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Trace metal speciation in the Pieman River catchment, western Tasmania.

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A thesis submitted in total fulfilment of the requirements for the degree of Doctor of Philosophy

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October 2000
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submitted for the degree of: **Doctor of Philosophy**

is the result of my own research, except where otherwise acknowledged, and that this thesis in whole or in part has not been submitted for an award, including a higher degree, to any other university or institution.

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<table>
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<th>Abbreviation</th>
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<tr>
<td>AAS</td>
<td>Atomic Absorption Spectrophotometry</td>
</tr>
<tr>
<td>AHS</td>
<td>Aquatic Humic Substances</td>
</tr>
<tr>
<td>AMD</td>
<td>Acid Mine Drainage</td>
</tr>
<tr>
<td>ANZECC</td>
<td>Australian and New Zealand Environmental Conservation Council</td>
</tr>
<tr>
<td>ASV</td>
<td>Anodic Stripping Voltammetry</td>
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<tr>
<td>ARMCANZ</td>
<td>Agricultural and Resource Management Council of Australia and New Zealand</td>
</tr>
<tr>
<td>$C_L$</td>
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<td>Diffusive Gradients in Thin-films</td>
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<td>Fulvic Acid</td>
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<td>FIAM</td>
<td>Free Ion Activity Model</td>
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<td>Flame Atomic Absorption Spectrophotometry</td>
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<td>Fourier Transform Infra Red</td>
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<td>$K'$</td>
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Summary

The Pieman River catchment has seen continuous mining of economic deposits of gold, silver, lead, copper, zinc and tin since the 1870’s. Tributaries of this river which receive mining effluent, either directly or from acid mine drainage (AMID), have total metal concentrations considerably above background levels and are of regulatory concern. The lower Pieman River is however classified as a State Reserve in which recreational fishing and tourism are the major activities. It is therefore important that water entering the lower Pieman River from upstream hydroelectric impoundments is of high quality.

Metals in natural waters exist in a variety of dissolved, colloidal and particulate forms. The bioavailability and hence toxicity of heavy metal pollutants is very dependant on their physico form. Knowledge of the speciation of a metal in natural aquatic environments is therefore necessary for understanding its geochemical behaviour and biological availability.

Complexation of metal ions by natural ligands in aquatic systems is believed to play a significant role in controlling their chemical speciation. This study has investigated temporal and spatial variation in complexation of metal ions in the Pieman River. The influence of pH, temperature, organic matter, salinity, ionic strength and time has been investigated in a series of field studies and in laboratory-based experiments which simulated natural and anthropogenic disturbances.

Labile metals were measured using two techniques in various freshwater and estuarine environments. Diffusive gradients in thin-films (DGT) allowed in situ measurement of solution speciation whilst differential pulse anodic stripping voltammetry (DPASV) was used to measure labile metal species in water samples collected from the catchment.

Organic complexation was found to be a significant regulating mechanism for copper speciation and the copper-binding ligand concentration usually exceeded the total copper concentration in the river water. Complexation was highly dependent on pH and at the river-seawater interface was also regulated by salinity, probably as a result of competitive complexation by major ions in seawater (eg. Ca\(^{2+}\) ions).
Zinc complexation was also evident, however total zinc concentrations in the water column often far exceeded the potential binding capacity of available ligands. In addition to organic complexation, Zn speciation may also be associated with adsorption by flocculated or resuspended colloidal Mn and/or Fe oxyhydroxides.

Metal ion complexation and hence speciation was found to be highly variable within the Pieman River catchment. This presents major difficulties for environmental managers, as it is therefore not possible to make catchment-wide assumptions about the bioavailability of these metals. These results emphasise the importance of site-specific sampling protocols and speciation testing, ideally incorporating continuous, in situ monitoring.
CHAPTER 1

Introduction

1.1 Trace metal speciation in natural waters

Trace metals in natural waters may exist in a variety of dissolved, colloidal and particulate forms (Table 1.1) depending on the physical and chemical characteristics of the water and sediments (Campbell and Tessier 1987). The various forms or “species” can coexist and may or may not be in thermodynamic equilibrium with one another (Florence 1992).

Changes to metal speciation may occur in response to changes in various environmental parameters. For example, sediment I water exchange, periodic de-oxygenation of deep-water impoundments, pH changes from acid mine drainage (AMD) or salinity changes where river water mixes with seawater in an estuary may significantly alter the speciation of heavy metals and hence alter their toxicity and ability to enter food chains. A proper assessment of the degree of environmental pollution and the threat to public health posed by heavy metals requires a detailed understanding of these processes.

Table 1.1: Possible physico-chemical forms of trace metals in natural waters

<table>
<thead>
<tr>
<th>Physico-chemical form</th>
<th>Possible example</th>
<th>Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free (aquo) metal ions</td>
<td>Zn(H₂O)₆²⁺</td>
<td>Dissolved</td>
</tr>
<tr>
<td>Inorganic complexes</td>
<td>ZnCl₂⁻, PbCO₃⁻</td>
<td>Dissolved</td>
</tr>
<tr>
<td>Weak organic complexes</td>
<td>Zn ²⁺ - fulvate</td>
<td>Dissolved</td>
</tr>
<tr>
<td>Strong ligand complexes</td>
<td>Fe - siderophores</td>
<td>Dissolved</td>
</tr>
<tr>
<td>Mixed ligand complexes</td>
<td>Fulvic acid - Fe-PO₄⁻</td>
<td>Dissolved</td>
</tr>
<tr>
<td>Colloidal metal hydroxides</td>
<td>Cu(OH)₂</td>
<td>Colloidal</td>
</tr>
<tr>
<td>Inorganic colloids</td>
<td>Cu ²⁺ - colloidal Fe(OH)₃</td>
<td>Colloidal</td>
</tr>
<tr>
<td>Organic colloids</td>
<td>Cu ²⁺ - humic acid</td>
<td>Colloidal</td>
</tr>
<tr>
<td>Mixed colloids</td>
<td>Cu ²⁺ - organic coatings</td>
<td>Colloidal</td>
</tr>
<tr>
<td>Adsorbed</td>
<td>Zn ²⁺ - clay</td>
<td>Particulate</td>
</tr>
<tr>
<td>Carbonate bound</td>
<td>MnCO₃</td>
<td>Particulate</td>
</tr>
<tr>
<td>Occluded in Fe/Mn oxides</td>
<td>Cu ²⁺ - MnO₂</td>
<td>Particulate</td>
</tr>
<tr>
<td>Sulphide bound</td>
<td>ZnS</td>
<td>Particulate</td>
</tr>
<tr>
<td>Matrix bound</td>
<td>Aluminosilicates</td>
<td>Particulate</td>
</tr>
<tr>
<td>Differing valency states</td>
<td>Fe(II), Fe(III)</td>
<td>Various</td>
</tr>
<tr>
<td>Organometallic complexes</td>
<td>CH₃HgCl</td>
<td>Lipid-soluble</td>
</tr>
</tbody>
</table>

(adapted from Florence et al. 1992; Campbell and Tessier 1987).
1.1.1 Bioavailability and toxicity

Although many trace metals are essential nutrients to aquatic organisms, they can also be toxic when present in elevated concentrations (Driscoll et al. 1994). Two of the most important features which distinguish metals from other toxic pollutants, is that they are not biodegradable and their bioavailability and potential toxicity is largely controlled by their physico-chemical form (Hart 1981; Florence 1982; Connell 1993; Muller 1996; Teasdale et al. 1996).

Figure 1.1 demonstrates possible pools and transformations of trace metals in aquatic environments (Driscoll et al. 1994). Measurement of the total concentration of a trace metal in a water sample will provide little indication of the metal’s potential interactions with other abiotic or biotic components of the system (Campbell and Tessier 1987) and may therefore overestimate the toxicity of the sample if it is assumed that all metal is in the most toxic form (Florence 1992).

![Figure 1.1: Possible pools and reactions of trace metals in aquatic environments (adapted from Driscoll 1994).](image)

For a trace metal to produce an effect on, or accumulate within an organism, it must be able to cross or interact with a cell membrane (Campbell 1995). Whereas ionic Cu has been found to be highly toxic for example, Cu complexes formed with natural organic
ligands have been found to be essentially non-toxic (Teasdale et al. 1996). The major mechanism for the transport of hydrophilic metal ions across a cellular membrane is believed to be by facilitated diffusion (Figure 1.2). This process involves a receptor molecule (e.g. a protein) on the outer membrane surface that binds to the metal ion, diffuses across the membrane as a complex and releases the metal ion into the cytosol (Florence et al. 1992).

![Figure 1.2: Representation of the transport of metal ions through a biomembrane.](image)

Studies of toxicity of heavy metals to fish and other aquatic organisms have shown that in many environmental samples, the uptake of certain trace metals is primarily a function of the free metal ion activity (Florence 1986). To explain the interactions between the free metal ion and the cell surface Morel (1984) devised the free ion activity model (FIAM). This model assumes that equilibrium exists between all forms of metal in the bulk solution and metal bound at surface sites of the organism (Campbell 1995). The interaction between a metal ion (M) or a metal-ligand complex (ML) with a ligand at the cell surface (X) resulting in the formation of a complex at the cell surface may be represented by the following simplified equations:
The FIAM, which assumes that the plasma membrane is the primary site for metal interaction with living cells has been supported by many toxicity studies (Campbell 1995). Exceptions to the model have also been observed and have been discussed in detail by Campbell (1995). For example, predictions for toxicity by the FIAM in the presence of natural “dissolved” organic matter (DOM) are not always supported by experimental observations. This may be partly attributed to the difficulties associated with the measurement of metal speciation in such waters. Another contributing factor may be that the role of DOM is not limited to metal complexation and that it may also act directly on biological surfaces (Campbell 1995).
1.1.2 Regulation of trace metal speciation

The equilibrium concentration of a free metal ion varies, not only with the total metal concentration, but also with physico-chemical factors including:

- nature and concentration of all competing ligands
- stability of the various metal ligand forms
- the rate at which equilibrium is attained (reaction kinetics)
- concentration of competing cations
- redox potential
- temperature
- pH
- salinity
- hardness

The chemistry of Cu for example is dominated by changes in organic matter, pH, salinity and redox potential in natural waters. Adsorption and complexation largely control dissolution and precipitation processes that lead to partitioning of Cu between the water column and the sediments. Fe and Mn oxyhydroxides and natural organic matter (NOM) are thought to be responsible for the majority of trace metal sorption. Although aluminosilicates are a major constituent of particles in most natural waters, it is thought that they provide only a physical support for surface coatings of the sorbing phases, but do not contribute significantly to direct sorption processes (Teasdale et al. 1996).

In natural waters, complexation of metal ions by DOM is thought to play a key role in controlling the free metal ion concentration (Apte et al. 1988; Iyer and Sarin 1992; Brealt et al. 1996). DOM is ubiquitous in natural waters (Campbell et al. 1997). Approximately 20% of the DOM in natural waters consists of carbohydrates, carboxylic acids, amino acids and hydrocarbons (Leenheer 1994). Up to 80% of the remaining DOM consists of aquatic humic substances (Leenheer 1994; Campbell et al. 1997). These relatively stable complex compounds arise from chemical and biological degradation (humification) of plant and animal tissues and from synthesis activities of micro-organisms (Sihombing 1990; Leenheer 1994).
Humic acid (HA) is an operationally defined fraction of DOM that precipitates at pH < 2 but is soluble at higher pH values. Fulvic acid (FA) is defined as the low molecular weight fraction that is soluble at all pH values (Morel 1984; Lu 1995). Aquatic humic substances (AHS) which include humic and fulvic acids, are heterogeneous and complex mixtures of organic macromolecules. They occur as a size continuum ranging from small dissolved molecules through to colloids and particles (Sihombing 1990). They have been described as polyelectrolytes or hydrophillic colloids (Duinker 1980) which can be coiled, long chain molecules or two or three-dimensional cross-linked molecules. Their shape and size is influenced by environmental conditions (Sihombing 1990). The structural differences between humic molecules are so varied that they cannot be represented by a single structural formula (Sihombing 1990). Although there is controversy over the structure of AHS, it is well known that these molecules possess a range of metal complexing sites of differing affinities (Hawke et al. 1996). Aliphatic and aromatic compounds are integrated with a variety of oxygen-containing groups dominated by carboxylic acids and phenols (Sihombing 1990; Campbell et al. 1997).

