smallest film having the fastest sorption.

Table 22 Initial adsorption kinetic coefficient of different metals using 170-210 µm *Azolla filiculoides* at 250 RPM

<table>
<thead>
<tr>
<th>Metal</th>
<th>γ (L/mg min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0.16</td>
</tr>
<tr>
<td>Cd</td>
<td>0.19</td>
</tr>
<tr>
<td>Hg</td>
<td>0.37</td>
</tr>
</tbody>
</table>
Table 22 describes the differences in initial sorption kinetics of the three metals under identical conditions. As described earlier copper and cadmium are very similar with copper the fastest and mercury is by far the slowest sorbate molecule.

The Adam-Bohart-Thomas relation used as analysis tool in this research to compare the initial sorption rate for the various scenarios is limited to some extent. The empirical equation does not take into account film and intraparticle diffusion.

5.8 Packed column trials

Commercial ion exchange processes involving resins or granular activated carbon are typically run in a fixed bed column process. These columns will be generally be of a considerable size containing a large quantity of sorbent material. For research purposes mini-columns can be used, thereby scaling down the industrial process. Therefore columns of 12mm diameter were used to house an adsorbent bed of 5ml. The particle size range used was 170-210µm. Feed solutions were made up at 0.25mM and the flow through the column was set at 10 bed volumes (BV) per hour, which equated to 0.83ml min⁻¹. The low flow rate is important to allow for the sorbate to contact with active sites on the adsorbent and come to an equilibrium. In a recent study Strelko¹⁴⁵ concluded that by increasing the flow through an activated carbon column by 3 times reduced the adsorption efficiency for Pb by 25%.

The mini-column experiments conducted show the biosorbent can be used in an industrially applicable process. Single metal breakthrough curves have been plotted and selectivity has been investigated with multi-metal solutions.

Tel-Or et al 1996,¹⁴⁶ Attempted a crude column experiment using a funnel lined with A. filiculoides biomass. They passed a fixed volume of highly concentrated cadmium solution and reported a 70% removal of the metal in the effluent. Flow rate through the filter and feed pH were not mentioned in the paper. They also used A. filiculoides to remove mixed metal solutions containing cadmium and nickel but treated the metals together and did not try to show any preference for one over the other. A complimentary study to polish Cd and Ni wastes after metal base precipitation was
hindered by the high pressure applied to the filter, which created channelling and reduced the efficiency of the contact process.

Fig. 65 Copper normalised breakthrough curves for *A. filiculoides* in H⁺ form. Feed metal concentration 0.25mM at pH 6

The pH profile of the effluent provides useful information regarding metal-sorbent surface interactions. The pH of the effluent was significantly lower than the influent pH throughout the duration of the sorption experiments. If we look at the copper and cadmium breakthrough curves (Fig. 65 and Fig. 66), we can see a trough of low pH where the adsorption of the metal ions is taking place which gradually rises simultaneously with the metal concentration in the effluent. This suggests that the removal of these two metals by *A. filiculoides* is of an ion exchange nature whereby cations replace protons in acidic surface functional groups. The effluent pH rises as a consequence of less hydrogen ions being released from the sorbent as it becomes increasingly saturated with metal sorbate.
Fig. 66 Cadmium normalised breakthrough curves for *A. filiculoides* H⁺ form. Feed metal concentration 0.25mM at pH 6

Fig. 67 Copper and cadmium normalised breakthrough curves for *A. filiculoides* in H⁺ form. Total metal ion concentration in feed 0.25mM at pH 6
The concentration profile of the effluent leaving the Cu/Cd column (see Fig. 67) shows a dynamic equilibrium being set up between the cadmium and copper ions. The copper ions are preferred and so the cadmium ions are the first to exit the column. However they are competing for the same adsorption sites and so a small number of cadmium ions exchange with copper ions and vice versa. When the cadmium ions are adsorbed there is a dip in the cadmium exit concentration. This reverses when the copper replaces the adsorbed cadmium and the cadmium concentration momentarily exceeds the feed concentration. The oscillations in the cadmium concentration exiting the column are mirrored by inflexions in the copper concentration and also by the pH profile. These oscillations reduce in amplitude and will reach an equilibrium (stable plateau). There is a preference of the adsorbent for Cu over Cd. This result reveals that the sorption groups responsible have a higher affinity for the copper ion.

![Fig. 68 Mercury normalised breakthrough curve for A. filiculoides in H\(^+\) form. Feed metal concentration 0.25mM at pH 6](image-url)
Fig. 69 Cadmium and mercury normalised breakthrough curves for *A. filiculoides* in H⁺ form. Total metal ion concentration in feed 0.25mM at pH 6.

Mercury breaks through first when a mixed metal solution containing cadmium and mercury was passed through a column of *A. filiculoides* biomass (see Fig. 69). This may indicate that the sorbent has selectivity towards cadmium. The cadmium exhibits a very sharp breakthrough curve, which is preferential in column processes. This contrasts markedly with the concentration of mercury leaving the column. There is a rapid increase to approximately 50% of the inlet concentration and then a gradual ramp to C₀ = C₁ which matches its single metal breakthrough curve (see Fig. 68). This may be an indication that there is more than one mechanism responsible for mercury sorption. The steep section indicates ion exchange reactions or simple chelation with surface groups on the sorbent. The graph then follows a slow rise to a state of equilibrium, which it is suggested results from the reduction and precipitation of Hg₂Cl₂ occurring on the biomass. Another explanation might be that as the pH of the column increases more of the adsorbents ionogenic groups become available for reactions by dissociation. For example the pKₐ of carboxyl groups on pectin is 3.5. The pH profile mirrors the sorption of the two metals, a low pH existing in the effluent from the column whilst metals are being removed from solution and as breakthrough occurs there is a simultaneous increase in pH.
For the efficient and economic performance of an adsorption column process a rapid and effective regeneration step is essential. For cation exchange resins the regeneration process would typically involve an acid or sodium feed. Using several cycles operating with the same ion exchange resin reduces working costs of the treatment plant. However regeneration maybe ineffective if the removal mechanism involves a chelation or chemical reaction. In such instances for example mercury removal by a commercial resin then a high capacity for the sorbate is important and it becomes a one use medium.

The elution process is very efficient for copper and cadmium (see Fig. 70 and Fig. 71). Using 0.1M HCl as the eluant 95% and 97% were removed respectively. The same eluant was used with the mercury loaded biomass and only achieved a 53% removal (see Fig. 73). This result suggests that Cu and Cd are primarily bound by reversible ion exchange reactions whereas Hg is probably removed from aqueous solution by several different mechanisms.

![Fig. 70 Copper elution profile for A. filiculoides using 0.1M HCl](image)
Similar results were observed when attempting to remove copper and cadmium simultaneously (see Fig. 72) with equally high desorption rate and quantity as in the individual experiments described above.
This was contrasted by the difficulty in removing Hg. 55% was removed in 20 BV (see Fig. 73). It may be more suitable to try a different eluant for the removal of Hg, for example, it has great affinity to KI. Previous work trying to elute mercury from activated carbons showed little promise the most effective eluant being 0.5M HCl\textsuperscript{123}. The removal of mercury increased from 47% to 80% by increasing the concentration of eluting agent HCl from 0.1M to 0.5M. This improvement in desorption can to some extent be explained by the increased solubility of the precipitate Hg\textsubscript{2}Cl\textsubscript{2} in that media.

![Fig. 73 Mercury elution profile for A. filiculoides using 0.1M HCl](image)

For the desorption of the mixed metal column containing cadmium and mercury (see Fig. 74), again the removal of cadmium was simple and rapid. 98% being removed in 5 bed volumes of 0.1M HCl. The mercury profile is similar to its individual one where 50% is removed in a slow process.
Fig. 74 Cadmium and mercury elution profile for *A. filiculoides* using 0.1M HCl

### 5.9 Stirred Cell

In Fig. 75 there is more evidence of a reversible ion-exchange type reaction with copper sorption by *A. filiculoides*. The graph shows a pH profile during the sorption run that dips as H+ ions are replaced on the solid by copper ions. As the sorbent becomes saturated the pH in the stirred cell slowly increases to match that of the feed. The concentration curve presented follows that of a typical CSTR. The dotted horizontal line through the graph represents a change of feed from 0.25 mmol/L copper to 0.1M HCl. This elution process increases the concentration of copper in the cell in excess of the original feed solution. This is indicated by the peak immediately after the dotted line where Co/Ci is greater than unity.
Fig. 75 Cu sorption and elution by *A. filiculoides* in a stirred cell
6. Commercial Resins, Characterisation and Comparison

This chapter is included to compare the uptake of heavy metals by the natural materials discussed in this study and commercially available ion exchange resins specifically tailored for metal removal. The resins chosen for comparison are: Purolite S-930, this has iminodiacetic functionality and is recommended for cationic metal ion removal. Rohm and Haas GT-73 has thiol groups and is targeted at cadmium and mercury removal. Purolite S-920 is a thiouronium resin that is specifically applied to mercury sorption.

6.1 Chemical characterisation

6.1.1 Total Exchange Capacity

The sulphur containing resin S-920 is quoted by the manufacturer as having a lower exchange capacity (0.75eq/l) compared to S-930 (1.1eq/l) due to the number and type of functional groups introduced in the manufacturing process. It is therefore expected to have a lower total exchange capacity in these experiments. The ion exchange capacity of each resin has been determined and is given in Table 23.

<table>
<thead>
<tr>
<th>Material</th>
<th>Ion-Exchange Capacity [mmol/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rohm and Haas GT-73</td>
<td>2.75</td>
</tr>
<tr>
<td>Purolite S-920</td>
<td>3.16</td>
</tr>
<tr>
<td>Purolite S-930</td>
<td>4.28</td>
</tr>
</tbody>
</table>

All the resins are first washed with 0.1M HCl and then rinsed with DDW. For S-920 this treatment results in the protonation of the secondary amino group (see Fig. 76). This reaction depends on the pK of the group in question. The pH titration data presented below shows a dissociation between pH 6 and 8 giving an approximate pK
of 7 attributable to the secondary amino group then it could not have dissociated during the DDW wash (pH ~5.5). However on contact with a highly alkali solution used for the total ion exchange capacity experiment, this group will hydrolyse to give the original free base structure and resultant acidification of the solution. This pH change is what is measured by the experiment and termed “ion exchange capacity”.

\[
R\text{-CH}_2\text{-S-C}^{\text{\#}}\text{-NH}{_2}
\]

(i)

\[
R\text{-CH}_2\text{-S-C}^{\text{\#}}\text{-NH}^+\text{Cl}^{-}\text{NH}{_2}
\]

(ii)

Fig. 76 Functional groups present on S-920 (i) neutral state (ii) following acid and DDW wash

6.1.2 pH Titrations

pH titrations were performed on the commercially available chelating resins; Purolite S-920, S-930 and Rohm and Haas thiol resin GT-73. With the resulting data proton binding curves were plotted (see Fig. 77).

S-930 and GT-73 show curves that are characteristic of weak acid cation exchangers with slow dissociation of groups giving a flat curve. S-920, in contrast, shows an inflexion point as the pH increases meaning the dissociation of a surface functional group. As discussed in the previous section it is supposed that this is the dissociation of the acid group that is associated with the secondary amino groups present. The pKₐ of amino groups is generally in the region of 9, but the electrophilic nature of the sulphur atom in the thiouronium group can increase charge separation and reduce the pKₐ for any proximal groups.
6.1.3 Electrophoretic Mobility Measurements

The IEP is most negative for the thiol resin GT-73, which suggests that it has the strongest acidic groups present. The thiouronium resin (S-920) has the highest IEP ~ 6 which indicates dissociation of cations in near neutral solution. The data for A. filiculoides particles has been included for comparison and to help identify some of its functionality. A. filiculoides exhibits a similar IEP to the iminodiacetic acid resin S-930 therefore the groups responsible for the surface charge must dissociate at similar values. The gradient showing change in zeta potential per pH unit is clearly sharper in the commercial ion exchange resins. This is expected as it would suggest a greater density of functional groups.
Fig. 78 Electrophoretic mobility measurements using *A. filiculoides* and several commercially available adsorbents

It is also observed in Fig. 78 that the IEP of the three sorbents follows the same trend as the PZC (see Table 8), however the PZC is displaced slightly to the right. This is most likely due to the inclusion of internal surface area, increasing the total number of functional groups available for the reaction. The surface charge below and above the IEP can be explained in relation to the protonation and dissociation of acidic groups.