Humic substances play an important role in the chemical speciation of natural waters, sediments and soils (Tipping 1994). Because of their broad spectrum of potential binding sites, AHS can interact in various ways with metal ions in aqueous solutions (Burba et al. 1994). They can form complex linkages by ion exchange, surface adsorption and chelation by neighbouring carboxyl and phenolic groups (Duinker 1980; Stumm and Morgan 1996). The mathematical description of the complexation equilibrium data of AHS is complicated by the heterogeneity of binding sites (Perdue 1989).

The interaction of AHS with metal ions in natural waters has become an important area of study in environmental chemistry (Lu 1995). Metal complexation capacities and the associated mechanisms of AHS in aquatic environments cannot be ignored when discussing the influence of mining discharges on water quality for the development of water quality regulations.
1.1.3 Water quality guidelines

Water quality criteria were initially developed to introduce objectivity into decisions concerning water quality management (Hart 1980). The Australian and New Zealand Environment and Conservation Council (ANZECC) water quality guidelines for the protection of marine and freshwater aquatic ecosystems were based on the ecologically sustainable development philosophy where the goal is to protect biological diversity and maintain ecological integrity (ANZECC 1992).

Establishing what is an adequate level of protection for biodiversity is not simple (ANZECC 1992) and the 1992 guidelines were based on measurement of total metal concentrations. However, regardless of whether they are acting as a toxicant or nutrient, the bioavailability of trace metals to aquatic organisms depends on their physico-chemical form. Knowledge of the chemical speciation is therefore essential for understanding metal geochemical cycling, bioavailability and toxicity (Florence 1982; Florence et al. 1992; Tercier and Buffle 1993; Allen and Hansen 1996).

The Australian water quality guidelines (ANZECC 1992) are currently being reviewed and it is apparent that management of water quality, with respect to heavy metals, has shifted its focus from total metal concentrations to metal speciation and bioavailability. It is now recognised that site-specific metal speciation information is necessary to more accurately determine trigger levels for management action that will provide appropriate protection for aquatic environments (ANZECC and ARMCANZ 1999).
1.2 Analytical techniques for speciation measurements

1.2.1 Traditional methods and problems

Filtration is the first step in the preparation of water samples for trace metal analysis in most speciation studies. By tradition, this filtration is usually performed with a 0.45 µm membrane filter, the resultant fractions being termed “particulate” and “dissolved” (Filella et al. 1995). This is an arbitrary distinction that disregards the fact that colloidal material, which can account for a significant proportion of trace metal binding, exists as a size continuum between particulate and truly dissolved forms (Florence and Batley 1980; Laxen and Chandler 1982; Morgan and Stumm 1991; Filella et al. 1995).

Various speciation schemes incorporating physical separation methods and analysis with suitable detection techniques have been developed (Figura and McDuffie 1979; Hart and Davies 1981; Laxen and Harrison 1981; Florence 1986). One of the main limitations of most speciation techniques is their inability to measure concentrations of individual ionic species (Florence 1986). Thus, many speciation schemes only allow the classification of metal forms into various operationally defined categories according to their physical or chemical reactivity (Florence 1982; Campbell and Tessier 1987).

Metal speciation studies are complicated by the fact that total metal concentrations in natural waters are often very low (Filella et al. 1995). Individual chemical species are therefore present in even lower concentrations, often at nano- and pico-molar levels (Morgan and Stumm 1991). As a result, contamination from a variety of sources can be a significant problem throughout sampling, storage and analysis. At the same time, losses of metal can occur by adsorption on the walls of sample bottles and other equipment if appropriate precautions are not employed (Filella et al. 1995).

Characterisation of the chemical species in natural waters is also complicated by the possibility that the species distribution may change during sampling and storage of the water sample (Batley 1989; Tercier and Buffle 1993; van den Berg and Achterberg 1994). Within a water sample there are many simultaneous equilibria affecting any particular species. Alteration of the concentration of one species may thus effect others. For example, a change in gaseous equilibrium can be significant in regulating the pH.
and composition of natural waters which in turn, affects solubility and adsorption of metals (Stumm and Morgan 1996).

Therefore, in situ speciation measurements are particularly desirable (Benes and Steinnes 1974; Tercier and Buffle 1993). In situ speciation techniques tend to fall into one of three categories (Davison et al. 2000) which are discussed below:

1. Continuous or discrete in situ measurements can be performed using ion selective electrodes (ISE). Although electrodes offer potential for in situ studies, few metals of current concern are amenable to direct determination at realistic concentrations in natural waters (Campbell and Tessier 1987). This technique has therefore been mainly applied to the determination of free Cu ions in polluted waters (Apte and Batley 1995; Mota and Correia Dos Santos 1995). It is also important to note that studies by ISE carried out close to the analytical detection limit, in low ionic strength media and in the presence of variable hydrogen ion concentrations (e.g. unbuffered Pieman River water) are particularly difficult (Campbell and Tessier 1987).

2. A series of discrete analysis can be performed either directly or after periodic collection of discrete samples using techniques such as Anodic Stripping Voltammetry (ASV) or Cathodic Stripping Voltammetry (CSV). The former method is adaptable for in situ use (Tercier and Buffle 1993), however as relatively sophisticated on-site equipment is required, reports of in situ measurement of metals in freshwaters are limited (Davison and Zhang 1994). CSV cannot generally be used in situ on undisturbed water samples as a ligand and usually a buffer must be added.

3. Fractionation of chemical species may be performed in situ with analysis delayed until return to the laboratory. Dialysis techniques have been developed for this purpose but these have poor sensitivity and long equilibration times (Davison and Zhang 1994; Apte and Batley 1995). Diffusive gradients in thin films (DGT) is a relatively new speciation procedure that can also be used to measure in situ fluxes of metals in natural waters (Davison and Zhang 1994; Davison et al. 1994; Zhang and Davison 1995; Zhang et al. 1995).
1.2.2 Measurement of the labile metal fraction

An ideal speciation technique for detection of the free metal ion activity would be selective for the metal of interest, with suitable sensitivity (0.1 nM to 1 M) to be used directly on natural water samples. It would create minimum disturbance to the sample during the measurement, would produce an analytical signal proportional to the concentration of the element of interest and be adaptable to measure a suite of metals (Campbell and Tessier 1987).

Most analytical techniques with suitable sensitivity for speciation measurements in natural waters (i.e. voltammetry, polarography, ion-exchange and chelation exchange resins) cannot measure the true free-ion concentration because the act of performing the measurement actually disturbs the sample equilibrium.

Ion selective electrode (ISE) potentiometry is the only method that can measure the activity of an individual ion, however this technique was not considered suitable for use during this study (Section 1.2.1).

Most speciation techniques such as ASV for example produce an “operationally-defined” labile fraction (Florence 1986) which still provides a useful measure for comparison between samples (Batley 1989).

Labile metal speciation measurements in aqueous solutions can be appreciated by considering a simple equilibrium between a free metal ion (M) and a ligand (L), represented by Eqn. (4).

\[ M + L \rightleftharpoons ML \]  \hspace{1cm} (4)

Measurement of a metal-ligand complex (ML) only occurs if it can dissociate during the measurement time. The extent to which metal dissociates from the ML complex and contributes to the measurement defines its lability.
For weakly bound labile complexes, where a rapid equilibrium exists between M and ML, both the free metal ions and the metal-ligand complexes will contribute to the flux of accumulating metal (M) to be measured.

For non-labile, inert complexes, where a very slow equilibrium exists between M and ML (i.e. strongly bound complexes), only the free metal ion concentration (M) will be detected (Davison and Zhang 1994).

A labile metal measurement will therefore include the free metal ion concentration and the proportion of metal released from weakly bound complexes that dissociate in the measurement time of the technique used. Inert complexes will not be measured.

1.2.3 Anodic stripping voltammetry (ASV)
Anodic stripping voltammetry is an extremely sensitive electrochemical technique commonly used for the measurement of labile trace metal species in natural waters (Florence and Batley 1977; Donat and Bruland 1990; Iyer and Sarin 1992; Tercier and Buffle 1993; Apte et al. 1995; Deaver and Rodgers Jr 1996; Muller 1996; Stauber et al. 1996; Wu et al. 1997). CSV is another highly sensitive electrochemical technique that is not subject to the kinetic dissociation problems often encountered in ASV studies (van den Berg 1984; Apte et al. 1988; van den Berg and Achterberg 1994). CSV is an equilibrium technique that can be used to determine the free metal ion concentration in natural waters (Florence 1986) but has mainly been applied in marine or estuarine studies.

Voltammetric techniques such as ASV do not appreciably disturb the bulk sample and thus are useful for obtaining information about in situ speciation (Mackey and Zirino 1994). The method is kinetically based and is operationally defined by the thickness of the diffusion layer at the working electrode (Morgan and Stumm 1991; Hawke et al. 1996).

A major form of metal accumulation in an organism occurs by dissociation of a metal-complex at a membrane surface with facilitated diffusion of a metal through the membrane and deposition in the cytosol (Figure 1.2). This process has been likened to
that of electro-deposition where the metal-ligand complex dissociates at the diffusion layer boundary. The metal ion then travels through the diffusion layer to the electrode where it is deposited (Florence et al. 1992). In terms of measuring toxicity to a biological cell, a kinetic-based measurement by ASV for example, may be more realistic than an equilibrium concentration obtained by ISE, which gives no clue about the lability of metal-ligand species within the diffusion layer of biological cell walls (Hawke et al. 1996).

Furthermore, if an analytical method is to produce useful information for ecotoxicological studies it should be shown to give a reasonable correlation with relevant bioassay techniques (Florence 1986). Studies to investigate the correlation between ASV-labile measurements and toxicity have produced variable results, ranging from good correlation between toxicity and ASV measurements, to ASV-labile concentrations measured as half that measured in a bioassay (Florence 1986). Florence (1992) showed a good correlation between metal concentration measured by ASV and the toxicity of the metal to algae, in various synthetic and polluted waters.

The application of ASV is restricted to those metals that form an amalgam with mercury (Filella et al. 1995). In addition to this restriction, interpretation of data can be complicated by adsorption of AHS onto the hanging mercury drop electrode (HMDE), by accumulation of excess metal during the stripping stage (Bugarin et al. 1994; Labuda et al. 1994; Filella et al. 1995) and by directly reducible metal-ligand complexes (Florence 1986). ASV has been applied in this study as a speciation tool in fresh and estuarine waters and the relevance of these dissociation issues to this work is investigated in Chapter 3.

1.2.4 Diffusive gradients in thin films (DGT)

DGT accumulates labile metal species in situ by immobilising them in a layer of Chelex resin after they have diffused through a layer of polyacrylamide gel (Zhang et al. 1995). The accumulated metal is later measured using conventional techniques (i.e. Atomic Absorption Spectrophotometry; AAS) in the laboratory. The technique can theoretically be applied to any element that can diffuse through the gel and be bound by an active component in a backing layer (Davison and Zhang 1994). DGT has been used as a speciation tool in this study.
Extensive laboratory research into the application of polyacrylamide gels by Davison, Zhang and co-workers has demonstrated the potential of DGT for trace element studies in both the water column and bottom sediments (Zhang and Davison 1995; Zhang et al. 1995; Davison et al. 1997). Polyacrylamide gels that are extensively cross-linked have been widely used in electrophoresis and have found a new application in DGT. These “hydro-gels” are over 95% water and have effective pore spaces of 2 - 5 nm when prepared according to the method of Zhang and Davison (1995). Hydrated ions (diam ≈ 0.2 - 0.3 nm) can diffuse through these gels at the same rate as through liquid water. Organic molecules with molecular weights up to 100,000 Dalton can also diffuse through the gels, although with increasing retardation as size increases (Davison et al. 1994). The ability of the structurally coherent gels to mimic the diffusive properties of natural waters is the key to their application for trace metal analysis and has led to the development of DGT.

In this technique two gel layers are used. A clear diffusive hydro-gel is laid on top of another thin layer of hydro-gel containing Chelex resin (75-100 µm diam) as a binding agent. The outer surface of the diffusive gel is covered by a 0.45 µm membrane filter in order to protect it from adhering particles. The gels are placed in a plastic holder and immersed in the water to be sampled. Cations diffuse across the filter and diffusive gel layer and are concentrated on the resin. In the vicinity of the resin the free cation concentration is effectively zero and a linear concentration gradient is quickly (~ mins) established across the diffusive gel layer. Ion diffusion across the gel is governed by Ficks Law from which Eqn. (5) is derived:

\[ J = \frac{D \left( C_b - C_{r*} \right)}{\left( \Delta g + \delta \right)} \]  

(5)

Where

- \( J \) = Flux of metal ion
- \( D \) = Diffusion coefficient of metal ion
- \( C_b \) = Bulk concentration of metal ion
- \( C_{r*} \) = Metal ion concentration at the boundary between the gel layers
- \( \Delta g \) = Thickness of diffusive gel layer
- \( \delta \) = Diffusion boundary layer thickness on the outside of the hydrogel
Davison and co-workers have shown that both $C_r^*$ and $\delta$ can be neglected in Eqn. (5) for waters moving above a minimum threshold velocity. Natural waters appear to provide sufficient natural convection to meet these criteria (Zhang and Davison 1995). Thus Eqn. (5) simplifies to Eqn.(6).

$$ J = \frac{D C_b}{\Delta g} \quad (6) $$

Following deployment, the resin layer is retrieved and placed in a known volume of dilute FINO to extract the metal ions off the resin. The acid extract is then analysed after suitable dilution to determine the mass of accumulated metals using Eqn.(7).

$$ M = C_e (V_{HN03} + V_{gel}) / f_e \quad (7) $$

Where

- $C_e$ = measured concentration in acid extract solution
- $V_{gel}$ = volume of gel
- $V_{HN03}$ = volume of added acid
- $f_e$ = extraction efficiency of acid for the metal (typically 0.80 for many metals; Zhang and Davison 1995).

The measured mass $M$, can be used to calculate the flux through the diffusive gel using Eqn. (8).

$$ J = \frac{M}{A t} \quad (8) $$

Where

- $t$ = deployment time
- $A$ = exposure area

Using Eqn. (6) and Eqn. (8) and rearranging gives Eqn. (9). The bulk concentration of metal in the original sample ($C_b$) can then be calculated from the measured mass of metal in the resin bed layer, the thickness of the diffusive gel layers, the time of immersion and ion diffusion coefficients (which are available from the literature; Zhang 1997).

$$ C_b = \frac{M \Delta g}{(D t A)} \quad (9) $$
Laboratory and field studies by Davison and co-workers have established the utility of the DGT method and calculated concentrations are consistent with determinations using other techniques.

Whereas ASV measures free metal ions and metal that can dissociate from complexes within < 100 ms (Davison and Zhang 1994), DGT measures free metal ions and labile metal complexes that can pass through the pores of the diffusive gel and dissociate in ~2 minutes. Both inorganic metal species and metal complexed by lower molecular weight humic substances can diffuse through the gel, but large colloidal species cannot. If small inert species can bind directly to the resin, they will also be measured by DGT (Zhang and Davison 1995).

Measurement by ASV relies (for organic complexes) on the reduction of Cu from complexes at the electrode whilst accumulation by chelex involves a competitive complexation reaction. Thus, the different measurement principles of the two techniques would also be expected to impact significantly on what is measured.

1.2.5 Measurement of metal ion complexation

The ability of natural ligands such as AHS and colloidal MnO₂ and Fe₂O₃ to react with metal ions is an important factor in aquatic environments (Hart 1981; Campbell and Tessier 1987; Iyer and Sarin 1992; Einax and Kunze 1996; Hawke et al. 1996; Teasdale et al. 1996; Turoczy and Sherwood 1997). The measurement of this parameter, known as the complexation capacity, is often used to describe the ability of receiving water to detoxify added metal (Hawke et al. 1996).

The capacity of water to complex metals is determined by titration of a water sample with a metal ion and measurement of the remaining unbound ionic metal concentration (Florence 1986). The ligand concentration (C_L) and a conditional stability constant (K’L) are usually determined using the van den Berg / Ruzic transformation (Ruzic 1982; van den Berg 1982; van den Berg 1984). Practical problems associated with these measurements and with the interpretation of data have been reviewed by several authors (Apte et al. 1988; Perdue 1989; Turoczy and Sherwood 1997) and are discussed in Chapter 3 of this thesis.
1.2.6 Equilibrium modelling

The aim of equilibrium chemical speciation modelling is to develop accurate mathematical models to describe chemical processes in natural waters (Turner 1995). Chemical modelling based on thermodynamic calculations offers the potential to understand and predict the behaviour of metal ions in natural waters (Ohman and Sjoberg 1988). The underlying assumption that forms the basis of chemical equilibrium calculations in natural waters is that equilibrium exists in the system chosen to study (Nordstrom and Ball 1984; Ohman and Sjoberg 1988; Turner 1995). Speciation calculations usually involve the solution of a set of simultaneous equations (Turner 1995). Since 1965, over 50 published computer programs have been developed for this purpose (Nordstrom and Ball 1984). A weakness of many chemical speciation models to date, has been the reliability or availability of necessary thermodynamic data (Nordstrom and Ball 1984; Ohman and Sjoberg 1988; Turner 1995).

One chemical speciation model has been applied in this study. The Windemere Humic Aqueous Model (WHAM) is a chemical equilibrium model and computer code designed to calculate equilibrium chemical speciation in surface and ground waters, sediments and soils (Tipping 1994). This model is designed especially for problems where the chemical speciation is dominated by organic matter present in dissolved or particulate form.

1.3 The Pieman River

1.3.1 Geographical location

The Pieman River, formed by the confluence of the Macintosh and Murchison Rivers, is located in central western Tasmania between 41°15’S and 41°50’S and 145° 10’E and 146°00’E. The total catchment area is approximately 3800 km (Figure 1 3) and covers terrain ranging from high mountains of the Cradle Mountain-Lake St Clair National Park to beach dunes near Pieman Heads.
Figure 1.3: Pieman River catchment (● = Tributary sampling site; ○ = Mine site).
1.3.2 Geology

Tasmania’s west coast is dominated by ancient Precambrian rocks, some of which are at least 700 million years old (Williams 1974). The continual weathering of the Precambrian and Paleozoic bedrock has resulted in the exposure of very old surfaces consisting mostly of quartzite. These weathering resistant rocks yield very few dissolved chemical species as water percolates through them. The lack of easily eroded mineral particles is responsible for the very low suspended sediment load found in the rivers and lakes of the Pieman catchment and few mineral particles are available to contribute to soil development in the region (Koehnken 1992).

Pleistocene glaciation in the higher altitudes of the catchment has resulted in steep, craggy exposed peaks, such as Mount Murchison, Cradle Mountain and Barn Bluff. Little or no soil has developed on the steep slopes. Glacial deposits occur in the mountainous eastern part of the catchment with flat-lying organic-rich soils more common on valley floors and the western area surrounding Lake Pieman (Koehnken 1992).

The Cambrian Mt Read Volcanics are situated in the Dundas Trough, a north-south oriented geologic feature located in the central part of the catchment. The Mt Read belt is approximately 20-30 km wide and contains important deposits of base and precious metals in volcanic-hosted massive sulphide deposits. All of the major lead-zinc-silver mines in the Pieman catchment are located in the Mt Read Volcanics (Koehnken 1992).

Small outcrops of carbonate deposits are present in a few tributaries feeding the Pieman lakes. Where these Paleozoic limestones are exposed, such as in the Vale River and Huskisson Basin, alkalinity and conductivity increase as additional salts are contributed to the rivers (Koehnken 1992).

1.3.3 Climate and hydrology

Average yearly rainfall increases from west to east within the catchment, with average total precipitation ranging from 1120 mm near the coast to over 3000 mm in the mountainous eastern region. The wettest period occurs between April and October although high rainfall (> 200 mm) has been recorded in all months (Koehnken 1992).
The high rainfall in the east of the catchment feeds the Murchison and MacIntosh Rivers that contribute over 40% of the total water flow measured at Pieman Heads. Average discharge of the Pieman River is approximately 190 cumecs (Koehnken 1992). Lake Mackintosh is the primary storage in the catchment, followed by Lake Pieman. Average residence time of water in Lakes Mackintosh, Pieman, Murchison and Rosebury are 146 days, 55 days, 23 days and 19 days respectively (Koehnken 1992).

Below Reece dam, the Pieman River flows unrestricted to its mouth at Pieman Heads (Koehnken 1992). Despite the intermittent and controlled discharge of water from the Reece Power station into the estuary, the water level of the lower Pieman River remains relatively constant because the riverbed is below sea level. This allows the incursion of a salt wedge. The salt wedge is always present near Pieman Heads but its extent upstream depends on water discharge from Reece Dam, the discharge of the Whyte, Savage and Donaldson Rivers, and the strength of the tides (Koehnken 1992).

On the central plateau, mean July air temperature ranges from 10.5°C at sea level to 0.5°C. Mean January temperatures range from 19°C to 9°C. Temperature extremes recorded over most of the Central Plateau are -15°C to >38°C (Williams 1974).

1.3.4 Water chemistry

Buckney and Tyler (1973) have discussed the general factors that control major ion chemistry of waters in this area. Essential features include the predominance of old, inert rocks, a mantle of peat isolating waters from rock contact, and proximity to an ocean coast with strong prevailing winds bringing high rainfall (Buckney and Tyler 1973).

The ionic ratios of the Pieman waters resemble those found in seawater (Koehnken 1992). The seawater order of cationic and anionic dominance (i.e. Na⁺ > Mg²⁺ > Ca²⁺ > K⁺ ; Cl⁻ > SO₄²⁻ > HCO₃⁻) is characteristic of surface water in this part of Tasmania when local geochemical influences are absent or minimal (Buckney and Tyler 1973; Bowling et al. 1986).

Lake Murchison and Lake Rosebury have been limnologically described as moderately dystrophic reservoirs with non-turbid waters (Bowling et al. 1986; Bowling and Tyler...
1990). In this type of lake, the bulk of the organic matter is derived from the surrounding catchment and internal organic carbon production is generally low (Chapman 1992). Breakdown of highly humic vegetation on geologically unreactive bedrock, combined with high rainfall, produce high concentrations of “dissolved” organic compounds in the waters of the Pieman system. These compounds give the water a characteristic clear brown colour, typical of many West Tasmanian waters (Bowling et al. 1986; Koehnken 1992).

Tributaries in the Pieman catchment generally receive little input from carbonate-rich strata (Section 1.3.2). Because of this, the overall water chemistry is dominated by the presence of organic compounds with the pH of the water decreasing with increasing “dissolved” organic carbon (DOC) concentrations. In tributaries where DOC exceeds 10 mg/l, the alkalinity drops to almost zero, although hardness is still present. This suggests that all hardness is non-carbonate hardness, and therefore is not a measure of the carbonate buffering capacity of the water (Koehnken 1992).

Overall, the lake waters are characterised by low alkalinity (< 21.4 mg/L), low pH (pH 3.6 - 6.7), low conductivity (usually < 100 µS/cm) and relatively high concentrations of “dissolved” organic matter (DOC range: 3.5 - 14 mg/L). Mean major ion concentrations measured at the most downstream Lake Pieman site studied by Koehnken (1992) are shown in Table 1.2.

**Table 1.2:** Average concentrations (mean ± standard error) of major ions and other water quality parameters measured at Reece Dam

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Concentration a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>96</td>
<td>5.0 ± 0.1 mg/L</td>
</tr>
<tr>
<td>K⁺</td>
<td>65</td>
<td>0.50 ± 0.01 mg/L</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>96</td>
<td>2.3 ± 0.1 mg/L</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>96</td>
<td>1.4 ± 0.1 mg/L</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>96</td>
<td>7.9 ± 0.1 mg/L</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>99</td>
<td>10.0 ± 1.0 mg/L</td>
</tr>
<tr>
<td>DOC</td>
<td>32</td>
<td>7.0 ± 0.3 mg/L</td>
</tr>
<tr>
<td>pH</td>
<td>106</td>
<td>5.54 ± 0.04</td>
</tr>
<tr>
<td>Conductivity</td>
<td>103</td>
<td>56 ± 1 µS/cm</td>
</tr>
<tr>
<td>Alkalinity (as CaCO₃)</td>
<td>102</td>
<td>4.3 ± 1.1 mg/L</td>
</tr>
</tbody>
</table>

a Average data from Pieman River Monitoring data; 1900 to 1997.
1.3.5 Thermal and chemical stratification

The thermal structure and characteristics of established reservoirs are the same as those described for lakes (Chapman 1992). The physical and chemical characteristics of Lake Murchison, Lake Mackintosh and Lake Rosebury are dominated by thermal stratification in the summer and thorough mixing during winter, typical of temperate lakes (Koehnken 1992). The reservoirs are moderately dystrophic, with most solar radiation being absorbed in the first few metres (Bowling et al. 1986; Bowling and Tyler 1990). This results in strong thermal gradients at shallow depths with a large hypolimnetic volume. (Bowling and Tyler 1990).