The comparison of PZC – IEP for the samples GT-73 and S-930 reveals a higher density of acidic groups on the external surface. S-920 has only a small difference between PZC and IEP. This can be accounted for, in part, by the practical difficulty in assessing pH in the neutral region.
The weakly basic thiouronium groups present on the surface of Purolite S-920 dissociate between pH 5 and 7. It has been suggested that thiouronium resins\textsuperscript{147} (e.g. Ionac SR-3, Sybron Chemicals Inc. and S-920, Purolite) have a useful working range at pH values up to 6. Beyond this value it is claimed that thiouronium groups are converted to thiol groups. A suggested mechanism for this is as shown in Fig. 79:

Figure 79 Suggested mechanism for the irreversible conversion of thiouronium groups on resin to thiol

The mechanism is initiated by OH\textsuperscript{-} ion attack. As the pH increases it is clear that the number of OH\textsuperscript{-} ions in solution will increase dramatically and increase the likelihood of the reaction occurring. Purolite's technical data sheet\textsuperscript{148} for this resin claims an operating pH range of 1-13. Therefore the above reaction is probably very slow. The
kinetics of the degradation could be studied in future work by analysis of the increasing concentration of urea produced.

6.2 Mercury Sorption

![Graph of Mercury sorption isotherms for Rohm and Haas GT-73 resin]

GT-73 displays a Freundlich modelled adsorption trend (parameters shown in Table 27) and a very large uptake. There is little or no effect of pH on the uptake. There is great affinity of the adsorbent for the adsorbate as described by the extremely sharp isotherm at low metal concentration.

Table 25 Model parameters for mercury sorption by GT-73

<table>
<thead>
<tr>
<th>pH</th>
<th>Langmuir/Freundlich Fit [$R^2$]</th>
<th>Freundlich parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.850 0.974</td>
<td>k = 4.764</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 3.654</td>
</tr>
<tr>
<td>6</td>
<td>0.773 0.971</td>
<td>k = 4.853</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 4.083</td>
</tr>
</tbody>
</table>
Fig. 81 Mercury sorption isotherms for Purolite S-920 resin

Table 26 Model parameters for mercury sorption by S-920

<table>
<thead>
<tr>
<th>pH</th>
<th>Langmuir/Freundlich Fit $[R^2]$</th>
<th>Freundlich parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.971 0.981</td>
<td>k 6.139</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n 1.623</td>
</tr>
</tbody>
</table>

Fig. 81 shows the mercury sorption isotherm for S-920 at pH 4. It is shown to have a lower uptake than Rohm and Haas GT-73 and is also best fitted with the Freundlich equations (see Table 27).

Comparison of mercury sorption in Fig. 80 and Fig. 81 shows that the highest uptake is achieved by GT-73 resin with 2.99 mmol/g at 0.15mM/l equilibrium concentration and pH 4.
Table 27 Model parameters for mercury sorption by commercial resins

<table>
<thead>
<tr>
<th>Material / pH</th>
<th>Model</th>
<th>R² fit</th>
<th>Model Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-920 4</td>
<td>Freundlich</td>
<td>0.969</td>
<td>k 6.14  n 1.62</td>
</tr>
<tr>
<td>GT-73 4</td>
<td>Freundlich</td>
<td>0.960</td>
<td>k 4.51  n 4.57</td>
</tr>
<tr>
<td>GT-73 6</td>
<td>Freundlich</td>
<td>0.976</td>
<td>k 4.68  n 4.90</td>
</tr>
</tbody>
</table>

6.2.1 Scanning Electron Microscopy (SEM)

Ion exchangers S-920 and GT-73 are smooth polymeric spherical particles with no visible signs of porosity see Fig. 82 and Fig. 83. Neither of the resins show the speckles of light areas shown in Chapter 5 in the SEM images of the biosorbent following contact with HgCl₂. This suggests a more even distribution of any mercury adsorbed from solution.

Fig. 82 Backscatter SEM image of Purolite S-920 following contact with HgCl₂
6.2.2 Energy Dispersive Spectroscopy (EDS)

In a similar fashion to the biosorbent following mercury sorption trials, the commercial resins were examined by EDS to confirm the elements present on their surface. The results shown below, Fig. 84 and Fig. 85 for S-920 and GT-73 respectively indicate the presence of mercury and chlorine.

![Fig. 83 Backscatter SEM image of Rohm and Haas GT-73 following contact with HgCl₂](image)

![Fig. 84 EDS spectra of S-920 equilibrated with HgCl₂ at pH 6](image)
Fig. 85 EDS spectra of GT-73 equilibrated with HgCl₂ at pH 6

6.2.3 X-Ray Diffraction (XRD)

The XRD spectra for HgCl₂ contacted S-920 (Fig. 86) and GT-73 (Fig. 87) differ markedly from those shown for A. filiculoides in the previous chapter. They both have a broad undulation from around 18-25 degrees, attributed to organic groups, which would be expected for a polystyrene/DVB resin. The lack of distinguishing peaks in the 21.43 and 28.15 2theta range (refer to Table 13) suggest that these two ion exchangers remove mercury by a completely different mechanism from the one observed for the biosorbent.
Fig. 86 XRD spectra of S-920 contacted with HgCl₂ at pH 6

Fig. 87 XRD spectra of GT-73 contacted with HgCl₂ at pH 6
Table 28 Main X-Ray Diffraction lines for Mercury salts

<table>
<thead>
<tr>
<th>Compound</th>
<th>Main Lines (2θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg₂Cl₂</td>
<td>21.45, 28.15</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>20.45</td>
</tr>
</tbody>
</table>

It would be interesting to further this study by analysing mercury sorption by the cysteine enhanced biosorbent at different equilibrium pH values. It would be expected to follow a similar pattern as the GT-73 resin whereby there was very little if any influence of pH on the uptake of mercury. The extraction of mercury from solution has also been shown to be independent of hydrogen ion concentration by other researchers. In both cases the adsorbent used contained a thiol group and the reason given was that the sorption mechanism is not ion exchange and that it was due to the extremely high affinity of mercury to the thiol group.

### 6.3 Cadmium sorption

The ion exchange resins were challenged with cadmium solution to compare their performance and elucidate the mechanism of adsorption. Purolite S-930 shows a large uptake of cadmium with uptake of 1.4 mmol/g at pH 6 and 1mmol/l equilibrium concentration (Fig. 88). The resin uptake was compared at equilibrium pH values of 4 and 6. There is almost a 100% increase in cadmium uptake at the higher pH. The carboxyl groups present on the resin are responsible for the sorption of the metal and typically have pK values of 2-5. The inference that can be made from this sorption data is that the pK lies at approximately pH 4, where 50% of its groups will have dissociated. Therefore at pH 6 99% of the carboxyl groups will be in the deprotonated form.
The cadmium sorption data for Purolite S-930 is fitted by the Langmuir equation. The resin is mono-functional and the model fitting suggests that the sorption is also mono-layer.

Rohm and Haas GT-73 resin has also been evaluated for cadmium removal from aqueous solution. The isotherms generated show a high capacity but noticeably less than S-930. The greatest sorption is observed at pH 6 and equilibrium concentration 1mmol/l of 0.95mmol/g. There is a similar effect of pH with GT-73 exhibiting greater cadmium uptake at pH 6 than 4. If the thiol groups introduced onto the resin during the manufacturing process partake in ion exchange then this increase could be explained by a greater quantity of groups being in the dissociated form. However the pK for thiol groups is typically around 8. The sulphur atom can also form coordinate bonds with the electron deficient metal ion. In this situation there will be greater mobility of the metal cations and less shielding of sites in the reduced hydrogen ion environment.
Comparing the cadmium uptake by all three materials shows that S-930 has the greatest uptake. However the biosorbent has a significant capacity that can be entirely applicable in a water treatment process.
6.4 Copper sorption

S-930 was contacted with a copper solution in order to study the pH effect on the isotherm. When comparing the sorption of copper to cadmium a similar result was observed as that for A. filiculoides in the previous chapter. The copper uptake for S-930 is greater than its ability to adsorb cadmium. For example at a pH of 6 and equilibrium solution concentration of 0.8 mmol/l it can adsorb 1.70 mmol/g of copper but only 1.40 mmol/g cadmium (see Fig. 93). The resin consists of a single type of functional group that adsorbs via ion exchange (see Fig. 92) and the adsorbate molecules are both divalent cations. It is expressed in the manufacturers data sheet that the selectivity order is Cu>Ni>Zn>Co>Cd>Fe(II)>Mn>Ca and there can be several explanations for this. The smaller hydrated ionic radii means that it has more mobility in relation to surface active sites. The iminodiacetic acid resin also has a nitrogen atom. As discussed earlier the lone pair of electrons on nitrogen can form coordinate bonds with metal ions and produce more stable bonds with copper than cadmium.

![Fig. 91 Copper sorption isotherm for Purolite resin S-930](image)

The copper sorption isotherms also show a much sharper curve at low copper concentrations displaying a strong affinity of the adsorbent for the metal. As with the
cadmium isotherms for this resin the experimental data is fitted with the Langmuir model. The difference in stability of transition metal ion complexes may be due to the fact that as the metal ion decreases in radius, the metal-ligand interactions increase in magnitude.\textsuperscript{151}

The donor atoms in the ligands which form the most stable complexes with hard metals tend to be small, electronegative and only slightly polarisable. They are also difficult to oxidise. These are defined as hard bases.\textsuperscript{152} The donor atoms of ligands which tend to form the most stable complexes with soft metals are larger, less electronegative and highly polarisable. They are easier to oxidise and are referred to as soft bases. Cadmium is a soft metal and as such forms stable complexes with soft bases such as H\textsuperscript{+} and CO. Copper however is a borderline Hard/Soft metal and as such has a better interaction with amino groups and OH\textsuperscript{−}. The terms hard and soft are very imprecise but do provide a guide to predicting complex stability.

\[ 
\ce{R-CH2-N(CH2-C==O) + M^{2+} -> R-CH2-N(CH2-C==O-M) + 2H^{+}} 
\]

\textbf{Fig. 92 Mechanism for Ion exchange with Purolite S-930 resin}\textsuperscript{153}
1.8-1
1.6 ý
1.4 -I
jl. 2I
[Image 0x0 to 2172x3494]
[870x3406]Fig. 93 A comparison of copper and cadmium sorption by Purolite S-930 at pH 6

6.5 Chapter conclusions

The characterisation of the resins helps to explain their behaviour in near neutral solution. The resins, as expected, show high capacity for the target sorbate molecules. They are also presented in a reasonably suitable form, as rigid small diameter spheres. There are however several disadvantages associated with using ion-exchange resins. Resin beads swell when changing their loaded form and the beads are damaged if the change in volume occurs rapidly. The loading kinetics depend on the surface area in contact with the solution, and so in order to obtain fast kinetics, small diameter beads are required. If the ion exchange beads are used in a column, there is a very substantial pressure drop across it, and so pumping costs can be appreciable. Fine solids may block the fine pores of the beads thus reducing their capacity. The thiol resins, as discussed earlier in this chapter, suffer from oxidation and consequent reduced reactivity. The largest disadvantage the resins have in comparison with the biosorbents is that of cost. At the time of writing the following prices were obtained for the resins:
Purolite S-930 £12.50 /l
Purolite S-920 £15 /l
Rohm and Haas GT-73 £22 /l

The biosorbent used in this study was primarily gratis and due to its prevalence and weed status could be harvested on a larger scale at small cost. The various subsequent processing steps required by this study to produce a homogeneous hydrogen form adsorbent of known particle size would not necessarily all need to be carried out for large scale operation.
7 Conclusions and Recommendations for Future Work

7.1 Conclusions

The plant *A. filiculoides* has been processed to create hydrogen form adsorbent particles. The biosorbent has been successfully applied to the removal of selected heavy metals e.g. copper, cadmium and mercury from aqueous solution. The sorption capacity was highest for copper (0.63 mmol/g) then mercury (0.53 mmol/g) and finally cadmium (0.31 mmol/g) at pH 6 and 0.5 mmol/l equilibrium concentration.

Adsorption experiments have been run in both batch and column mode. Single metal uptake has been evaluated and the effect of pH studied in the range of 4-6. For each metal studied the adsorption on *A. filiculoides* was greater at higher pH with 355, 206 and 228% higher uptake at pH 6 than at 4 for copper cadmium and mercury respectively.

The sorption capacity of the biosorbent for the selected metal ions was significantly increased by chitosan attachment (cadmium) and by cysteine impregnation for mercury. The chitosan attached material exhibited a 388% increase in cadmium uptake at pH 4. When the cysteine impregnated biomass was challenged with a mercury solution a 270% increase in uptake was observed. The Freundlich model fitted experimental data well for copper and cadmium sorption on *A. filiculoides*, whereas mercury was best described by the Langmuir model. Following cysteine impregnation the shape of the mercury isotherm changed to better match the Freundlich model. This was due to a change in the sorption mechanism and matches the shape of the commercially produced resin with similar functionality.

Quantification of the ion exchange capacity of the biosorbent was carried out by potentiometric titrations. The results show an applicable concentration of weakly acidic surface functional groups that compare favourably with as-received activated carbon. The difference between PZC and IEP show that the biosorbent has a reasonably homogeneous surface charge distribution in comparison to, for example,
activated carbon. This is due to the material having no defined pore structure, merely an external surface with large voids and surface cracks due to the mechanical material processing.

The mechanical properties of the \textit{A. filiculoides} are similar to the brown marine algae previously studied in their mechanical rigidity. However they do not show considerable swelling on rewetting and therefore are unlikely to cause the bed expansion problems associated with swelling.

Kinetic coefficients were used to compare the sorption of the different metal ions by \textit{A. filiculoides}. Copper was the fastest bound metal with mercury the slowest. This was explained by the hypothesis that mercury is bound by a two-phase reduction precipitation reaction. In contrast copper and cadmium are considered to be primarily sorbed by an ion-exchange mechanism and of the two copper has the smallest hydrated radii and subsequently faster diffusion constant.

Packed column studies were carried out and acceptable breakthrough curves were obtained for the copper and cadmium target metals. Dual metal solutions were processed showing a dynamic equilibrium between the ionic copper and cadmium with copper being the preferred cation. When mercury was run through the column it displayed a much slower approach to equilibrium. Mercury and cadmium were run simultaneously and cadmium was the preferred metal. The pH profile of the effluent leaving the column matched the breakthrough profile of the metal ions copper and cadmium. The release of hydrogen ions from the sorbent was a consequence of metal removal from solution. The pH profile generated from the mercury column experiment had a much smaller initial dip in effluent pH. A slow, much flatter curve was then plotted showing less dependence on the exchange of hydrogen ions than the ion exchange reactions involved for copper and cadmium. Regeneration experiments were performed and copper and cadmium were successfully eluted with hydrochloric acid to 97%. Conversely mercury was poorly eluted with no more than 50% recovered indicating a different mechanism of removal.

The \textit{A. filiculoides} biomass may be successfully employed in treating mildly acidic copper, cadmium and mercury bearing wastewater streams. Its performance is
Conclusions and Recommendations for Future Work

comparable with conventional iminodiacetic resin and oxidised active carbon which possess similar reactive sites for metal sorption. The biomaterial can be re-generated if required or due to its lower cost simply stored or destroyed.

7.2 Recommendations for future work

It is clear from the work presented in this thesis that the biosorbent particles prepared from *Azolla filiculoides* has great potential for removal of trace toxic metals from aqueous solutions. The suitable metal sorption capacity and relatively low cost suggest that this material could be competitive in the water treatment market. Development of new biosorption materials would be of interest and benefit to water treatment industries. It would require examining biomass types for metal sorption properties and evaluating the uptake of the target toxic metals, precious metals, radionuclides, etc.

There is still not great enough understanding of biosorption metal-binding mechanisms. For different biosorbents the process by which it can remove pollutants can vary widely, e.g. ion exchange, chelation, microprecipitation, etc. An extension to this work could investigate the sorption mechanism of copper, cadmium and mercury in more detail.

The work completed in this study could be extended by placing emphasis on mathematical modelling and metal binding simulation. This might include mapping the conformation of active sites that enable the adsorption process to take place.

In the processing, pre-treatment and formulation of new biosorbent materials it would be pertinent to consider the shape and mechanical rigidity of the final solids so as to be applicable within industrial technology such as packed columns.

Desorption of adsorbed species was studied in this work but an extension to include: process modelling, simulation and scale up studies may lead to take up of the concept at an industrial scale. To this end it would be beneficial to trial sample waste streams
where the added complications of ionic strength, additional ligands and even suspended solids will affect the sorption process.

There is plenty of interest and scope for further work into the exact mechanisms of mercury removal from solution. The results in this thesis show quite different uptakes of the metal by the various adsorbents and evidence of difference in the bound species. The adsorption studies could be extended to investigate the removal of organic mercury species such as methyl mercury since these species are highly toxic and often predominate in wastewaters.

Increasing an adsorbents uptake for a target pollutant is an attractive aim, particularly if it can be achieved with little incurred cost. The metal uptake enhancement experiments performed as a part of this thesis could be optimised by altering the experimental parameters such as temperature and solid/liquid ratio.
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Purolite, Technical Data Sheet, Macroporous Iminodiacetic Chelating Resin S-930
The sorption isotherms for pectin show that this solid is proficient at removing trace toxic metals from aqueous solution. There are several salient points that can be elucidated from the graphs. In the case of all three metals tested there is very little or no uptake at low metal concentration. Initially this looks odd particularly when you compare it to the Langmuir (mercury) and Freundlich (copper/cadmium) models for *Azolla filiculoides*. This phenomena can be explained, however, by reference to the food industry where they use low-methyl pectin as a thickener in reduced sugar products. When used as a thickener it requires divalent cations of calcium to form a network of pectin molecules crosslinked by calcium as described in Fig. 48 below. It is suggested that at low divalent ion concentration there are not enough crosslinking atoms to produce the network and the pectins solubility reveals the limits of the experimental technique.

Generally, pectins do not possess exact structures. D-galacturonic acid residues form most of the molecules, in blocks of 'smooth' and 'hairy' regions. The molecule does not adopt a straight conformation in solution, but is extended and curved ('worm like') with a large amount of flexibility. The carboxylate groups tend to expand the
Appendix

List of publications


This paper investigates the use of commercially available and modified activated carbon and a natural biosorbent for the removal of cadmium from water. A wood-based activated carbon, AUG WHK, was acid oxidised to enhance its metal binding capacity. The leaves of a water fern, Azolla filiculoides were separated from the roots and ground into particles and acid washed to create a uniform hydrogen form adsorbent. These materials were subsequently studied for the removal of cadmium ions from aqueous solution. The sorption performance of these materials for cadmium is compared. The physical structure of the adsorbents has been investigated using scanning electron microscopy, nitrogen and amino acid content and BET surface area. Carbon adsorbents were characterised by N2 adsorption at 77K before and after oxidation, and a quantitative determination of weak-acid surface groups was carried out by direct titration. The BET surface area decreased considerably after oxidation, however, the total amount of oxygen-containing surface groups was 3.3 times higher compared to the untreated adsorbent. Cadmium adsorption isotherms were performed at pH values of 4, and 6 showing an increase in capacity as pH increases. The maximum capacity for the sorbents was 0.08, 0.33, 1.40 mmol/g for the three adsorbents: unoxidised WHK, Azolla filiculoides and acid oxidised WHK, respectively. Kinetic experiments showed that the materials were all rapid adsorbents of cadmium, with 80% of capacity reached in 0.2 hours for all three materials.

Keywords: cadmium, biosorbent, activated carbon, granular carbon, adsorbents, kinetics, oxidation.

INTRODUCTION
The presence of heavy metals in effluents is a world-wide environmental problem. There are a wide range of industries that produce heavy metal waste, therefore efficient and cost-effective methods of water treatment are essential. Cadmium is prominent on the EU Black List of priority pollutants that are highly toxic and a serious threat to life. It is a carcinogen and causes damage to the kidneys. Cadmium is used extensively in electroplating due to its corrosion resistance and is a component in the expanding market for rechargeable batteries. Concentrations of the metal can reach 100ppm in surrounding areas adjacent to mines, smelters and Ni-Cd battery plants. Therefore, cost-effective methods of removing this trace metal are in great demand. At present a number of technologies, such as chemical precipitation, electroplating, evaporation, adsorption and ion exchange, are used to treat heavy metal containing wastewaters. Conventional chelating ion exchange resins can be effective
but their production costs are a limiting factor. The above methods, other than adsorption and ion exchange, are not efficient or cost effective when the concentrations of metal ions are as low as 100ppm and the required concentration in the treated water is almost at the limit of detection 1.

Adsorption has been widely applied for the removal of trace contaminants from potable water, domestic water and industrial effluents. Sorption of heavy metals on activated carbon is not a simple process because it depends on several factors such as water chemistry and the surface reactivity of the adsorbent material. Granular activated carbons are extensively used in wastewater treatment for the removal of a wide range of contaminants. They possess high mechanical rigidity, well defined pore size distribution and offer extensive surface area for sorption of metal ions from aqueous solutions.

The use of naturally occurring plants as biosorbents for the removal of trace toxic metals is extensively studied on the laboratory scale but has not yet found widespread industrial application. Biosorption defines processes that remove contaminants from wastewater by either metabolic or physico-chemical pathways 2. Many biological materials have been investigated for their ability to remove cadmium ions from solution. These include bacteria 3, fungi 4 and most commonly algae 5,6,7. When considering biomass as a commercial process, the abundance and availability of the material are important considerations. In the case of algae, seaweeds can be harvested directly or received as recycled waste from the algin production industry. Azolla filiculoides is a fast free-growing “weed” that re-produces prodigiously, covering and blocking many waterways around the world. Biosorbents generally have a lower capacity than commercial ion exchange resins and modified activated carbons, however, they are regenerable and low-priced. A. filiculoides has already been shown to be very effective in repeatedly removing many pollutants from waste waters 8,9,10, including cadmium 11.

It is the aim of this work to compare the cadmium sorption capacity abilities of a commercial and modified granular activated carbon and a natural biosorbent. Sorption isotherms and kinetic experiments were performed to describe their performance. Samples were characterised, chemically and physically, by acid/base titration, pH titration, nitrogen and amino acid content, BET surface area and Scanning Electron Microscopy.