Water release from Bastyon Power Station, seasonal air temperature variations and cold density currents caused by in-flowing tributaries draining high land to the north are three major factors influencing the chemical and physical characteristics of Lake Pieman (Bowling and Tyler, 1990). In regions affected by Power Station outfalls, water is thermally and chemically homogenous. However, Lake Pieman becomes stratified from near the Huskisson River inlet to Reece Dam in summer, with minimum temperatures decreasing downstream. Stratification is usually substantially weakened by June. Winter turnover and mixing has been confirmed by water quality measurements performed during August (Koehnken 1992).

1.3.6 Water release for power generation

Deep reservoirs can be subjected to major changes to their thermal and chemical structure by the design of the water release system at the dam site (Chapman 1992). All Pieman reservoirs have high-level takeoffs (Bowling and Tyler 1990). Extraction of water via such outlets creates extensive withdrawal currents near the surface but leaves the bottom waters relatively undisturbed, leaving a considerable depth below the takeoff for establishment of chemical gradients (Bowling and Tyler 1990).
1.3.7  Vegetation
The Pieman River catchment is largely uncleared and contains a variety of vegetation types characteristic of low altitude, high rainfall regions. About half the catchment is covered by temperate rainforest. On low-lying areas, where the underlying soils are generally organic-rich peaty deposits, extensive button grass (*Gymnoschoenus sphaerocephalos*) moors are found (Bowling et al. 1986; Koehnken 1992).

1.3.8  Ecosystem disturbances in the catchment
The Pieman River is located in central western Tasmania, a region that is internationally recognised for its outstanding natural features. The river also lies in one of Australia’s rich mineralogical provinces and is presently the site of gold, copper, lead, zinc, tin and Fe mining. Many disused mines also exist in the catchment.

The organic-rich waters of the Pieman River have acted as a receiving environment for discharge of wastewater from mining operations for over a century (Koehnken 1992). Release of metals from mining sites occurs primarily through acid mine drainage (AMD) and erosion of waste dumps and tailings deposits (Salomons 1995). Depending on the nature of the tailings, AMD, which occurs as a result of oxidation of sulphidic ore and formation of sulphuric acid by reaction with water, can contain elevated levels of metals (Salomons 1995). Other significant sources of heavy metal pollution to the Pieman River include the early mining practices of dumping tailings directly into the river and in some cases, mine sites and their associated tailings were inundated by impoundments. Quantitation of the influence of these submerged tailings deposits to water quality in the now flooded river valley is not possible (Koehnken 1992).

Before damming, the Pieman River flowed through narrow, steep-sided, heavily vegetated valleys (Bowling and Tyler 1990). Significant modifications have taken place within the catchment over the past century. The major alteration of the river system has been the construction of the Pieman River Hydroelectric Power Development, consisting of the Mackintosh, Rosebury and Lower Pieman Schemes commissioned in the 1980’s, and more recently, the Anthony Power Development (Koehnken 1992).
Since the creation of the lakes, mining discharges are maintained for longer periods in a lake environment. Because the dynamics of lakes differs considerably from river systems, the behaviour of mining discharges in the aquatic environment will also differ. Discharges may encounter low oxygen waters and interact with sediments, instead of being diluted and dispersed rapidly as they might in a river system. Due to the lack of earlier monitoring data for Pieman waters, it is difficult to distinguish ecosystem alterations caused by damming from ecosystem changes resulting from mining discharges (Koehnken 1992).

Below the final power station (Reece Dam), the river is used extensively for tourism and recreation, which require good water quality and undisturbed natural settings. The designation of the lower Pieman River as a State Reserve and Conservation area necessitates the discharge of high quality water from Lake Pieman to ensure the protection of ecosystems in this region of the river.

1.4 Previous water quality monitoring in the Pieman River

The Pieman River Environmental Monitoring Program was initiated in 1990 when a fish kill occurred in the lower Pieman River. The fish kill was attributed to gas-bubble disease caused by the release of water that was supersaturated with air from the Reece Power Station. Although the “kill” was not directly linked to heavy metal concentrations in the water, the limited understanding of riverine and lake processes affecting water chemistry within the catchment became obvious at this time (Koehnken 1992).

The aim of the Pieman River Monitoring Program was to gain an understanding of the physical and chemical processes within the catchment as a result of both natural and human-induced activities and to document the dynamics of the lakes and rivers within the system (Koehnken 1992). Through the collection of physical and chemical data, the on-going program has established base-line information about water quality in the rivers and lakes, documenting background levels and seasonal variations.

Measurement of heavy metal concentrations and other water chemistry parameters in Lakes Murchison, Mackintosh and Rosebury indicate that mining has had little impact
on background water quality in these lakes. This is probably because mining discharges are diluted considerably by river water feeding these lakes (Koehnken 1992). Tributaries of the Pieman River receiving mining effluent from waste discharge or acid mine drainage however, have total metal concentrations considerably above background levels (Koehnken 1992; Denney 1999; Denney et al. 1999) and are of environmental and regulatory concern. Mining discharges are also detectable in Lake Pieman. In particular, Zn is present in concentrations currently considered detrimental to ecosystems ([Zn\textsubscript{T}] must be \( \leq 50 \) µg/L; ANZECC 1992). About 80% of the Zn entering Lake Pieman can be attributed to mining activities and most of this input is contributed via the Ring and Huskisson Rivers (Koehnken 1992).

1.5 Aims and objectives of this study

The dynamics of processes controlling metal speciation in aquatic environments are not fully understood and little work has been done for waters having the properties of those found within the Pieman catchment.

Thus, the aims of this project were to:

- gain an understanding of the processes controlling metal speciation in these organic rich, poorly buffered, low ionic strength waters.
- investigate the response of metal speciation in Pieman River water to changes in environmental conditions such as pH, salinity, temperature and organic carbon concentrations.
- determine whether activities which may occur within the catchment have the potential to change the metal speciation and hence its toxicity. These activities may include the release of water from the hydroelectric dams for power generation, dredging of silt from deep impoundments, or salinity changes where the river mixes with seawater in the estuary.
- determine the status of heavy metal speciation in various aquatic environments within the Pieman catchment and assess their compliance to current water quality guidelines.
- provide information which will be useful for the environmental management of the river’s aquatic resources with regard to heavy metal occurrence and distribution.
To fulfil these aims, the following project design was implemented:

Cu ion complexation titrations were performed in Pieman River water samples collected from a range of sites across the catchment to determine the natural spatial and temporal variability in complexation parameters.

Complexation parameters were determined in Pieman River water samples that were manipulated in laboratory experiments to simulate natural and anthropogenic physico-chemical changes. The influence of pH, salinity, ionic strength and temperature has been investigated.

Metal speciation studies were undertaken in mine-affected freshwater tributary and lake sites using two speciation techniques. As part of this research, measurements made by in situ application of DGT have been compared with ASV laboratory measurements.

Metal speciation studies were also performed in the Pieman River estuary. Both vertical and horizontal variation was assessed. During this research, the potential of ASV as a surrogate indicator of bioavailable Zn, measured using an algal enzyme-inhibition (β-D-galactosidase) bioassay was investigated.

Finally, recommendations for future research are proposed.
CHAPTER 2

Materials and Methods

2.1 Reagents used in this study

2.1.1 Reagent list

The commercially available reagents used in this study are listed in Table 2.1.

Table 2.1: Commercially available reagents used in this study.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Grade</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid (glacial)</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Acrylamide (Electran)</td>
<td>Molecular biology</td>
<td>BDH Lab Supplies</td>
</tr>
<tr>
<td>Ammonium persulphate</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Cadmium (II) nitrate AAS standard</td>
<td>Spectrosol</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Chelex 100 (100-200 mesh; Na form)</td>
<td>Analytical reagent</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>Copper (II) nitrate AAS standard</td>
<td>Spectrosol</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Di-potassium hydrogen orthophosphate</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Extran 300</td>
<td></td>
<td>BDH Chemicals</td>
</tr>
<tr>
<td>Hydrochloric acid (36 %)</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Iron (II) nitrate AAS standard</td>
<td>Spectrosol</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Lead (II) nitrate AAS standard</td>
<td>Spectrosol</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Manganese (II) nitrate AAS standard</td>
<td>Spectrosol</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Nitric acid (65 %)</td>
<td>Suprapur</td>
<td>Merck</td>
</tr>
<tr>
<td>Nitric acid (70 %)</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>High Purity</td>
<td>BOC Gases</td>
</tr>
<tr>
<td>Perchloric acid (70 %)</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>PIPES (Piperazine-N,N'-bis[2-ethanesulfonic acid] disodium salt)</td>
<td>Not stated</td>
<td>Sigma</td>
</tr>
<tr>
<td>Polyacrylamide (Electran)</td>
<td>Molecular biology</td>
<td>BDH Lab Supplies</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Potassium chromate</td>
<td>Analytical reagent</td>
<td>BDH Lab Supplies</td>
</tr>
<tr>
<td>Potassium di-hydrogen orthophosphate</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Potassium hydroxide</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>Analytical reagent</td>
<td>BDH Lab Supplies</td>
</tr>
<tr>
<td>Sodium acetate (anhydrous)</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>Analytical reagent</td>
<td>APS Ajax Finechem</td>
</tr>
<tr>
<td>Sulphuric acid</td>
<td>Analytical reagent</td>
<td>Ajax Chemicals</td>
</tr>
<tr>
<td>TEMED (N,N,N',N'-tetramethylethylenediamine)</td>
<td>Electrophoresis reagent</td>
<td>Sigma</td>
</tr>
<tr>
<td>Titanium dioxide P25</td>
<td>TOC catalyst</td>
<td>SGE</td>
</tr>
<tr>
<td>Zinc (II) nitrate AAS standard</td>
<td>Spectrosol</td>
<td>Ajax Chemicals</td>
</tr>
</tbody>
</table>
2.1.2 Deionized water
Ultrapure water (MQ) was prepared by passing singly distilled water through a Milli-Q (Millipore) water purification system. The resistivity of freshly produced water was always $\geq 18 \text{ M}\Omega \text{ cm}^{-1}$.

2.1.3 Preparation of buffers and standard solutions
All solutions were prepared with MQ water. Buffer solutions were stored at 4°C in pre-cleaned HDPE (Nalgene) bottles or polystyrene (Sterilin) bottles.

a) Phosphate buffers
Phosphate buffers were prepared at the required pH by mixing appropriate volumes of 1 M potassium di-hydrogen orthophosphate solution with 1 M di-potassium hydrogen orthophosphate solution.

b) Acetate buffers
Acetate buffers were prepared from a 1 M sodium acetate solution and a 1 M acetic acid solution, which were mixed in appropriate proportions to give the required pH.

c) PIPES buffers
PIPES buffers were prepared by dissolving 4.33 g of Piperazine-N,N’-bis[2-ethanesulfonic acid] disodium salt in 25 mL of MQ water. The pH was adjusted by addition of HNO$_3$ (Suprapur).

d) Metal standard solutions
Metal standard solutions were prepared by serial dilution of atomic absorption spectrophotometry standards (Spectrosol grade). To improve their stability, the standard solutions were made 0.01M in HNO$_3$ (Suprapur) and stored at 4°C.
2.2 Sampling sites

The map locations for Pieman River catchment sampling sites selected for individual experiments performed during this study are listed in Table 2.2 and are described in more detail in the relevant chapters.