EXPERIMENTAL

MATERIALS
A. filiculoides was received from The University of Liverpool, Department of Biological Sciences. This was frozen in liquid nitrogen and the leaves separated from the roots. The leaves were then selected as they had shown a significantly greater cadmium sorption capacity than the roots. These were then ground into particles using a mortar and pestle. The particles were dried and sieved to 170-210µm. A wood based granular activated carbon WHK, supplied by AUG Germany, was sieved to a particle size fraction of 170-210µm, washed carefully with distilled water and then dried in an oven at 378K until no change in weight was observed. Cadmium solution was prepared using CdCl2·H2O laboratory grade purchased from May & Baker Ltd., Dagenham, England. Sodium hydroxide, nitric acid, hydrochloric acid and potassium chloride were prepared from analytical reagents supplied by Fisher, UK. Aldrich Chemicals, USA, supplied volumetric standard solutions of sodium hydroxide, sodium carbonate, and HPLC grade ethanol. Sodium hydrogen carbonate and sodium ethoxide solutions were prepared from analytical reagents purchased from Aldrich Chemicals, USA.
CHARACTERISATION

Surface Area
The Surface area of granular activated carbons was obtained by nitrogen adsorption and desorption at 77 K using a Micromeritics ASAP2010 automatic surface area analyser. The samples were outgassed for 24 hours at 378 K under a vacuum of <10 µmHg.

Scanning Electron Microscopy (SEM)
Scanning Electron Microscopy pictures of granular carbons and A. filiculoides were taken using a Cambridge Stereoscan 360 operated at an accelerating voltage of 10 kV.

Nitrogen Analysis
Nitrogen analyses for biosorbent were conducted using the Kjelhdahl Method. Duplicate 1g samples of A. filiculoides and native and dealginated seaweeds: Ascophyllum nodosum and Lessonia flavicans (both supplied gratis by Kelco, UK) were weighed on filter paper and placed into digestion vessels on a Buchi B435 digestion unit. The nitrogen control sample was a known weight of ammonium sulphate (to calculate process efficiency). The samples were then heated for 45 minutes at 623 K with sulphuric acid and catalyst pellets, to complete the hydrolysis stage. The hydrolysed product was steam distilled for 3 minutes using a Buchi 323 Kjeldahl distillation unit. The resulting distillate was titrated against a 0.1M hydrochloric acid solution, using screened methyl red indicator in a 2% boric acid solution.

Amino Acid Analysis
Samples of A. filiculoides were hydrolysed in order to liberate the amino acids. This was achieved by adding 0.5ml of 0.1M phenol (to reduce oxidation) and 4.5ml of 6.6M HCl to 100mg of the sample. The samples were heated at 383 K for 24 hours and then allowed to desiccate until dryness. Distilled water was twice added and evaporated to ensure removal of all HCl. The amino acids were then obtained as hydrochlorides. The samples were analysed by ion exchange High Performance Liquid Chromatography (HPLC) using a Kontron Analytical Chromakon 500. The results were compared against standards and quantities of each amino acid were ascertained.

pH Titration
15 ml of a 0.1M NaCl solution was added to 25ml Erlenmeyer flasks. The solution pH was varied by adding, a total volume of 5 ml, 0.1M NaOH, HCl and/or distilled de-ionised water. Then, 10mg of neutrally buoyant adsorbent particles, <90 µm, were added to the flasks. The samples were stirred for 48 hours at room temperature to allow them to reach equilibrium. The initial (before the addition of adsorbent) and final pH were measured. Blank samples, under the same conditions, were titrated at the same time for comparison. The electrophoretic mobility of the equilibrated samples was measured using a Malvern Instruments Zetasizer 3000HSA.

Acid/Base Titration
The distribution of oxygen-containing groups was analysed by direct titration using the Boehm method. The samples were contacted with bases of different strength, NaOH, NaCO3, NaHCO3 and NaOC2H5 (dissolved in HPLC grade ethanol). A pre-determined amount of adsorbent was placed in a 50 ml conical flask and then contacted with 20 ml of each alkali solution. The flask was sealed and stirred using an orbital shaker at 300 min⁻¹ for seven days. The solution was filtered using a 0.2 µm PTFE syringe top filter to remove adsorbent particles. Finally a 5 ml aliquot was titrated with volumetric standard solution of
HCI, using a glass burette (tolerance ± 0.02ml), with methyl red as indicator. A simple mass balance was used to determine the ion exchange capacity of each oxygen-containing group.

**Batch Sorption**

A pre-determined amount of adsorbent was added to a 100ml conical flask containing 50ml of cadmium solution, of known initial concentration and pH. Samples were agitated by an orbital shaker at 300 min⁻¹ at room temperature. The cadmium solution pH was checked and adjusted daily by addition of 0.1M NaOH or HCl until a constant pH was attained. The samples were deemed to have achieved equilibrium when no significant change in pH was observed (± 0.1 units) in a 24-hour period. The equilibrated samples were filtered using a 0.2µm PTFE syringe top filter to remove the adsorbent particles and then analysed for cadmium concentration, using a Varian SpectraAA-200 atomic adsorption spectrophotometer in flame mode at 228.8nm wavelength. Blank samples using the same solutions under the same conditions without adsorbent were prepared for comparison.

**Kinetic Experiments**

990ml of distilled water was added to a round-bottomed flask. Then, 1g of adsorbent was placed into a rotating basket made of perspex and plastic mesh (opening 50 µm)¹⁴. The basket containing adsorbent was placed in the reactor and connected to a stirrer. The adsorbent was contacted with distilled water for 1 hour prior to the start of the experiment to allow trapped air to diffuse out and in the case of the biomass for particle swelling. 10ml of cadmium solution, of known initial concentration, was added to the reactor and the timer and the stirrer motor (set at 250 min⁻¹) started immediately. This was noted as the zero-time of the experiment. Samples were collected at certain time intervals and analysed for cadmium concentration. The experiments were run for up to 3 hours and the temperature was kept at 298 K by a temperature control unit.

**RESULTS AND DISCUSSION**

**NITROGEN CONTENT AND AMINO ACID ANALYSIS**

Previous metal sorption experiments on seaweed algae have attributed metal removal to functional groups present as part of the polysaccharide algin¹⁵. However, a significant residual metal sorption capacity remains after the alginates have been chemically removed¹⁶. It was suggested that this residual capacity can be attributed to functional groups associated with protein in the material. Protein is composed of a polymer of amino acids joined by primary amine and carboxyl groups. It is the functionality of the side chain that is of importance in metal binding. Amino acids contain a wide variety of side chains but only two are ionised in the pH range of interest i.e. the carboxylic groups on aspartic and glutamic acid (see Figure 1).
Table 1 Nitrogen content of several biomaterials

<table>
<thead>
<tr>
<th>Material</th>
<th>Percent Nitrogen</th>
<th>Estimated Percent Protein*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Azolla filiculoides</em></td>
<td>3.80</td>
<td>23.76</td>
</tr>
<tr>
<td>De-alginate <em>Lessonia flavicans</em></td>
<td>3.20</td>
<td>20.02</td>
</tr>
<tr>
<td><em>Lessonia flavicans</em></td>
<td>1.90</td>
<td>11.86</td>
</tr>
<tr>
<td>De-alginate <em>Ascohyllum nodosum</em></td>
<td>1.66</td>
<td>10.38</td>
</tr>
<tr>
<td><em>Ascohyllum nodosum</em></td>
<td>0.79</td>
<td>4.94</td>
</tr>
</tbody>
</table>

* Using AOAC international protein factor of 6.25

The biomaterials were analysed for nitrogen and this value was converted to a protein concentration using the general Association of Official Analytical Chemists (AOAC) factor of 6.25, which assumes the nitrogen content of the protein is 16%. Table 1 shows a high nitrogen content per unit mass for the dealginated seaweeds. *A. filiculoides* contains more than twice the nitrogen content of native *L. flavicans* and *A. nodosum*.

Figure 2 shows that *A. filiculoides* has a high concentration of the useful amino acids that may be involved in metal binding. 14.2% of the amino acids were aspartic acid and 10.2% glutamic acid.

**OXYGEN-CONTAINING GROUPS**

Figure 3 shows the concentration and type of functional groups on activated carbons. It can be seen that the concentration of oxygen-containing groups increases considerably after acid oxidation, but not in equal proportion. As-received granular activated carbon (WHK) contains carbonyl surface groups in the highest concentration. Acid oxidation results in an increase of 2, 3, 5 and 9 times higher for carbonyl, lactonic, phenolic and carboxyl groups, respectively. It is clear that carboxyl groups are introduced in the highest concentration, which will render acid oxidised carbon (WHK) more efficient in the treatment of drinking water since carboxyl groups are completely dissociated at near-neutral pH.

**PH TITRATION**

The surface chemistry of the adsorbents is extremely important in the sorption of metal ions and has to be studied in detail. The point of zero charge (PZC) is a useful parameter and can be determined by pH titration. PZC is the pH at which the net surface charge (internal and external) is zero. This point can be deduced in Figure 4. The PZC for commercial granular carbon is at pH 4.5 whereas after acid oxidation it is shifted to pH 3.5. This behaviour is attributed to an increase in acidic surface groups, e.g. carboxyl, phenolic and carbonyl. The increase of these functional groups is also reflected in high concentration of ions released, \( H^+ \), with increasing pH (see Figure 4). The surface is positively charged in conventional and modified granular carbon WHK at pH values below the PZC where the oxygen-containing groups are undissociated and the adsorbent is able to remove anionic species. On the other hand, at pH values greater than the PZC, the sorbent surface becomes increasingly negative due to the dissociation of weakly acidic oxygen-containing groups. Hence, the adsorbent surface is able to attract and exchange cations in solution.

Alternatively, *A. filiculoides* has a proton binding curve that does not show a PZC within the experimental range (above pH 2). This means that the charge on the surface is always negative which is characteristic of a weak acid cation exchanger.
ELECTROPHORETIC MEASUREMENTS

The zeta potential (ZP) obtained by electrophoretic measurements at different pHs is reported in Figure 5. ZP is an index of the magnitude of interaction between colloidal particles. Colloidal suspensions/dispersions of fine particles in a liquid phase possess an electric charge that depends on the nature of the solid surface and the surrounding medium. The point of zero net external surface charge is defined as the isoelectric point (IEP), which is located at the crossover point shown in Figure 5. The IEP for commercial and modified granular carbon is at pH 2.19 and 0.90 respectively, whereas for A. filiculoides it is at a pH of 1.42. The surface charge below and above the IEP can be explained in terms of the protonation and dissociation of oxygen-containing groups. It has already been mentioned that the PZC relates to the internal and external surface, whereas the IEP refers only to the external surface of the adsorbent. Hence, it can be deduced that the distribution of acidic surface groups is not homogeneous since the IEP is located at lower pH values. This indicates that the concentration of acidic groups is higher at the external surface as compared to the interior of the adsorbent.

SCANNING ELECTRON MICROGRAPHY

The SEMs presented in Figure 6 show the surface morphology of commercial and modified carbons, respectively. Un-oxidised carbon shows a well-defined and regular distribution of pores, whereas the oxidised sample shows irregular openings and roughness produced by chemical erosion. This is reflected in the loss of surface area. In comparison, the SEM of A. filiculoides leaves shows no sign of porosity.

BATCH EXPERIMENTS

Natural biosorbent, A. filiculoides, commercial and oxidised granular activated carbons, WHK, were tested for the removal of cadmium from aqueous solution. The sorption of cadmium at an equilibrium concentration of 0.8mM and pH 6 was 3.7 times higher for A. filiculoides than for commercial WHK (see Figure 7). Under the same conditions acid oxidised WHK showed 4 times higher cadmium capacity than A. filiculoides. This was expected since the concentration of oxygen-containing groups, found by acid/base titration, increased after chemical modification. However, BET surface area of the oxidised carbon decreased from 1912 to 714m$^2$/g due to the chemical reaction. An adsorbent with this surface area is entirely suitable for water treatment. The tendency for cadmium uptake is also reflected in the proton binding curves and electrophoretic mobility measurements. The concentration of ions released and zeta potential versus pH increases in the following order: commercial WHK, A. filiculoides and acid oxidised WHK. These results are in total agreement with the amount of cadmium removed by the adsorbents investigated in this research.