Table 2.2: Pieman River catchment sampling sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Description</th>
<th>Map location</th>
<th>Map^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAB</td>
<td>Pieman above Bobodil</td>
<td>CP772782</td>
<td>S</td>
</tr>
<tr>
<td>PBH</td>
<td>Pieman below Huskisson R. inlet</td>
<td>CP685743</td>
<td>P</td>
</tr>
<tr>
<td>PRD</td>
<td>Pieman at Reece Dam</td>
<td>CP447787</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tributary site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal Creek</td>
<td>Murchison Hwy</td>
<td>CP845897</td>
<td>S</td>
</tr>
<tr>
<td>Baker Creek</td>
<td>Above junction with Ring R.</td>
<td>CP755677</td>
<td>S</td>
</tr>
<tr>
<td>Farm Creek</td>
<td>Pieman Rd</td>
<td>CP828810</td>
<td>S</td>
</tr>
<tr>
<td>Hatfield River</td>
<td>Murchison Hwy</td>
<td>CP884018</td>
<td>S</td>
</tr>
<tr>
<td>Marionoak River</td>
<td>Pieman Rd</td>
<td>CP762785</td>
<td>S</td>
</tr>
<tr>
<td>Que River</td>
<td>Murchison Hwy</td>
<td>CP903963</td>
<td>S</td>
</tr>
<tr>
<td>Ring River</td>
<td>Murchison Hwy</td>
<td>CP712712</td>
<td>P</td>
</tr>
<tr>
<td>Savage River</td>
<td>Tarkine Rd.</td>
<td>CP398908</td>
<td>P</td>
</tr>
<tr>
<td>Stanley River</td>
<td>Pieman Rd</td>
<td>CP578185</td>
<td>P</td>
</tr>
<tr>
<td>Sterling River</td>
<td>Murchison Hwy</td>
<td>CP844746</td>
<td>S</td>
</tr>
<tr>
<td>Stilt River</td>
<td>Rosebury Park</td>
<td>CP787732</td>
<td>S</td>
</tr>
<tr>
<td>Vale River</td>
<td>Road to Cradle Mountain</td>
<td>DP064990</td>
<td>S</td>
</tr>
<tr>
<td>Wilson River</td>
<td>Pieman Rd</td>
<td>CP640815</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estuary site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAC</td>
<td>Bend above Corinna</td>
<td>CP402864</td>
<td>P</td>
</tr>
<tr>
<td>BAN</td>
<td>Bend below Nancy Ck. inlet</td>
<td>CP440858</td>
<td>P</td>
</tr>
<tr>
<td>Corinna</td>
<td>Midstream</td>
<td>CP398868</td>
<td>P</td>
</tr>
<tr>
<td>E1</td>
<td>Below Savage R. inlet</td>
<td>CP387889</td>
<td>P</td>
</tr>
<tr>
<td>E2</td>
<td>Below Donaldson R. inlet</td>
<td>CP353905</td>
<td>P</td>
</tr>
<tr>
<td>E3</td>
<td>East bank</td>
<td>BP322895</td>
<td>N</td>
</tr>
<tr>
<td>E4</td>
<td>East bank</td>
<td>BP292868</td>
<td>N</td>
</tr>
<tr>
<td>Nancy</td>
<td>On straight below Nancy Ck. inlet</td>
<td>CP426880</td>
<td>P</td>
</tr>
<tr>
<td>Whyte</td>
<td>Bend upstream of Whyte R. inlet</td>
<td>CP413868</td>
<td>P</td>
</tr>
</tbody>
</table>

2.3 Sampling

2.3.1 Sample bottles

Sample bottles were made of high-density polyethylene (HDPE, Nalgene). All sample bottles used for collection of samples for trace metal analysis were cleaned by initially soaking in 0.1 % Extran 30 detergent in hot water. They were then soaked for 1 week in 50 % HCl followed by 1 week in 10 % HNO₃ rinsed well with MQ water and stored in plastic zip-lock bags until required (Gledhill and van den Berg 1995). Where bulk water samples were required, river water was collected into acid-cleaned (10 % HNO 25 L carboys (HDPE).

For determination of alkalinity and gilvin (g440), water samples were collected into 1 L HDPE bottles which had previously been detergent-cleaned (0.1 % Extran) and rinsed well with MQ water. All bottles were rinsed 3 times with the sample before a sample was retained.

2.3.2 Sample collection

Water samples were collected using a custom-built, all-plastic, acid-cleaned, close-interval sampler (Jorgensen et al. 1979; Rouse 1998) which was held at the required water depth by a pre-calibrated nylon rope. The sampler was attached to the end of acid-cleaned polyvinylchloride (PVC) tubing through which water was pumped using a 12 V variable speed peristaltic pump (Masterflex model 7533-40). The tubing was flushed at each new depth for 5 to 10 minutes, prior to retention of sample. This time was based on flow rate (1200 mL/min) and tubing capacity measurements (maximum length = 90 m; inside diam = 10 mm).

At shallow tributary sites, sample bottles were immersed below the surface by hand either by reaching from the riverbank or by wading into the stream. Samples were collected manually in the upstream direction to avoid contaminating the sample. Powder free polyethylene gloves were worn at all times.
2.3.3 Filtration

Filtration was generally performed under vacuum through acid washed polycarbonate membranes (Poretics Corporation) of pore size 0.4 µm, held in a Millipore Sterifil Aseptic filtering unit. Prior to use, the filtration unit was soaked in 10 % HNO₃ for 24 hours, rinsed well with MQ water and stored in a zip-locked polyethylene bag. Filters were cleaned by soaking in 0.5 % HNO₃ (Suprapur) for 24 hours and then in MQ water for two 24 hour periods. They were stored in fresh MQ water until required and were then transferred to the filter holders using plastic tweezers.

Field and laboratory based filtration blanks performed throughout this study showed that the filtration apparatus and adopted procedures did not contribute significantly to metal concentrations.

For toxicity studies, samples were filtered using sterile disposable filtration units containing 25 mm cellulose acetate membranes of pore size 0.2 µm (Micro Filtration Systems; MFS). The filtration units were rinsed with sample (20 mL) before a filtered sample was retained for analysis.

In order to test for sample contamination by 0.2 µm disposable filtration units (MFS), three pre-treatments were tested (Table 2.3).

Table 2.3: Treatments for contamination control of MFS disposable filtration units.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No pre-treatment, 120 mL aliquot of filtered MQ retained for analysis</td>
</tr>
<tr>
<td>B</td>
<td>Units rinsed with MQ (1 x 20 mL) before retaining 120 mL aliquot of filtered MQ for analysis</td>
</tr>
<tr>
<td>C</td>
<td>Units rinsed with 10 % HNO₃ (2 x 20 mL) and MQ (2 x 20 mL) before retaining 120 mL aliquot of filtered MQ for analysis</td>
</tr>
</tbody>
</table>
Each treatment was performed in triplicate and measurement of peak currents in the resulting filtrates were performed in duplicate using DPASV (Section 2.5.1). Metal standard solutions were added to a blank MQ solution to determine the analytical sensitivity. This value was used to determine the Zn or Cu concentration in each of the filtrates (Figure 2.1). Analysis of variance (ANOVA) showed that filter pre-treatment did not significantly effect the Zn and Cu concentrations measured in the resultant filtrates (p > 0.05). Thus, for subsequent sample filtration, the disposable filtration units (MFS) were pre-rinsed (20 mL) once with the sample, before an aliquot was retained for analysis.

Figure 2.1: Zn and Cu concentrations in MQ blank filtrates with various filter pre-treatments (Mean ± standard deviation of replicate measurements).
2.4 Water quality measurements

Where possible, water quality parameters were measured in situ in the Pieman River catchment using a Hydrolab Surveyor®3 (SVR3) Data Logger in conjunction with an H2O® Multiprobe, calibrated as recommended in the SVR3 Operating Manual (1995). On occasions when this instrument was not available other meters were used and have been described in the following sections. All instruments were calibrated independently and measurements were crosschecked between meters.

2.4.1 Depth

Water depth was measured using a standard ruler, a pre-calibrated rope or by using the Hydrolab zeroed in air near the surface of water to be measured.

2.4.2 pH

pH was measured in situ using the Hydrolab data logger. A two buffer calibration (high conductivity pH 7 and pH 4 buffers) was used and the instrument was tested on a control sample with low conductivity (595 µS cm\(^{-1}\)) at pH 6.85 before being taken into the field.

An Orion Sureflow combination electrode connected to a Hanna Instruments (HI8519N) pH Meter, calibrated with a 0.05 M phosphate buffer of pH 6.88 and a 0.05 M potassium hydrogen phthalate buffer of pH 4.00, was used to perform pH measurements during laboratory-based experiments. The precision of the pH measurements was approximately ± 0.02 pH units.

2.4.3 Temperature

During field studies, water temperature was measured in situ using the Hydrolab, a Yeo Kal (Model 602 MK II) Salinity Temperature Bridge or a WTW Microprocessor Conductivity Meter (LF 96) with WTW TetraCon 96 conductivity probe. Crosscheck measurements between meters agreed closely. In laboratory experiments, water temperature was measured using a calibrated mercury thermometer.

2.4.4 Dissolved oxygen

Dissolved oxygen was measured in situ using the Hydrolab, a Yellow Springs Instrument (YSI; Model 57) Dissolved Oxygen Meter or a WTW Microprocessor Oximeter (OXI 96) with WTW Oxical—S probe (EO 96). All instruments were
calibrated independently in air and crosscheck measurements between meters were shown to agree closely.

2.4.5 Redox potential
Redox potential was measured *in situ* using the Hydrolab which had been calibrated using a standard Zobell solution containing ferric- and ferrous- cyanide in KC1, as recommended in the SVR3 Operating Manual (1995).

2.4.6 Salinity and conductivity
The Hydrolab, a Yeo-Kal Model 602 MK II Salinity Temperature Bridge or a WTW Microprocessor Conductivity Meter (LF 96) with WTW TetraCon 96 conductivity probe was used to determine salinity or conductivity during field studies. Cross-check measurements between meters agreed closely. For low ionic strength water samples, a TPS (Model 2100) Digital Conductivity Meter, calibrated using 0.01 M KC1, was used to measure conductivity.

This thesis adheres to the Practical Salinity Scale of 1978 for seawater and estuarine water samples. Thus salinities in the range of 2 - 43 parts per thousand on the old scale are reported as dimensionless values.

2.4.7 Alkalinity
Total alkalinity was determined in non-filtered samples using the standard titration method (APHA 1995). Analyses were performed within 24 hours of sample collection.

2.4.8 Suspended solids
Suspended solids were determined using Whatman Glass Fibre (GF/C) filters and the standard method (APHA 1995).

2.4.9 Gilvin (g440)
A Varian Techtron UV-VIS Spectrophotometer (Model 635) was used to perform all absorbance measurements. Wavelength was checked using a Didymium filter (BS5713; \( \lambda = 807.5 \pm 0.1 \) nm). Photometric repeatability was checked using a BS5715 screen at 800 nm and stray light was checked using a solution of potassium chromate (0.16 g/L in 0.05 M KOH) at 374 nm. Gilvin (g440) values were determined using 4 cm cells and Eqn. (10) as outlined by (Kirk 1976).
\[ g_{440} = 2.303 \times A_{440} / l \]  
where \( l \) = pathlength (m)

### 2.4.10 Organic carbon

Water samples for organic carbon determinations were collected in 125 mL HDPE bottles. As soon as possible after sample collection (< 24 hours), water samples for determination of “dissolved” organic carbon (DOC) were filtered through 0.4 µm polycarbonate membranes (Poretics Corporation). All samples were then preserved by acidification to pH 1-2 using H\(_2\)SO\(_4\) and stored in the dark at room temperature (21.5°C). Total organic carbon (TOC) was determined in unfiltered water samples.

For freshwater samples, organic carbon concentrations were measured using an SGE Anatoc Total Organic Carbon Analyser with UV detection, according to the method recommended by SGE Anatoc Instruction and Operation Manual (1994). The instrument was calibrated with 20 mg/L and 200 mg/L benzoic acid standards and showed linear behaviour within this concentration range. Replicate measurements of the 20 mg/L standard produced a precision of ± 0.5 mg/L.

Organic carbon concentrations in estuarine samples were analysed using a Skalar Total Organic Carbon Analyser by the Division of Environment and Planning Laboratory (Department of Primary Industries, Water and Environment), Chemistry Department, University of Tasmania. After the initial removal of inorganic carbon by mixing with dilute sulphuric acid and sparging with nitrogen, the sample is oxidised and exposed to UV irradiation in a reaction coil. The CO produced is removed by mixing with dilute HCl and nitrogen sparging. The CO is mixed with H and passed over a Ni catalyst at 400°C to produce methane. This passes to a flame ioniser that has been calibrated using tartaric acid standards (4 - 20 mg/L). A precision of ± 0.5 mg/L was determined by analysis of replicate samples.

### 2.4.11 Turbidity

Turbidity was measured using the Hydrolab turbidity sensor operated in nephelometric mode.
2.5 Analytical methods used for trace metal detection

All laboratory procedures and experiments were carried out in a small specialist laboratory (Deakin University, Warrnambool) supplied with filtered air. Critical manipulations were performed in an Airpure UVS Laminar flow cabinet.

2.5.1 Anodic stripping voltammetry

Voltammetric measurements were performed using a Metrohm 646 VA Processor in conjunction with a Metrohm 647 VA Stand unless indicated differently. A conventional three-electrode arrangement consisting of a Metrohm multi-mode electrode (MME) used in the hanging mercury drop electrode (HMDE) mode (drop size = 5), a Ag/AgCl (3.0 M KCl) reference electrode and a platinum wire auxiliary electrode was used. All samples were purged initially for 5-7 minutes with high purity nitrogen (BOC gases) followed by a rest period of 30 s. Five mercury drops were dispensed with the fifth drop being retained for the measurement. For single metal measurements, a deposition potential of -0.60 V (Cu) or 1.2 V (Zn) was applied for 2 minutes with the stirrer set at 2500 rpm, followed by 5 s rest period with the stirrer off. Metal ions reduced at the electrode were re-oxidised by scanning to 0.20 V (Cu) or to 0.60 V (Zn) in the differential pulse mode at a scan rate of 10 mV/s and a pulse height of 50 mV. For simultaneous determinations of total Zn, Cd, Pb and Cu, similar conditions were applied except a deposition potential of -1.2 V was employed, followed by an anodic scan to 0.20 V.

A Metrohm Polarecord E506 in conjunction with a 633 VA stand equipped with the same electrode system as described above, was used to investigate buffer effect and stirrer speed on ASV-labile metal concentration. Samples were purged as previously described. A deposition potential of -1.2 V was applied for 2 minutes with the stirrer set at 3 (1500 rpm). Metals reduced at the HMDE were re-oxidized during an anodic scan to 0.20 V.
2.5.2 Flame atomic absorption spectrophotometry
An Hitachi 6000 Polarized Zeeman Atomic Absorption Spectrophotometer (flame-AAS) was used in absorption mode for flame atomic absorption measurements. An air-acetylene flame was used for all analyses at the pressures recommended for this instrument. Standard conditions for this instrument (Hitachi 6000 Polarized Zeeman Spectrophotometer Operating Manual) were used for all elements analysed.

2.5.3 Graphite furnace atomic absorption spectrophotometry
An Hitachi 7000 Polarized Zeeman Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS) was used with Hitachi tube cuvettes to make all graphite furnace atomic absorption measurements. An Hitachi auto-sampler was used to inject samples (typically 20 µL). Standard conditions and temperature programs for this instrument were used (Hitachi 7000 Polarized Zeeman Graphite Furnace Spectrophotometer Operating Manual) for all elements analysed however atomisation times were reduced where possible to enhance the life of the graphite tubes.

2.5.4 Detection limits
The limits of detection for the analytical techniques used in this study (Table 2.4) were determined as three times the standard deviation of multiple (7 to 10) measurements of blank solutions or samples close to the detection limit (Willard et al. 1988). GF-AAS was found to be too sensitive for the determination of Zn as suitable blank values could not be achieved.

Table 2.4: Detection limits for analytical methods used in this study.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Method</th>
<th>Detection limit (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>ASV</td>
<td>0.3</td>
</tr>
<tr>
<td>Cu</td>
<td>ASV</td>
<td>0.5</td>
</tr>
<tr>
<td>Pb</td>
<td>ASV</td>
<td>0.5</td>
</tr>
<tr>
<td>Zn</td>
<td>ASV</td>
<td>1.0</td>
</tr>
<tr>
<td>Fe</td>
<td>flame-AAS</td>
<td>200</td>
</tr>
<tr>
<td>Mn</td>
<td>flame-AAS</td>
<td>50</td>
</tr>
<tr>
<td>Zn</td>
<td>flame-AAS</td>
<td>20</td>
</tr>
<tr>
<td>Cd</td>
<td>GF-AAS</td>
<td>0.1</td>
</tr>
<tr>
<td>Cu</td>
<td>GF-AAS</td>
<td>0.5</td>
</tr>
<tr>
<td>Fe</td>
<td>GF-AAS</td>
<td>2.0</td>
</tr>
<tr>
<td>Mn</td>
<td>GF-AAS</td>
<td>0.8</td>
</tr>
<tr>
<td>Pb</td>
<td>GF-AAS</td>
<td>0.5</td>
</tr>
</tbody>
</table>
2.6 Trace metal speciation procedures

2.6.1 Speciation scheme

Analysis of water samples was based on the scheme outlined in Figure 2.2. Time critical water quality parameters were measured in situ at the time of sample collection.

Figure 2.2: Analytical scheme for Pieman River water samples.
2.6.2 Total and “dissolved” metals

Samples collected for total metal analysis were preserved by acidification to pH 1-2 by addition of HNO₃ (Suprapur) and were stored in the dark, at room temperature.

Water samples collected for “dissolved” metal analysis were filtered within 24 hours of collection (Section 2.3.3). Following filtration, these samples were also preserved by addition of HNO₃ to pH 1 - 2 and stored in the dark at room temperature.

Prior to analysis for both total and “dissolved” metal fractions, the acidified samples were made 0.1 % in H₂O₂ and were irradiated with ultra-violet (UV) light for ~ 5 hours in quartz tubes arranged around a 1000 W Oliphant UV lamp held in a fan-cooled chamber. Metal concentrations were determined using GF-AAS or flame-AAS with calibration curves constructed using standards prepared in a similar matrix, or by ASV and the method of standard additions.

a) Metal recoveries following sample digestion

To investigate recoveries of trace metals when using the UV digestion method, four aliquots of Pieman River water were spiked using a 10 mg/L mixed-metal standard solution and were UV irradiated using the method previously described. They were then analysed using DPASV following addition of 40 µL of 5 M NaNO₃ to a 20 mL aliquot of each digested sample. Recoveries of Cu, Zn, Cd and Pb are shown in Table 2.5.

Recovery ranged from 97 % to 117 %. Recoveries in Tube 4 were systematically high for all metals indicating that the volume of standard added to this tube was greater than intended or else the sample volume was less than expected. Recoveries ranged from 97 % to 109 % if results from Tube 4 are ignored. Recoveries of Cu, Zn, Cd and Pb averaged 105 %, 104 %, 100 % and 100 % respectively if data from Tube 4 was removed from the calculations. As standard additions were not added for routine sample digestions, the random error associated with recoveries in Tube 4 can be eliminated for later experiments. The recoveries obtained using this method are therefore considered satisfactory.
Table 2.5: Recoveries of metal standard additions following sample digestion by UV irradiation.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Tube</th>
<th>Initial [M] (μg/L)</th>
<th>Added [M] (μg/L)</th>
<th>Final [M] (μg/L)</th>
<th>Recovery [M] (μg/L)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>1</td>
<td>0.59</td>
<td>25.4</td>
<td>28.2</td>
<td>27.6</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.59</td>
<td>25.7</td>
<td>27.2</td>
<td>26.6</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.59</td>
<td>27.1</td>
<td>28.7</td>
<td>28.1</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.59</td>
<td>24.6</td>
<td>28.6</td>
<td>28.0</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>μ±stdev 27.4±0.8</td>
<td>105±3</td>
</tr>
</tbody>
</table>

| Zn    | 1    | 22.0               | 25.4             | 49.6             | 27.5                | 108        |
|       | 2    | 22.0               | 25.7             | 47.6             | 25.6                | 99         |
|       | 3    | 22.0               | 27.1             | 50.2             | 28.1                | 104        |
|       | 4    | 22.0               | 24.6             | 50.9             | 28.8                | 117        |
|       |      |                    |                  |                  | μ±stdev 27.1±1.2     | 104±4.5    |

| Cd    | 1    | 0.05               | 25.4             | 25.9             | 25.8                | 102        |
|       | 2    | 0.05               | 25.7             | 25.4             | 25.3                | 98         |
|       | 3    | 0.05               | 27.1             | 27.1             | 27.0                | 100        |
|       | 4    | 0.05               | 24.6             | 26.6             | 26.5                | 108        |
|       |      |                    |                  |                  | μ±stdev 26.0±0.5     | 100±2      |

| Pb    | 1    | 0.98               | 25.4             | 26.8             | 25.9                | 102        |
|       | 2    | 0.98               | 25.7             | 26.0             | 25.0                | 97         |
|       | 3    | 0.98               | 27.1             | 28.2             | 27.2                | 101        |
|       | 4    | 0.98               | 24.6             | 26.9             | 26.0                | 106        |
|       |      |                    |                  |                  | μ±stdev 26.0±0.6     | 100±2.5    |

a: data from tube 4 not included

2.6.3 ASV-labile metals

Labile metals measured by ASV were determined using the method described in Section 2.6.5 or in 20 mL aliquots of non-acidified water sample to which 40 μL of 5 M NaNO₃ solution was added (Stauber et al. 1996; Davies et al., 1998).

For the latter method, standard addition curves were constructed by addition of standard metal solutions to an equivalent sample which had previously been UV irradiated (0.1 % H₂O₂ to destroy organic matter. In the case of ASV-labile Zn measurements, where the Zn binding capacity was usually exceeded, standard additions were added directly to the sample aliquot being analysed.
2.6.4 DGT-labile metals

a) Preparation and assembly of DGT units

Diffusive and Chelex gel sheets were purchased from DGT Research Ltd (Skelmorlie, Quenmore, Lancaster, UK) or prepared and stored as recommended (Zhang 1997), using Polyacrylamide (Electran, Molecular Biology grade) obtained from BDH Laboratory Supplies, TEMED (Electrophoresis reagent) obtained from Sigma and an agarose-derived cross-linker obtained from DGT Research. Other reagents used were obtained from Ajax Chemicals and were of analytical grade. The gel sheets were cut into discs using a disc gel cutter, assembled into the gel holders and stored in MQ water for up to several hours before deployment in the field. Acid-cleaned cellulose nitrate membrane filters of pore size 0.45 µm (MFS) were used in all DGT units.

b) DGT precision and recoveries

The performance of the DGT technique was tested in the laboratory to determine the precision and recoveries in two aqueous solutions. A 3 L solution of MQ water, buffered to pH 5.7 ± 0.1 by addition of a sodium acetate buffer solution (final concentration 0.01 M) was prepared in a polyethylene container with a well fitting lid. This solution was spiked with 150 µL of a 1 mg/mL standard solution of Cu(NO₃)₂ and 30 µL of a 1 mg/mL Cd(NO₃)₂ standard solution (all Ajax Chemicals, Spectroso grade) to give final concentrations of approximately 50 µg/L for Cu and 10 µg/L for Cd. The solution was stirred well and then allowed to equilibrate at 18°C (± 0.5°C) in the dark for 48 hours.

A 3 L solution of Pieman River water was prepared in the same way. The river water used was collected in February 1998 from a well-mixed and relatively unpolluted site in Lake Pieman known as Pieman above Bobodil (PAB). Five DGT assemblies were immersed for approximately 24 hours in each of the prepared solutions. During the deployment period, the solutions were stirred continuously using a magnetic stirrer. Water temperature was maintained at 18°C in an air-conditioned room. A Data Logger placed in an equivalent adjacent water sample (2 L) was used to monitor temperature throughout the deployment period. Results showed that temperature did not vary by more than ± 0.5°C during this period. Aliquots of the immersion solutions were
collected for metal analysis at the start of the deployment period and when the DGT units were retrieved.