The effect of pH on adsorption was investigated and is reported in Figure 8. An increase of 53.12 and 58.33 % in cadmium uptake at 0.8mM was found when the solution pH was increased from 4 to 6 for A. filiculoides and oxidised granular carbon WHK, respectively. This is attributed to increased dissociation of acidic surface groups as the pH increases. For example the pK values of carboxylic groups lies between 2 and 5.
Table 2 Freundlich isotherm parameters for the adsorption of cadmium

<table>
<thead>
<tr>
<th>Material</th>
<th>pH</th>
<th>$k_f$ ( \frac{[\text{mg}^1\text{L}^{-1}]}{\text{mg}^1\text{g}^{-1}} )</th>
<th>$n$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. filiculoides</td>
<td>4</td>
<td>0.161</td>
<td>2.915</td>
<td>0.995</td>
</tr>
<tr>
<td>A. filiculoides</td>
<td>6</td>
<td>0.350</td>
<td>5.495</td>
<td>0.989</td>
</tr>
<tr>
<td>Acid-ox. WHK</td>
<td>4</td>
<td>0.726</td>
<td>3.401</td>
<td>0.995</td>
</tr>
<tr>
<td>Acid-ox. WHK</td>
<td>6</td>
<td>1.267</td>
<td>7.937</td>
<td>0.988</td>
</tr>
<tr>
<td>Un-ox. WHK</td>
<td>6</td>
<td>0.081</td>
<td>5.208</td>
<td>0.919</td>
</tr>
</tbody>
</table>

The isotherms (Figures 7 and 8) were fitted using the Freundlich adsorption model, which had the best correlation of the experimental data compared with the Langmuir model. The parameters are shown in Table 2.

It has been mentioned that the surface chemistry and the metal speciation in solution are essential parameters to an understanding of the sorption mechanism. The speciation diagram for 0.1M CdCl$_2$ in aqueous solution (see Figure 9) was calculated using the equilibrium constants reported by Stumm and Morgan. Cadmium appears as Cd$^{2+}$, CdCl$^+$ and CdCl$_2$(aq) below pH 7.6 in the approximate proportions of 58, 39 and 3 %, respectively. Cadmium precipitates above pH 7.6 as Cd(OH)$_2$. Therefore cation exchange and/or complexation with surface functional groups is the most likely sorption mechanism.

The results presented in this section show that natural biosorbent, A. filiculoides, has 3.7 times higher cadmium capacity than commercial granular carbon WHK. Biosorbents are potentially useful for water treatment since they possess satisfactory capacities for metal ions and have a distinct economic advantage. However, it is shown that by oxidising the granular carbon WHK it is possible to obtain a cadmium sorption capacity greater than A. filiculoides. The drawbacks are that this process incurs extra cost and reduces the mechanical strength of the material. Oxidised carbons may also leach humic substances during subsequent use in water treatment.

**KINETICS**

Kinetic data are plotted in Figure 10 and this shows that the adsorption rate for cadmium is extremely fast for all the adsorbents. A significant difference is observed after 0.2 hours, when 94% capacity is reached with activated carbons compared to 82% for the biosorbent. Rapid sorption kinetics in these experiments can be attributed to the relatively small and close size distribution of particles and well-defined pore size distribution for the carbons. It has been shown that there is little or no porosity in the biosorbent, hence there are no internal diffusion constraints in the sorption mechanism.

**CONCLUSIONS**

The capacity of biomass for cadmium is 4 times greater than as-received commercial granular carbon WHK. The oxidation of commercial activated carbon increases sorption capacity for cadmium by a factor of 15 compared with the as-received material. There is, however, a subsequent loss in surface area due to the chemical reaction. Biosorbents are potentially useful for water treatment since they possess satisfactory capacities for metal ions and have a distinct economic advantage. All the materials displayed fast sorption kinetics, more than
80% capacity was reached in 0.2 hours, making them suitable for conventional column techniques.

ACKNOWLEDGEMENTS
The authors would like to thank for financial assistance during the period of this research, Severn Trent Water PLC and EPSRC. J. R. Rangel-Mendez appreciates the scholarship (ref. 70767/125253) from Consejo Nacional de Ciencia y Technologia (CONACyT), Mexico.
Fig. 1 Amino acids, (a) aspartic acid and (b) glutamic acid
Fig. 2 Amino acid profile of *A. filiculoides*
Fig. 3 Oxygen containing groups on conventional and modified granular carbon WHK
Fig. 4 Proton binding curves for granular carbon WHK and *A. filiculoides*
Fig. 5 Electrophoretic mobility measurements using granular carbon WHK and *A. filiculoides*
Fig. 6 Scanning Electron Micrographs of: (a) conventional WHK, (b) Acid oxidised WHK, (c) *A. filiculoides*
Fig. 7 Equilibrium cadmium sorption isotherms for granular carbon and *A. filiculoides* at pH 6 and room temperature.
Fig. 8 Equilibrium cadmium sorption isotherms for oxidised granular carbon and A. filiculoides at pH 4 and 6, and room temperature.
Fig. 9 Speciation diagram of 0.1 M CdCl$_2$ in aqueous solution at 298.15 K
Fig. 10 Comparison of kinetics for granular carbons and the biosorbent
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CHARACTERISATION, EVALUATION AND APPLICATION OF ECONOMICAL ADSORBENTS IN CADMIUM REMOVAL FROM AQUEOUS SOLUTIONS

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ABSTRACT

This paper investigates the use of more economical adsorbents: commercially available and modified activated carbon and a natural biosorbent for the removal of cadmium from water. A commercial wood based activated carbon, AUG WHK, was modified using nitric acid to enhance its ion exchange capacity. Biosorbents offer considerable potential but have yet to be exploited in any particular niche for the removal of trace toxic metals in wastewater treatment. Samples of Azolla filiculoides were harvested and the roots separated from the leaves. Results showed that the maximum cadmium capacity for conventional as-received WHK was 0.07mmol/g whereas for A. filiculoides it was 0.3mmol/g at pH 6. BET surface area of WHK decreased slightly after oxidation but there was a substantial increase in cadmium uptake to 1.09 mmol/g at pH 6. Sorption experiments were conducted at pH 4 and 6 and these showed that there was an increase in the adsorption of cadmium with increase in pH for both the granular activated carbon and biosorbent. Kinetic experiments with oxidised WHK revealed a rapid sorption rate for cadmium (sorption half-time of 2.5 minutes). This was significantly faster than the biosorbent, which gave a half-time of 16 minutes. Packed column runs gave sharp concentration profiles with breakthrough (10%) occurring at 15 bed volumes (BVs) for the biosorbent and 5 and 140 BVs for the as-received and oxidised carbon. Regeneration was readily achieved for both adsorbents in excess of 95 % using 0.1M HCl.

Keywords: cadmium, biosorbent, activated carbon.

INTRODUCTION

The presence of heavy metals in effluents is a world-wide environmental problem. There are a wide range of industries that produce heavy metal waste, therefore efficient and cost effective methods of water treatment are essential. Cadmium is prominent on the EU Black List of priority pollutants that are highly toxic and a serious threat to life. It is a carcinogen and causes damage to the kidneys. Cadmium is used extensively in electroplating due to its corrosion resistance and is a component in the expanding market for rechargeable batteries. Concentrations of the metal can reach 100ppm in areas adjacent to mines, smelters and Ni-Cd battery plants. At present a number of technologies, such as chemical precipitation, electroplating, evaporation, adsorption and ion exchange, are used to treat heavy metal containing wastewaters. Conventional chelating ion exchange resins can be effective but their production costs are a limiting factor. Other than adsorption and ion exchange these methods are not efficient or cost effective when the concentrations of metal ions are as low as 100ppm and the required concentration in the treated water is almost at the limit of detection [1].
Adsorption has been widely applied for the removal of trace contaminants from potable water, domestic water and industrial effluents. Sorption of heavy metals on activated carbon is not a simple process because it depends on several factors such as water chemistry and the surface reactivity of the adsorbent material. Granular activated carbons are extensively used in wastewater treatment for the removal of a wide range of contaminants. They possess high mechanical rigidity, well defined pore size distribution and offer extensive surface area for sorption of metal ions from aqueous solutions. The use of naturally occurring plants as biosorbents for the removal of trace toxic metals is extensively studied on the laboratory scale but has not yet found widespread industrial application. Biosorption defines processes that remove contaminants from wastewater by either metabolic or physico-chemical pathways [2]. Many biological materials have been investigated for their ability to remove cadmium ions from solution. These include bacteria [3], fungi [4] and most commonly algae [5,6,7]. When considering biomass as a commercial process, the abundance and availability of the material are important considerations. *Azolla filiculoides* is a fast free-growing "weed" that re-produces prodigiously, covering and blocking many waterways around the world. Biosorbents generally have a lower capacity than commercial ion exchange resins and modified activated carbons, however, they are regenerable and cheap. *A. filiculoides* has already been shown to be very effective in removing many pollutants from waste waters [8,9,10], including cadmium [11].

It is the aim of this work to compare the cadmium sorption capacity abilities of a commercial and modified granular activated carbon and a natural biosorbent. Sorption isotherms and kinetic experiments were performed to describe their performance. Samples were characterised by pH titration, zeta potential and BET surface area.

**EXPERIMENTAL**

**Materials**

*A. filiculoides* was received from The University of Liverpool, Department of Biological Sciences. This was frozen in liquid nitrogen and the leaves separated from the roots. The leaves were then selected as they had shown a significantly greater cadmium sorption capacity than the roots. The leaves were ground into particles using a mortar and pestle and then dried and sieved to 170-210µm. A wood based granular activated carbon WHK, supplied by AUG Germany, was sieved to a particle size fraction of 170-210µm, washed carefully with distilled water and then dried in an oven at 378K until no change in weight was observed. Cadmium solution was prepared using CdCl₂·H₂O laboratory grade purchased from May & Baker Ltd., Dagenham, England. Sodium hydroxide, nitric acid, hydrochloric acid and potassium chloride were prepared from analytical reagents supplied by Fisher, UK. Aldrich Chemicals, USA, supplied volumetric standard solutions of sodium hydroxide, sodium carbonate, and HPLC grade ethanol. Sodium hydrogen carbonate and sodium ethoxide solutions were prepared from analytical reagents purchased from Aldrich Chemicals, USA.

**pH Titration**

15 ml of a 0.1M NaCl solution was added to 25ml Erlenmeyer flasks. The solution pH was varied by adding, a total volume of 5 ml, 0.1M NaOH, HCl and/or distilled de-ionised water. Then, 10mg of neutrally buoyant adsorbent particles, <90 µm, were added to the flasks. The samples were stirred for 48 hours at room temperature to allow them to reach equilibrium. The initial (before the addition of adsorbent) and final pH were
measured. Blank samples, under the same conditions, were titrated at the same time for comparison. The electrophoretic mobility of the equilibrated samples was measured using a Malvern Instruments Zetasizer 3000HSA.

**Batch Sorption**
A pre-determined amount of adsorbent was added to a 100ml conical flask containing 50ml of cadmium solution, of known initial concentration and pH. Samples were agitated by an orbital shaker at 300 min\(^{-1}\) at room temperature. The cadmium solution pH was checked and adjusted daily by addition of 0.1M NaOH or HCl until a constant pH was attained. The samples were deemed to have achieved equilibrium when no significant change in pH was observed (± 0.1 units) in a 24-hour period. The equilibrated samples were filtered using a 0.2µm PTFE syringe top filter to remove the adsorbent particles and then analysed for cadmium concentration, using a Varian SpectraAA-200 atomic adsorption spectrophotometer in flame mode at 228.8nm wavelength. Blank samples using the same solutions under the same conditions without adsorbent were prepared for comparison.