At the end of the deployment period, the DGT units were removed from the immersion solutions and rinsed well with MQ water. The resin layers were retrieved and placed in Sterilin tubes, each containing 2 mL of 1 M HNO$_3$ solution. These solutions were left to equilibrate for approximately one week to allow extraction of metals from the resin prior to analysis by AAS.

Additionally, ASV-labile metals were measured immediately in a 20 mL aliquot taken from the 3 L container (buffer already added). Peak heights were measured in triplicate and the instrument sensitivity was determined in a similar sample that had been UV irradiated in presence of H$_2$O$_2$ to destroy organic matter.

Total metal and ASV-labile metal concentrations were measured in both the MQ and river water immersion solutions at the start of the DGT deployment period and at the time the DGT units were retrieved from the solutions (Table 2.6). Organic carbon analysis in the river water revealed a TOC concentration of 6.9 mg/L. Steady state conditions were maintained in these solutions during the deployment period.

**Table 6.2:** Speciation measurements (in MQ and river water solutions. Precision estimates based on data in Table 25.
In the MQ solution, where strong complexation was absent, measurements of labile Cu by DGT agreed well with labile concentrations measured using ASV (Table 2.6). A similar result was observed for labile Cd measurements in both the MQ and river water immersion solutions indicating that Cd was not strongly complexed by natural ligands in the river water. In the river water solution, where Cu was found to be strongly complexed, measurements of labile Cu by DGT and ASV also agreed well.

A precision of 10 % or better has been claimed for the DGT method (Zhang and Davison 1995). The biggest uncertainty is usually associated with the precision of the analytical measurement, but there are also uncertainties in the diffusion coefficient (D), proportion of metal extracted from the resin (\(f_e\)) and the gel thickness (\(\Delta g\)). Results from this study produced a precision of 13 % and 8 % for Cu and 10 % and 6 % for Cd, each measured in MQ solution and river water respectively (Table 2.6). Analysis of replicate DGT deployments (\(n = 5\)) is thus consistent with a precision of 10 % for Cu and Cd.

c) Field deployment protocols

In each of the Pieman River tributaries, the water at the sites selected for DGT deployment was fast flowing and well aerated with a maximum depth of 0.5 to 1 m. Builder’s twine was tied to a branch or log over-hanging the stream. The other end of the twine was tied to a rock that was placed on the bottom of the stream, downstream of the over-hanging branch, so that the twine remained taut. Pairs of DGT assemblies were suspended in-stream from the twine at a depth of 0.1 to 0.2 m below the water surface.

At deep sites (depth > 1 m), DGT units were deployed at various depths based on water quality measurements. The DGT assemblies were attached to a weighted polyethylene rope that was pre-calibrated for depth. Using a plastic hose-winder to lower the weighted rope into the water, the DGT units were attached to the rope using acid-washed modified Polytape Insulators (G669W HDPE; Gallagher Electronics Ltd, Hamilton, NZ) which held the DGT assemblies several centimetres away from the rope (Figure 2.3). These reusable units were easy to use whilst wearing gloves and could be attached to the rope at depths determined in the field on the day of deployment depending on relevant water chemistry measurements. The rope was kept afloat using polystyrene buoys.
On recovery, the DGT assemblies were rinsed well with MQ water and immediately placed in clean, plastic, zip-lock bags. As soon as possible upon return to the laboratory (≤ 4 hours) the caps of the DGT assemblies were carefully prised off and the resin layers were retrieved using plastic tweezers and placed in Sterilin tubes each containing 1 or 2 mL of 1 M HNO₃ solution. These solutions were stored in this way, usually for several weeks, until they could be analysed. By carefully prising the DGT cap away from the bottom piston, it was found that the DGT holders could be reused several times before the cap became too loose, at which time the units were discarded.

\[d\] Calculations

The theory and calculations involved in the measurement of DGT-labile metal concentrations have been previously described in Section 1.2.4. DOT concentrations reported in this thesis represent the mean ± 1/2 the range of measurements by duplicate DGT assemblies. Where greater than two units were deployed simultaneously, data is reported as the mean ± standard deviation.
2.6.5 Complexation capacity

a) Complexation titrations

Titrations were performed in 10 - 13 identical 125 mL acid-washed HDPE (Nalgene) bottles each containing an aliquot of sample (25 mL) which was buffered to the required pH using sodium acetate buffer, phosphate buffer or PIPES buffer. The volume of buffer added was selected to give an ionic strength of 0.01. The solutions were allowed to equilibrate at the required temperature (4 - 28°C) and were then spiked with additions of ionic Cu or Zn standard (250 µM) to give total metal concentrations in the range of 0 - 6 µM. Total metal was calculated as the sum of the added metal and the initial concentration present in the water sample. Thus $C_L$ is a “true” measure, taking into account already bound metal, rather than a residual binding capacity. Temperature was controlled (± 1.5°C) using an air-conditioner or by refrigeration. After a suitable equilibration period of 18 - 24 hours (determined by preliminary experiments described in Section 3.11) ASV was used to measure the oxidation current of the unbound or labile metal deposited at the HMDE. Solutions were analysed in ascending order of metal concentration. A titration curve was produced by plotting the duplicate current peak heights ($i_p$) against the total metal concentration for each sample aliquot (Figure 2.4).

![Figure 2.4: A typical metal ion complexation titration curve (The gradient of the solid line represents the instrumental sensitivity to free metal, S).](image)
Error bars associated with complexation titration results throughout this thesis represent
the 95 % confidence interval for each titration calculated using the uncertainty
associated with the slope and y-intercept of the van den Berg / Ruzic linearisation plots.

b) **Determination of complexation parameters**

The total ligand concentrations ($C_L$) and conditional stability constants ($K'$) were
determined from the experimental data using the van den Berg / Ruzic linearisation
method (Ruzic 1982; van den Berg 1982). The transformation, in its simplest form,
assumes that the heterogenous mixture of ligands in a natural water sample can be
considered as a single species, $L$, forming a 1:1 metal-ligand complex, $ML$, with the
metal titrant. From these assumptions, Eqn. (11) can be derived.

$$\frac{[M]}{[ML]} = \left(\frac{1}{C_L}\right) + \left(\frac{1}{K'C_L}\right) \quad (11)$$

where

$[M]$ = the concentration of uncomplexed metal (M)

$[ML]$ = the concentration of bound metal (M)

$K'$ = the conditional stability constant for the $ML$ complex (M')

$C_L$ = the total ligand concentration (M)

Unbound metal $[M]$ is calculated from the experimental titration data using Eqn. (12).
During this study, the slope formed by the final 4 points of the titration curve (Figure
2.4) was used to determine instrument response, $S$. In this linear region of the curve,
ligands are assumed to be saturated with metal ions (Turoczy and Sherwood 1997).

$$[M] = \frac{i_p}{S} \quad (12)$$

where

$S$ = the sensitivity of the analytical technique (AM$^{-1}$)

$i_p$ = the peak current (A)

The concentration of non-labile metal $[ML]$ is calculated by difference from the known
total metal concentration (CM) in the sample and the free metal ion $[M]$ concentration
using Eqn. (13).

45
If the single ligand model is appropriate, a plot of $[\text{M}]/[\text{ML}]$ vs. $[\text{M}]$ results in a straight line (Figure 2.5). Values for $C_L$ and $K'$ can then be obtained from the slope and the y-intercept (Apte et al. 1988; Donat and Bruland 1990). Only estimates of conditional stability constants can be obtained by ASV titrations because the fixed detection window of this technique usually underestimates the Cu binding strength (Apte et al. 1988; Apte et al. 1995).

Figure 2.5: The van den Berg / Ruzic transformation of data in Figure 2.4.

During this study, no significant curvature of the $[\text{M}]/[\text{ML}]$ vs. $[\text{M}]$ plot was observed indicating that samples could be treated as containing a single complexing ligand (or complexing site) over the range of metal concentrations studied (van den Berg and Dharmvanij 1984). The 1:1 model has often been found to be useful in describing experimental data. Other more complicated models are available to treat data that do not fit this model (Ruzic 1982; van den Berg 1982; van den Berg 1984; Ruzic 1996).
C) *Precision of parameters determined by complexation titrations*

The precision of Cu ion complexation titrations was determined from replicate titrations performed on separate sub-samples of a Lake Pieman water sample at pH 4.80 and 5.90 (Table 2.7). From five titrations performed at pH 4.80, the average measured $C_L$ was 320 nM (rsd = 13 %). A higher precision was achieved from five replicate titrations performed at pH 5.90, where the average measured $C_L$ was determined as 610 nM (rsd= 5%).

**Table 2.7:** Precision of complexation parameters determined by titration of Pieman River water with ionic Cu at pH 4.8 and pH 5.9

<table>
<thead>
<tr>
<th>pH</th>
<th>$C_L$ (nM)</th>
<th>$C_L$ (μg Cu/L)</th>
<th>$K'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>320</td>
<td>21</td>
<td>1.32 x 10^7</td>
</tr>
<tr>
<td></td>
<td>±40</td>
<td>±3</td>
<td>±7.05 x 10^6</td>
</tr>
<tr>
<td></td>
<td>±13</td>
<td>±13</td>
<td>±53</td>
</tr>
<tr>
<td></td>
<td>±5</td>
<td>±5</td>
<td>±5</td>
</tr>
<tr>
<td>5.9</td>
<td>610</td>
<td>39</td>
<td>1.07 x 10^7</td>
</tr>
<tr>
<td></td>
<td>±30</td>
<td>±2</td>
<td>±6.90 x 10^6</td>
</tr>
<tr>
<td></td>
<td>±5</td>
<td>±5</td>
<td>±64</td>
</tr>
<tr>
<td></td>
<td>±5</td>
<td>±5</td>
<td>±5</td>
</tr>
</tbody>
</table>

Measured over 6° or 18° months (see Section 3.13).

The method for determination of $K'$ from the van den Berg / Ruzic transformation is extremely susceptible to measurement variability produced by the analytical technique. Poor precision was achieved for replicate determinations of $K'$ at both pH 4.8 (rsd = 53 %) and pH 5.9 (rsd = 64 %). The variability in sequential measurements can sometimes produce enough variation in the line representing the transformed data to produce a negative value for $K'$ (Apte et al. 1988). Thus, $K'$ values determined throughout this thesis are used to estimate an average log $K'$ value for Pieman waters but are not used to compare variation in water samples under different experimental conditions.

**2.6.6 Toxicity tests**

Bioavailable Zn was estimated in mixed estuarine samples by an algal enzyme inhibition test. Bioassays using filtered Pieman River estuarine samples were performed by the Centre of Advanced Analytical Chemistry, CSIRO, Lucas Heights, NSW,
Australia. The bioassay is based on galactosidase activity in the green marine alga *Dunaliella tertiolecta* (Petersen and Stauber 1996; Stauber *et al.* 1996). The galactosidase enzyme is present in some algal species that exist in dark environments, allowing growth where photosynthesis cannot occur. It has also been found in other organisms such as sediment bacteria and algae (Petersen and Stauber 1996).

β-D-galactosidase enzymes present in *D. tertiolecta* are able to cleave the fluorogenic substrate 4-methylumbelliferone-β-D-galactoside (MU-gal) releasing the fluorescent compound 4-methylumbelliferone (MU). The presence of toxicants reduces enzyme activity, resulting in a proportional reduction in fluorescence. *D. tertiolecta* cells were exposed to various concentrations of ionic Zn, under defined conditions.

**a) Algal stock cultures**

Algal bioassays were performed according to the standard protocol described by Petersen and Stauber (1996) using *Dunaliella tertiolecta* Butcher (Strain CS-175). Algae were cultured axenically in a modified half strength medium with half strength Fe and trace element concentrations and maintained on a 12 h light : 12 h dark cycle at 21 °C (Petersen and Stauber 1996).

Cells in the logarithmic growth phase were used in the algal bioassays (Petersen and Stauber 1996). Prior to their use, culture medium was removed by washing and centrifuging the inoculum three times. The washed *Dunaliella tertiolecta* cells were counted using a Coulter Multisizer II Particle Analyser (70 µm aperture). The final concentration of cells in each assay tube was $10^5$ cells/mL.