**Kinetic Experiments**
990ml of distilled water was added to a round-bottomed flask. Then, 1 g of adsorbent was placed into a rotating basket made of perspex and plastic mesh (opening 50 µm) [12]. The basket containing adsorbent was placed in the reactor and connected to a stirrer. The adsorbent was contacted with distilled water for 1 hour prior to the start of the experiment to allow trapped air to diffuse out and in the case of the biomass for particle swelling. 10ml of cadmium solution, of known initial concentration, was added to the reactor and the timer and the stirrer motor (set at 250 min\(^{-1}\)) started immediately. This was noted as the zero-time of the experiment. Samples were collected at certain time intervals and analysed for cadmium concentration. The experiments were run for up to 3 hours and the temperature was kept at 298 K by a temperature control unit.

**Column Experiments**
Small plastic columns were packed with 0.5g of sorbent (170-210 µm particle size). A solution containing approximately 1 mM of cadmium at pH 6 was passed through the column, at 10 BVs/h, to obtain the breakthrough curves. Elution of cadmium was carried out using 0.1M HCl. Samples were collected by a fraction collector.

**RESULTS AND DISCUSSION**

**pH Titration**
The surface chemistry of the adsorbents is extremely important in the sorption of metal ions and has to be studied in detail. The point of zero charge (PZC) is a useful parameter and can be determined by pH titration (Figure 1). PZC is the pH at which the net surface charge (internal and external) is zero [13]. This point can be deduced in Figure 1. The PZC for commercial granular carbon is at pH 4.5 whereas after acid oxidation it is shifted to pH 3.5. This behaviour is attributed to an increase in acidic surface groups, e.g. carboxyl, phenolic and carbonyl. The increase of these functional groups is also reflected in high concentration of ions released, H\(^+\), with increasing pH (see Figure 1).
Figure 1. Proton binding curves for granular carbon WHK and A. filiculoides

The surface is positively charged in conventional and modified granular carbon WHK at pH values below the PZC where the oxygen-containing groups are undissociated and the adsorbent is able to remove anionic species. On the other hand, at pH values greater than the PZC, the sorbent surface becomes increasingly negative due to the dissociation of weakly acidic oxygen-containing groups. Hence, the adsorbent surface is able to attract and exchange cations in solution.

A. filiculoides has a proton binding curve that displays a low PZC of 2.7. This means that the charge on the surface is always negative in the experimental range which is characteristic of a weak acid cation exchanger.

Electrophoretic Measurements

The zeta potential (ZP) obtained by electrophoretic measurements at different pHs is reported in Figure 4. ZP is an index of the magnitude of interaction between colloidal particles. Colloidal suspensions/dispersions of fine particles in a liquid phase possess an electric charge that depends on the nature of the solid surface and the surrounding medium [14]. The point of zero net external surface charge is defined as the isoelectric point (IEP), which is located at the crossover point shown in Figure 2. The IEP for commercial and modified granular carbon is at pH 2.19 and 0.90 respectively, whereas for A. filiculoides it is at a pH of 1.42. The surface charge below and above the IEP can be explained in terms of the protonation and dissociation of oxygen-containing groups. It has already been mentioned that the PZC relates to the internal and external surface, whereas the IEP refers only to the external surface of the adsorbent. Hence, it can be deduced that the distribution of acidic surface groups is not homogeneous since the IEP is located at lower pH values. This indicates that the concentration of acidic groups is higher at the external surface as compared to the interior of the adsorbent.
Figure 2. Electrophoretic mobility measurements using granular carbon WHK and A. filiculoides

Batch Experiments
Natural biosorbent, A. filiculoides, commercial and oxidised granular activated carbons, WHK, were tested for the removal of cadmium from aqueous solution. The sorption of cadmium at an equilibrium concentration of 0.8mM and pH 6 was 3.7 times higher for A. filiculoides than for commercial WHK (see Figure 3).

Figure 3. Equilibrium cadmium sorption isotherms for granular carbon and A. filiculoides at pH 6 and room temperature.

Under the same conditions acid oxidised WHK showed 4 times higher cadmium capacity than A. filiculoides. This was expected since the concentration of oxygen-containing groups increased after chemical modification. However, BET surface area (determined by nitrogen adsorption at 77K) of the oxidised carbon decreased from 1912 to 714m²/g due to the chemical reaction. An adsorbent with this surface area is entirely suitable for water treatment. The tendency for cadmium uptake is also reflected in the proton binding curves and electrophoretic mobility measurements. The concentration of ions released and zeta potential versus pH increases in the following order: commercial WHK, A.
filiculoides and acid oxidised WHK. These results are in total agreement with the amount of cadmium removed by the adsorbents investigated in this research.

The effect of pH on adsorption was investigated and is reported in Figure 4. An increase of 53.12 and 58.33% in cadmium uptake at 0.8mM was found when the solution pH was increased from 4 to 6 for A. filiculoides and oxidised granular carbon WHK, respectively.

![Figure 4. Equilibrium cadmium sorption isotherms for oxidised granular carbon and A. filiculoides at pH 4 and 6, and room temperature.](image)

This is attributed to increased dissociation of acidic surface groups as the pH increases. For example the pK values of carboxylic groups lies between 2 and 5 [15]. The isotherms (Figures 3 and 4) were fitted using the Freundlich adsorption model, which had the best correlation of the experimental data when compared with the Langmuir model.

It has been mentioned that the surface chemistry and the metal speciation in solution are essential parameters to an understanding of the sorption mechanism. The speciation diagram for 0.1M CdCl₂ in aqueous solution was calculated using the equilibrium constants reported by Stumm and Morgan [16]. Cadmium appears as Cd²⁺, CdCl⁺ and CdCl₂(aq) below pH 7.6 in the approximate proportions of 58, 39 and 3%, respectively. Cadmium precipitates above pH 7.6 as Cd(OH)₂. Therefore cation exchange and/or complexation with surface functional groups is the most likely sorption mechanism.

The results presented in this section show that natural biosorbent, A. filiculoides, has 3.7 times higher cadmium capacity than commercial granular carbon WHK. Biosorbents are potentially useful for water treatment since they possess satisfactory capacities for metal ions and have a distinct economic advantage. However, it is shown that by oxidising the granular carbon WHK it is possible to obtain a cadmium sorption capacity greater than A. filiculoides. The drawbacks are that this process incurs extra cost and reduces the mechanical strength of the material. Oxidised carbons may also leach humic substances during subsequent use in water treatment.

**Kinetics**

Kinetic data are plotted in Figure 5 and this shows that the adsorption rate for cadmium is extremely fast for all the adsorbents. A significant difference is observed after 0.2 hours, when 94% capacity is reached with activated carbons compared to 82% for the biosorbent. Rapid sorption kinetics in these experiments can be attributed to the relatively small and close size distribution of particles and well-defined pore size
distribution for the carbons. We have observed that there is little or no porosity in the biosorbent, hence there are no internal diffusion constraints in the sorption mechanism.

Figure 5. Comparison of kinetics for granular carbons and the biosorbent

Column Experiments
The breakthrough curves reported in Figure 6 shows the difference in cadmium capacity between the commercial GAC WHK and A. filiculoides. The oxidised WHK sample shows a great difference in sorption performance that was expected due to the higher content of oxygenated surface groups introduced during the modification process. Mini-column studies also show that the biosorbent treated 15 BVs at 10% whereas as-received WHK and oxidised samples 5 and 140 BVs, respectively. The elution of cadmium was conducted using 0.1M HCl. A cadmium mass balance shows that more than 95% was recovered after 5 BVs. This results suggest that Cd is primarily bound by reversible ion exchange reactions.

Figure 6. Cadmium breakthrough curves for samples in H form. 1 mM cadmium feed solution at pH 6.
CONCLUSIONS
The capacity of biomass for cadmium is 4 times greater than as-received commercial granular carbon WHK. The oxidation of commercial activated carbon increases sorption capacity for cadmium by a factor of 15 compared with the as-received material. There is, however, a subsequent loss in surface area due to the chemical reaction. Biosorbents are potentially useful for water treatment since they possess satisfactory capacities for metal ions and have a distinct economic advantage. All the materials displayed fast sorption kinetics, also shown in packed columns, more than 80% capacity was reached in 0.2 hours, making them suitable for conventional column techniques. Cadmium adsorption by GAC WHK and A. filiculoides is highly reversible meaning that the sorption mechanism is mainly due to ion exchange.

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The authors would like to thank for financial assistance during the period of this research, Severn Trent Water PLC and EPSRC. J. R. Rangel-Mendez appreciates the scholarship (ref. 70767/125253) from Consejo Nacional de Ciencia y Tecnologia (CONACyT), Mexico.

REFERENCES
Mercury sorption from aqueous solution by chelating ion exchange resins, activated carbon and a biosorbent

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Abstract

This paper discusses Hg(II) sorption from aqueous solution onto different adsorbents e.g., a wood based granular activated carbon (WHK), two commercially available chelating ion exchange resins (Purolite S-920 containing isothiouronium functional groups and Rohm and Haas GT-73 containing thiol functional groups) and a biosorbent (Azolla filiculoides).

These sorbents were characterised using scanning electron microscopy (SEM), determination of nitrogen and amino acid content, BET surface area by N₂ adsorption at 77K, acid/base titration, ion exchange capacity and electrophoretic measurements. Samples were also characterised by energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD) after contact and equilibration with mercury solution. These

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techniques were used in an attempt to elucidate the mechanisms involved in mercury sequestration.

The reduction of Hg(II) to Hg(I), i.e., soluble mercuric to insoluble mercurous chloride (Hg₂Cl₂) on the adsorbent surface was found to be a controlling reaction mechanism for sorption on granular activated carbon and *Azolla filiculoides*. Kinetic experiments showed that mercury sorption was rapid on all of the materials. Batch equilibration experiments indicated that Rohm and Haas GT-73 has the highest mercury sorption capacity. Although mercury sorption was studied at elevated concentrations similar to those encountered in industrial effluents, it is suggested that the findings would also apply to final stage water treatment. As a general rule, it was found that mercury (II) removal increased with pH.

Keywords: mercury, adsorption, biosorbent, granular activated carbon, ion exchange resin, kinetics.
Introduction

Toxic heavy metals in water are a global problem and an increasing threat to the environment. There are many potential sources of heavy metal pollution that include the coal, natural gas, metal-finishing and mining industries. Specifically this paper is concerned with environmental pollution of mercury. Mercury is introduced to natural waters primarily from the discharges of the chlorine manufacturing industry but also arises in effluents from battery and plastics manufacture and oil refining (1).

Exposure to high levels of metallic, inorganic, or organic mercury can permanently damage the brain, kidneys, and developing foetus. Effects on brain function may result in irritability, tremors, changes in vision or hearing and memory problems (2).

The Environmental Protection Agency (EPA) has determined that mercuric chloride and methyl mercury are possible human carcinogens. Mercury from both natural and anthropogenic sources tends to bio-accumulate with increasing trophic levels.

Industrial organisations treat liquid effluents using conventional techniques to meet the strict legislation that regulates discharge to water bodies. Typical treatments include chemical precipitation, with and without the addition of soluble chelating agents, electroplating and evaporation. These methods are generally successful when the concentration of toxic metals is relatively high (> 100 ppm). However, adsorption and ion exchange are far more effective and cost-efficient when the concentration of trace toxic metals is less than about 100ppm (3). A variety of adsorbents and ion exchange
resins have been developed and used for this purpose, e.g., activated carbon, natural zeolites, biosorbents and polymeric ion exchange resins.

The term biosorption is used to describe a process whereby contaminants are removed from wastewater by either metabolic or physico-chemical pathways (4). The potential for metal concentration by certain types of biomass, either dead and alive, has been well established over the last two decades (5,6,7,8,9). Biosorption invariably uses raw materials that are either abundant in nature (e.g. seaweed) or waste materials from other industrial operations (e.g. fermentation). For example, *Azolla filiculoides* is a fast-growing weed that reproduces rapidly and floats on the surface in many waterways around the world. It is available in abundance since it is routinely removed by water utilities during maintenance to avoid blockages. In general, biosorbents have a lower sorption capacity than commercially available ion exchange resins and/or modified activated carbons.

The objective of this paper is to outline the physico-chemical properties and sorption characteristics of several different adsorbent materials capable of removing mercury from aqueous solutions at simulated effluent concentrations.

**Experimental**

**Materials**

Samples of *A. filiculoides* were originally obtained from the University of Liverpool, Department of Biological Sciences and subsequently cultivated in our laboratory. The plants were frozen in liquid nitrogen and the leaves separated from the roots. The leaves
were then ground into particulate form using a mortar and pestle. The particles were dried and sieved to 170-210µm. A wood based granular activated carbon WHK, supplied by AUG Germany, was sieved to a particle size fraction of 170-210µm, washed carefully with distilled water and then dried in an oven at 378K until no change in weight was observed. Commercial ion exchange resins, Purolite S-920 (containing isothiouronium functional groups) and Rohm and Haas GT-73 (containing thiol functional groups) were used in the hydrogen form in the bead size range 170-210µm.

Mercury solution was prepared using HgCl₂ purchased from Acros Chemicals. Sodium hydroxide, nitric acid, hydrochloric acid and potassium chloride were prepared from analytical reagents supplied by Fisher, UK. Aldrich Chemicals, USA, supplied volumetric standard solutions of sodium hydroxide, sodium carbonate, and HPLC grade ethanol. Sodium hydrogen carbonate and sodium ethoxide solutions were prepared from analytical reagents purchased from Aldrich Chemicals, USA. Rhodamine 6G was made up according to Ramakrishna (10) using the salt from Acros Chemicals, potassium iodide, sodium thiosulphate, both Fisher Chemicals, UK, and potassium hydrogen phthalate, BDH Chemicals Poole, England.

Sodium Capacity

A series of experiments was conducted to determine the total sodium exchange capacity of sorbents used in this study. 50mg of adsorbent was placed into a conical flask containing 25ml of 0.1M volumetric standard sodium hydroxide. This was agitated at 300 min⁻¹ for 48 hours. The solution was then filtered and 5ml of the sample was back-titrated with 0.1M HCl using methyl red as the indicator.
Batch Sorption Isotherms

10mg of adsorbent was added to a 50ml flask containing 500ml of mercury solution of known initial concentration and pH. Relevant personal protective equipment was worn at all times when handling mercury. Samples were agitated and the solution pH was checked and adjusted daily by addition of 0.1M NaOH or HCl until a constant pH was attained. The samples attained equilibrium when no significant change in pH was observed (± 0.1 units) in a 24-hour period. The equilibrated samples were filtered using a 0.2µm PTFE syringe top filter to remove the adsorbent particles, and then analysed at 575 nm using a UV/VIS spectrophotometer (Perkin Elmer lambda 12). Mercury analysis was performed using the method reported by Ramakrishna et al. using rhodamine 6G where the detection limit is 1ppb (5 x 10⁻⁶ mmol/l). Blank samples using the same solutions, under the same conditions, but without adsorbent were prepared for comparison.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy pictures of samples were taken, before and after the sorption process, using a Leica Cambridge Stereoscan S360 operated in electron backscatter mode at an accelerating voltage of 20 kV.

Energy Dispersive Spectroscopy (EDS)

A Stereoscan S360 instrument equipped with an energy dispersive spectroscopy system was used. This instrument focuses electrons at certain locations on the surface of an adsorbent sample in order to displace electrons in the orbital shells of the trace elements. A discrete quantity of energy is given out as electrons fall back into vacant orbitals thereby revealing the identity of the elements present.
X-Ray Diffraction (XRD)

A Bruker D8 X-ray diffractometer was used to examine the crystallography of precipitates formed on the surface of adsorbent samples subsequent to equilibration with mercury solutions.

Kinetic Experiments

980ml of distilled water was added to a round-bottomed flask. A known mass of adsorbent was placed into a rotating basket made of perspex and teflon mesh with an opening of 50µm (11). The basket containing adsorbent was connected to a stirrer and placed in the reactor. The adsorbent was contacted with distilled water for 1 hour prior to the start of an experiment to allow for any trapped air to escape and for any particle swelling to occur. 20ml of mercury solution, of known initial concentration, was added to the reactor and the timer and the stirrer motor (set at 250 min⁻¹) started immediately. This was noted as the zero-time of the experiment. Samples were collected at certain time intervals and analysed for mercury concentration. The experiments were performed for up to 3 hours and the temperature was kept at 298 K by a temperature control unit.

Other Techniques Used

The experimental procedures for acid/base titration, electrophoretic mobility measurements, surface area and biomass nitrogen and amino acid content have been reported elsewhere (12).

Results and Discussion

Several researchers have used live *A. filiculoides* as a heavy metal adsorbent (13,14). However, metal uptake is greatly enhanced by using the non-viable biomass of the plant (15). For example, Duncan et al (16), have used freshly harvested *A. filiculoides* dried at
60°C and ground to a specific particle size range. This, however, ignores the fact that freshly harvested biomass already contains some metals sequestered by the plant from its natural environment. Furthermore, heating to 60°C may denature some of the more sensitive structural parts of the plant. This methodology also ignores the fact that there are two distinctly different segments of the plant i.e., the leaves and the roots, with potentially quite different attributes. To confirm this, we performed an elementary experiment to measure the sodium uptake capacity of these two sections of the plant. The results show that the leaves of *A. filiculoides* have a distinctly higher ion exchange capacity (see Table 1). As a result, we performed subsequent experiments using only the leaves.

Sodium uptake is an indication of the cation exchange capacity of a sorbent. When an adsorbent or ion exchanger is contacted with sodium hydroxide, Na\(^+\) ions exchange with counterions at dissociable cationic surface functional groups. The results for all the adsorbents used in this work (given as mmol Na/g) are shown in Table 1. *A. filiculoides* leaves possess the highest Na capacity and this may be attributed to the large concentration of carboxylic and other weakly acid functional groups located within the cell walls of the biomass. Purolite S-920 has an unexpectedly large sodium uptake in alkaline solution. Rohm and Haas GT-73 and WHK have an appreciable sodium uptake capacity that is attributed to dissociation of thiol and carboxylic acid functional groups respectively.

Zeta potential (ZP) is an indicator of the magnitude of interaction between colloidal particles. Colloidal suspensions/dispersions of fine particles in a liquid phase possess an
electric charge that depends on the nature of the solid surface and the surrounding medium (17). The point of zero net external surface charge is defined as the isoelectric point (IEP), and is located at zero ZP. Electrochemical measurements have been widely used in the characterization of activated carbons and it is suggested that isoelectric point (IEP) values are in fact only indicative of external surface charges of particles in solution whereas the point of zero charge (PZC) varies in response to the total (external and internal) surface charge of the particles (18). We propose to apply the same argument to the various adsorbents used in this work.

The zeta potential (ZP) as a function of pH for each adsorbent is given in Fig 1. The shape of the ZP curves of WHK and A. filiculoides are similar and can be attributed to weakly acidic oxygen-containing surface functional groups. The IEP values are given in Table 2. The IEP value for Rohm and Haas GT-73 is 0.65 and this corresponds with the more acidic nature of the thiol functional group. The measured IEP value of Purolite S-920 is about 5.9 and this corresponds to the acid/base reaction of the isothiouronium functional group as follows:

\[
\begin{align*}
R-\text{CH}_2-\text{S}-\text{C} &\quad \text{acid HX} \\
&\quad \text{base} \\
\end{align*}
\]

The point of zero charge (PZC) is determined from characteristic pH titration curves. A. filiculoides and GT-73 show curves that are typical of a weak acid cation exchanger with slow dissociation of groups giving a flat curve (see Fig. 2). On the other hand, S-920 and WHK show inflexion points as pH increases implying dissociation of different
types of surface groups. It is also observed that the PZC of the four sorbents follows a similar trend to the IEP (see Fig 1), but the PZC is displaced slightly to the right. This may be attributed to additional internal surface area contributing to surface charge, thereby effectively increasing the total number of functional groups available for the reaction.

Table 2 reports the value of the difference \((\text{PZC-IEP})\) since this has often been interpreted as a measure of surface charge distribution of porous activated carbons. Values greater than zero indicate more negatively charged external than internal particle surfaces and values close to zero indicate a more homogeneous distribution of surface charges (18). It is clearly not possible to draw any conclusions from the results reported for S-920 due to the unreliability of pH determination close to pH 7. However, \((\text{PZC-IEP})\) values deduced for \(A.\ filiculoides\), WHK and GT-73 seem to indicate that the distribution of acidic groups in the structure are reasonably homogeneous.

SEM photographs are shown in Fig 3 (a-d). \(A.\ filiculoides\) does not seem to possess a regular porous structure. There are, however, occasional breaks in the surface due to mechanical processing and stomatal pores. In contrast granular carbon WHK exhibits a characteristic porous structure typical of all carbonaceous materials. Ion exchange resins S-920 and GT-73 are smooth spherical polymer particles with no visible indication of porous structure. It is interesting to observe that the backscatter images of mercury-equilibrated samples of \(A.\ filiculoides\) (Fig 3a) and WHK (Fig 3d) show the presence of many light areas of an apparent crystalline substance on the surface. It must be noted that molecules of high atomic number will show up as lighter areas.
Electron Dispersive Spectroscopy (EDS) was carried out in order to determine the chemical composition of the light areas present on the surface of the adsorbents. EDS profiles of each sample were generated using the detector associated with the microscope to analyse an incremental element of each image. The EDS spectra of the precipitate on each sorbent is presented in Fig 4(a-d). These spectra show that both mercury and chlorine are present and seem to suggest the existence of solid Hg₂Cl₂ on the surface of the adsorbent phase. The presence of silicon and gold is an artifact of the experimental methodology since samples were glued to aluminum platforms and sputter-coated in gold prior to analysis to enable them to conduct electricity. Mercury peaks from other displaced electrons falling back into their orbital shells can be seen clearly at higher eV values.

Further experiments using XRD were carried out in order to elucidate the crystallographic form of the precipitate observed by SEM. The spectra shown in Fig 5 for A. filiculoides and GAC WHK give two peaks at 2θ angles of 21.4 and 28.2, that correspond to the peaks determined for mercurous chloride (Hg₂Cl₂) as given in Table 3. Moreover, these spectra show no evidence of a peak at 20.5 that would indicate the presence of mercuric chloride (HgCl₂). These results seem to suggest that mercury is, at least in part, removed from solution by the reduction of Hg(II) (uncharged aqueous species HgCl₂ that predominates at pH values lower than 6, see the speciation diagram given in Fig 6) to Hg(I) followed by precipitation of Hg₂Cl₂. Adams (19) performed electron microscope analyses of an activated carbon surface that was contacted with HgCl₂ solution and confirmed the presence of both Hg and Cl thus also proposing the importance of the reduction mechanism. Lopez-Gonzales et al suggested the following
reaction to explain the reduction of HgCl₂ with surface hydroxyl groups on activated carbon (20):

$$2(-\text{OH}) + 2\text{HgCl}_2 (\text{aq}) \rightarrow 2(=\text{O}) + \text{Hg}_2\text{Cl}_2 (\text{s}) + 2\text{HCl}$$

There is still considerable contradictory evidence in the literature regarding the precise mechanism of mercury binding with activated carbon. For example, the removal of trace mercury from nitrate solutions by activated carbon cloth has recently been reported by Babic et. al. (21). Trace mercury speciation in nitrate solution shows that the predominant species at pH values in excess of 2 is Hg(OH)₂. Babic et al found that mercury uptake was almost constant between pH 2.5 and 6 and they concluded that "precipitation of mercury as Hg(OH)₂" is the dominant adsorption mechanism. Many authors have attributed mercury sorption on activated carbon to the presence of Hg(OH)₂ but have not discussed the precise sorption mechanism since speciation indicates its existence as an uncharged soluble hydroxide salt (22,23). We believe that if mercury precipitates during sorption in the presence of chloride ions, then it is likely to be as Hg₂Cl₂. In fact, Radovic et al in a recent extensive literature review article has also concluded that mercury sorption by activated carbon is probably due to adsorption and/or a reduction reaction (24).

On the other hand, The XRD spectra of GT-73 and S-920 show only a broad undulation from around 18-25 degrees. This seems to indicate that these two ion exchangers bind mercury by a different reaction mechanism, either by conventional ion...
exchange or by a chelation/coordination reaction. It is well known that mercury is strongly bound to thiol groups as evidenced by the strong interaction with proteins and enzymes, e.g cysteine (25). The uptake of Hg by the thiol groups in GT-73 is likely to be governed by the dissociation of the –SH functional group and the mechanism of sorption can be explained by conventional ion exchange. However, the binding of Hg to S-920 is much more complicated and probably involves a coordination of Hg with the sulphur and amine groupings in the functional group. The precise mechanism and stereochemistry of the reaction is unknown but also probably resembles the binding mechanisms found in protein systems (26).

Equilibrium sorption experiments show a marked difference in mercury uptake between polymeric ion exchangers and natural sorbents. The mercury uptake of the commercial polymeric adsorbents is at least an order of magnitude greater than activated carbon and biomass. Equilibrium mercury uptakes of approximately 0.1 and 0.2 mmol g$^{-1}$ were found for WHK and A. filiculoides, respectively, at a solution concentration of 0.15 mmol L$^{-1}$ and pH 4 (see Fig 7). The corresponding values for S-920 and GT-73 are 1.9 and 3 mmol g$^{-1}$ respectively.

Fig 6 shows the speciation diagram of $10^{-4}$M HgCl$_2$ solution and indicates that mercury is mainly present as the uncharged HgCl$_2$ molecule with only a trace of HgCl$^+$ at pH 4. The adsorption isotherm of mercury adsorption onto A. filiculoides and WHK activated carbon at pH4 is shown in Fig 7. The data is reasonably fitted by the Langmuir isotherm (see Table 4). The uptake of mercury attains a value of 0.23 mmol g$^{-1}$ on A filiculoides compared with a value of 0.11 mmol g$^{-1}$ for WHK at an equilibrium solution concentration of 0.45 mmol l$^{-1}$ and pH4. Both these adsorbents possess oxygen-
containing surface functional groups, e.g. carbonyl, carboxyl, hydroxyl, lactonic and phenolic. These functional groups tend to dissociate at pH4 and consequently interact with positively charged ionic species in solution. Moreover, it has also been reported that surface hydroxyl groups on activated carbon are capable of reducing HgCl₂ to Hg₂Cl₂²⁻ and that this may further promote the binding of mercury at the surface and within pores of the matrix by colloidal precipitation. Mercury adsorption is enhanced when the pH in solution is raised to 6 as seen in Fig 8. Langmuir coefficients for the data correlated at pH6 are given in Table 4. A significant enhancement of mercury uptake is observed for A. filiculoides at pH6. This is attributed to the enhanced dissociation of weakly acidic surface functional groups as pH rises.

The synthetic polymeric resin Purolite S-920 contains a chelating thiouronium functional group that comprising both nitrogen and sulphur atoms in the ligand whereas Rohm and Haas GT-73 resin contains a thiol functional group. The adsorption isotherms of S920 and GT-73 at pH4 are shown in Fig 9. Mercury uptake is an order of magnitude greater than with A. filiculoides and WHK and the data is best fitted by the Freundlich expression indicating a different reaction mechanism (see Table 4). Specific binding by chelation/coordination is most likely with S920 whereas cation exchange at the -SH thiol sites is likely with GT-73. There is little enhancement of mercury sorption as pH is raised from 4-6 on GT-73 as expected since the thiol group is completely dissociated at pH>3 (see Fig 2).

S-920 samples equilibrated with a solution of high mercury concentration at pH values in excess of 4 were found to contain a fine white solid precipitate at the surface. This solid did not appear in a blank solution in the absence of S-920 under the same conditions.
of pH and solution concentration. It appears that a mercury complex forms and is precipitated in the presence of S-920 at a certain combination of aqueous conditions, i.e., pH, mercury concentration, chloride concentration and redox potential.

It must be pointed out that GT-73 is rather unstable in the presence of air due to oxidation of the thiol functional groups and thus the resin must be fully immersed in solution at all times. This is an undesirable feature, despite its greater mercury uptake. We have not reported mercury elution characteristics in this paper but suffice it to say that regeneration of S920 is not straightforward and this is a disadvantage where recycling and reuse of resin is a process requirement. In most circumstances, especially for environmental pollution control, the use of A. filiculoides and/or WHK activated carbon is likely to be a cheap and viable alternative.

Kinetic studies show a clear difference in the rate of mercury uptake (see Fig 11). Mercury sorption by GT-73 is relatively slow whereas S920 is rapid. We observed that 25, 49, 56 and 78% of the fractional approach to equilibrium of mercury was attained for respectively: GT-73, A. filiculoides, WHK and S-920 after the first two minutes when diffusion and sorbate/sorbent affinity is predominant. After a period of ten minutes, we observe that the fractional approach to equilibrium has reached about 75% for all the samples and the difference in kinetics is not significant.

These results are evidently related to the physicochemical properties of sorbents. The nature of surface groups and the porosity of the matrix structure, plus the chemical form of the species in solution and molecular size will determine the kinetics of sorbate uptake by each adsorbent. For instance, the predominant mercury species at pH4 is uncharged
HgCl$_2$(aq) and this does not bind by electrostatic attraction to the negatively charged oxygen-containing surface groups of $A. filiculoides$ and WHK. The mechanisms thought responsible for mercury removal are predominantly physi-sorption of uncharged species coupled with a reduction reaction and subsequent precipitation on the surface and in the pores of the adsorbent. Moreover, we can conclude that ion exchange and/or chelation is the more likely mechanism for S-920 and GT-73. The mechanism responsible for mercury removal by the ion exchange resins is shown as being much faster than the two-phase reduction-precipitation reaction of mercury as observed with $A. filiculoides$ and WHK.

**Conclusions**

The mechanisms of mercury sorption by the sorbents studied in this research are strongly dependent on the physico-chemical characteristics of the solid sorbent and solution parameters such as concentration and pH. All the adsorbents studied show a reasonable uptake for mercury. The commercially manufactured ion exchange resins (S-920 and GT-73) with specific sulphur-containing functional groups showed about 15 times higher affinity for mercury than the activated carbon and biosorbent. The mercury uptake increases by 52 and 100% when pH increases from 4 to 6 for WHK and $A. filiculoides$, whereas no significant change is observed for GT-73. The kinetics of mercury sorption for WHK and $A. filiculoides$ is relatively fast, i.e., 49 and 56 % uptake respectively in the first two minutes. The chelating reactions of the commercial ion exchange resins differ in rate. Purolite S-920 has a very fast reaction whilst GT-73 is the slowest, 78 and 25 % removal respectively in the first two minutes.
Characterisation of the materials before and after sorption has revealed that the activated carbon and biosorbent can reduce mercury in chloride solution from the +2 valency state (HgCl$_2$) to an insoluble precipitate in the +1 valency state (Hg$_2$Cl$_2$). This phenomenon seemingly does not occur with the sorption of mercury by commercial chelating polymeric resins containing thiouronium and thiol functional groups.

Acknowledgements

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TABLE 1. Sodium capacity of sorbent materials

TABLE 2. A comparison of PZC and IEP for the different adsorbents

TABLE 3. Main X-Ray Diffraction lines for Mercury salts (27)

TABLE 4. Sorption isotherm model parameters
<table>
<thead>
<tr>
<th>Material</th>
<th>Sodium Capacity [mmol/g]</th>
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<tbody>
<tr>
<td><em>A. filiculoides</em> leaves</td>
<td>3.80</td>
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<tr>
<td><em>A. filiculoides</em> roots</td>
<td>2.63</td>
</tr>
<tr>
<td>Rohm and Haas GT-73</td>
<td>2.75</td>
</tr>
<tr>
<td>Purolite S-920</td>
<td>3.16</td>
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<td>GAC WHK</td>
<td>1.57</td>
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Table 1
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<th>Sample</th>
<th>PZC</th>
<th>IEP</th>
<th>PZC-IEP</th>
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<tr>
<td><em>A. filiculoides</em></td>
<td>2.77</td>
<td>1.57</td>
<td>1.20</td>
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<tr>
<td>S-920</td>
<td>6.6</td>
<td>5.9</td>
<td>0.7</td>
</tr>
<tr>
<td>GT-73</td>
<td>2.58</td>
<td>0.65</td>
<td>1.93</td>
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<tr>
<td>WHK</td>
<td>4.5</td>
<td>2.19</td>
<td>2.31</td>
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Table 2
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<td>Hg₂Cl₂</td>
<td>21.43, 28.15</td>
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<td>HgCl₂</td>
<td>20.45</td>
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Table 3
<table>
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<th>Parameters</th>
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<td>Langmuir</td>
<td>0.980</td>
<td>$q_m$ 0.11</td>
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<td></td>
<td></td>
<td>$b$ 261.5</td>
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<td>WHK 6</td>
<td>Langmuir</td>
<td>0.865</td>
<td>$q_m$ 0.18</td>
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<td></td>
<td>$b$ 80.3</td>
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<td>Langmuir</td>
<td>0.902</td>
<td>$q_m$ 0.23</td>
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<tr>
<td>AZL 6</td>
<td>Langmuir</td>
<td>0.998</td>
<td>$q_m$ 0.56</td>
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<td>$b$ 37.2</td>
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<td>S-920 4</td>
<td>Freundlich</td>
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<td>$k$ 4.91</td>
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<tr>
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<td>Freundlich</td>
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<td>$k$ 4.51</td>
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<td>0.976</td>
<td>$k$ 4.68</td>
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<td>$n$ 4.90</td>
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Table 4
FIGURES

FIG. 1. Electrophoretic mobility of different sorbent particles

FIG. 2. Proton binding curve for different sorbents

FIG. 3. SEM photographs of adsorbents equilibrated with HgCl₂ at pH 6. (a) A. filiculoides, (b) ion exchanger S-920, (c) ion exchanger GT-73, (d) GAC WHK

FIG. 4. EDS spectra of samples equilibrated with HgCl₂ at pH 6. (a) A. filiculoides, (b) ion exchanger S-920, (c) ion exchanger GT-73, (d) GAC WHK

FIG. 5. XRD spectra for adsorbents contacted with HgCl₂: (a) A. filiculoides, (b) ion exchanger S-920, (c) ion exchanger GT-73, (d) GAC WHK

FIG. 6. Speciation diagram for 1x10⁻⁴ M HgCl₂ in aqueous solution

FIG. 7. Isotherms for Hg uptake by A. filiculoides and WHK at pH 4

FIG. 8. Isotherms for Hg uptake by A. filiculoides and WHK at pH 4 and 6

FIG. 9. Isotherms for Hg uptake by S-920 and GT-73 at pH 4

FIG. 10. Isotherms for Hg uptake by GT-73 at pH 4 and 6

FIG. 11. Kinetics of Hg adsorption by adsorbent particles of 170 – 210µm at 25°C
Fig. 1
Fig. 2
Fig. 4
Fig. 5
Fig. 6
Fig. 7
Fig. 8
Fig. 9
Fig. 10
Fig. 11
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