**b) Algal enzyme inhibition bioassay**

0.0625 g of MU-gal was dissolved in 8 mL of dimethylformamide (DMF) to produce the fluorescent substrate, MU-gal. The solution was sonicated to dissolve the substrate prior to the addition of 8 mL of MQ water. To minimise background fluorescence, the solution was cleaned by passing it through a Waters Accell Plus QMA Sep-Pak Plus cartridge (pre-rinsed with 20 mL of 0.5 M NaOH and 40 mL of MQ water) at a flow rate of 4 mL/min. The Sep-Pak was then rinsed with 8 mL of MQ. The rinse solution was added to the MU-gal eluent and this combined solution was made up to 25 mL with
MQ water in a volumetric flask. The solution was filter-sterilised through 0.22 µm membrane filters immediately prior to performing the enzyme assay.

The algal inoculum was prepared so that a volume of 50 - 100 µL was sufficient to add enough cells to produce a final density of 1 x 10⁵ cells/mL. Bioassay test solutions were buffered to a pH of 7.2 ± 0.1, using PIPES buffer, pH adjusted by addition of 3 M HCl. Buffering at this pH was relevant for samples investigated during this study which were within a pH range of 6.9 to 7.9 (Table 4.6). A summary of the test protocol is given in Table 2.8.

**Table 2.8: Summary of the test protocol for the enzyme inhibition bioassay using marine alga**

(*Dunaliella tertiolecta*)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test type</td>
<td>Static</td>
</tr>
<tr>
<td>Temperature</td>
<td>44.5 ± 0.5°C</td>
</tr>
<tr>
<td>Test chamber size</td>
<td>12 mL</td>
</tr>
<tr>
<td>Test solution volume</td>
<td>9.1 mL</td>
</tr>
<tr>
<td>Renewal of test solutions</td>
<td>None</td>
</tr>
<tr>
<td>Age of test organisms</td>
<td>5 - 6 days</td>
</tr>
<tr>
<td>Initial cell density</td>
<td>1 x 10⁵ cells/mL</td>
</tr>
<tr>
<td>No. replicates per sample concen.</td>
<td>3</td>
</tr>
<tr>
<td>No. of concentrations for calibration curve</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Dilution water</td>
<td>Natural 0.22 µm filtered or autoclaved seawater</td>
</tr>
<tr>
<td>Test duration</td>
<td>1 hour</td>
</tr>
<tr>
<td>Endpoint</td>
<td>Enzyme activity (fluorescence)</td>
</tr>
<tr>
<td>Test acceptability</td>
<td>Control response: 260 – 1223 nM MU</td>
</tr>
</tbody>
</table>

A Perkin-Elmer LS-5 Luminescence Spectrometer, initially calibrated using MU standards (0, 80, 160, 320 and 640 nM) was used to measure fluorescence (excitation λ = 375 nm and emission λ = 465 nm). Fluorescence was reported as concentration of MU. Blank solutions of MU-gal with no algae were carried through the bioassay procedure to account for contribution to the fluorescence signal by chemical hydrolysis of the substrate, which may occur during the incubation period. A blank, containing algae plus toxicant (but no MU-gal) was also included to correct for any background fluorescence contributed by the algae.

The enzyme activity or toxicity of the water samples was determined by reduction in fluorescence of the algae in the presence of Zn compared to an appropriate control.
Fluorescence inhibition was calculated as a percentage of the control response (Petersen and Stauber 1996; Stauber et al. 1996). Bioavailable Zn was estimated from toxicity response calibration curves constructed at salinities of 20 and 34.

Statistical analysis was performed using trimmed Spearman Karber or Probit analysis. Data were initially tested for normality and homogeneity of variance. Dunnetts Multiple Comparison ANOVA Tests were then used to determine which test concentrations were significantly different to controls (U. S. EPA 1994). This enabled estimation of the sub-acute end-points of the algal enzyme inhibition assay which include an EC (Effective Concentration at which 50 % of test organisms are affected compared to the controls), the LOEC (Lowest Observed Effect Concentration) and the NOEC (No Observed Effect Concentration).

2.7 Chemical speciation modelling

Chemical speciation modelling was performed on an IBM compatible Pentium PC using the Windemere Humic Aqueous Model (WHAM; Tipping 1994).

2.8 Data processing and statistical analysis

Data processing and statistical analyses were performed using Microsoft ®Excel 97 or Systat Version 7.0, 1997, SPSS INC.

2.9 Units used throughout this thesis

Although trace metal concentrations are usually expressed in terms of molarity in many international journals (e.g. Marine Chemistry, Analytica Chimica Acta), a mixture of units are used throughout this thesis. Molar concentrations are a requisite of calculations involving equilibrium constants (i.e. the van den Berg / Ruzic transformation and the WHAM computer program) and are thus used accordingly. In most other situations, concentrations are expressed in terms of µg/L or mg/L in line with relevant water quality guidelines (ANZECC 1992; ANZECC and ARMCANZ 1999). On some occasions, both units are reported.
CHAPTER 3

Method Development

3.1 Introduction

Many studies of metal ion complexation have been performed on solutions prepared from isolated humic acids (e.g. Hering and Morel; Hoxey 1994; van den Hoop et al. 1994). Hoxey (1994) concluded that measured metal ion complexation parameters in these solutions was primarily a function of the extractant and its concentration during the isolation of the humic acid. It is therefore difficult to relate complexation parameters measured in these prepared solutions with processes that occur within natural water samples. For this reason, all complexation parameters measured during this study have been performed in natural water samples.

Where samples are collected for laboratory analysis for trace metal speciation and modelling studies, it is important that sample handling and analytical procedures have minimal effect on speciation within the sample. During this study, metal speciation and complexation were determined using ASV. Measurements were performed on samples manipulated in laboratory experiments to simulate changes in environmental conditions.

A series of experiments was therefore initially performed to test aspects of the ASV methodology and to establish that observed changes within a sample were a result of changes to the variable being investigated and not just artefacts of the analytical technique.

A bulk water sample from Lake Pieman (PAB 21296) was collected in February 1996 to investigate the potential of ASV for use in this project. Water quality parameters for this water sample are summarised in Table 3.1.
Table 3.1: Water quality of the Lake Pieman sample used for methodology development experiments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.90</td>
</tr>
<tr>
<td>Conductivity</td>
<td>31.2 μS/cm</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>2.0 mg/L</td>
</tr>
<tr>
<td>TOC</td>
<td>13.5 mg/L</td>
</tr>
<tr>
<td>DOC</td>
<td>11.1 mg/L</td>
</tr>
<tr>
<td>Total Fe</td>
<td>0.76 mg/L</td>
</tr>
<tr>
<td>Total Zn</td>
<td>15 μg/L</td>
</tr>
<tr>
<td>Total Cu</td>
<td>0.6 μg/L</td>
</tr>
<tr>
<td>Total Pb</td>
<td>1.1 μg/L</td>
</tr>
<tr>
<td>Total Cd</td>
<td>&lt; 0.1 μg/L</td>
</tr>
</tbody>
</table>

3.2 Influence of stirrer speed on peak current

For polarographic techniques such as ASV, the lability of a metal-ligand complex depends on the dissociation kinetics and on the effective measurement time of the technique. The measurement time depends on the time that a metal-ligand complex is in the diffusion layer surrounding the electrode, where it may dissociate and contribute to the flux of accumulating metal to be measured. For a HMDE, the thickness of the diffusion layer is controlled mainly by the rate at which the solution is stirred (Florence 1986).

The influence of stirring speed on Zn, Cd, Pb and Cu peak heights was examined simultaneously in a spiked Pieman River water sample. Sodium acetate buffer (pH 4.7) was added to a 20 mL aliquot of the sample to give an ionic strength of 0.01. Analyses were performed by DPASV using a Metrohm 633 VA Stand in conjunction with a Metrohm Polarecord E506. All stirrer settings were tested at least twice and were selected in a random order. Variation in peak current with respect to stirrer speed is shown in Figure 3.1.
Figure 3.1: Variation in peak height with change in stirrer speed (● = Zn; ■ = Cd; ▲ = Pb; ● = Cu).

Stirrer speed had similar effects on peak current for the four metals investigated (Figure 3.1). Stirring speeds greater than 1 500 revs/mm produced little variation in peak current. Based on these results, a stirring speed of 2500 revs/min was selected for use for all subsequent measurements.
3.3 Influence of deposition time on peak current

The linearity of peak current with respect to deposition time at the HMDE was investigated (Figure 3.2) in a Pieman River water sample which was made 5 µg/L in Cu by addition of a Cu(NO₃)₂ standard solution. Sodium acetate buffer (pH 4.7) was added to a 20 mL aliquot of the sample which was analysed by DPASV. The sample was purged initially for 5 minutes with high purity nitrogen. Cu was reduced at the HMDE using a deposition potential of -0.25V for 2, 5, 10 or 20 minutes. After 5 seconds rest period, the Cu was re-oxidized using an anodic scan from -0.25 V to 0.20 V.

![Figure 3.2: Change in peak current with deposition time.](image)

An increase in deposition time (E_D) produced a linear increase in peak current (Figure 3.2). From these results, the minimum deposition time of two minutes was chosen for use in all analyses throughout this thesis.
3.4 Linearity of peak current with metal ion concentration

Linearity of peak current with respect to metal concentration was investigated for Zn and Cu in Pieman River water (Figure 3.3). Measurements were performed using the method described in Section 2.5.1. For a deposition time of 2 minutes, Zn peak current was found to be linear with concentration to \([\text{Zn}_T] \geq 8 \mu\text{M}\). Cu peak current was found to be linear with respect to concentration up to \([\text{Cn}_T] \leq 6\mu\text{M}\). The maximum total metal concentration used in subsequent complexation titrations was therefore always \(\leq 6\mu\text{M}\).

Figure 3.3: Linearity of Cu and Zn DPASV peak current with variation in metal concentration.
3.5 Influence of pH on peak current

The influence of pH on peak current was investigated in a sample of UV irradiated MQ water (pH 2) that was spiked to a concentration of 5 µg/L with a Cu standard solution. pH was adjusted between measurements by 100 µL additions of a 1 M KOH solution. 200 µL of sodium acetate buffer (pH 4.8) was initially added to the reaction solution to swamp and therefore minimise ionic strength effects as a result of KOH additions. Following the measurement of ionic Cu at pH 11.5, the pH was adjusted back to pH 5.8 and pH 4.8 by two sequential additions of 200 µL of a 1 M acetic acid solution (represented by the empty red circles on Figure 3.4). Re-measurement at these two pH values demonstrated that “hysteresis” effects were negligible. Ionic Cu in solution was analysed by DPASV. Peak current was measured in duplicate for each pH (Figure 3.4). The solution pH was monitored in a duplicate aliquot of the same sample to which equivalent portions of sodium acetate buffer and 1 M KOH were added at the same times throughout the experiment.

Figure 3.4: Effect of pH on DPASV Cu peak current (see text for significance of open and closed points).

A decline in peak height was observed as pH increased. As the Cu concentration was quite low, a possible explanation for this effect may be removal of Cu ions from solution by adsorption processes (Davison et al. 1987), which might also be expected to
be reversible when the solution was re-acidified. For small changes in pH however, sensitivity of the analytical method did not differ significantly. For the pH range of 2.6 to 5.2, peak height ranged from 27 to 30 nA. This peak current range represents a sensitivity range of 0.34 nA/nM to 0.38 nA/nM (i.e. 1/2 range = ± 6 %).

### 3.6 A comparison of phosphate and acetate buffers

Because of the dilute nature of Pieman waters and the lack of natural pH buffering capacity due to low alkalinity, it is necessary to buffer samples prior to analysis so that disturbance to the equilibrium, as a result of the actual measurement, is minimised. Adding buffer can disturb the sample equilibrium, however such disturbance can be controlled and minimised by selection of suitable buffers and by addition of minimal volumes.

To study the effect of pH on metal speciation in Pieman waters, it was necessary to adopt a buffer system that would allow samples to be buffered over a wide pH range (i.e. pH 4.0 to pH 8.5) without introducing experimental artefacts. Most universal buffers described in the literature contain several components, the most common being the citric acid/citrate component. The citrate ion, is itself, a simple ligand, capable of metal complexation. Perdue (1989) used the behaviour of the citrate ligand to model metal complexation by humic substances. Universal buffers that contain citrate ions were avoided for this reason.

Two simple buffers were therefore selected for use in this study. Acetate buffer, which is commonly used in voltammetric speciation studies (Florence et al. 1992; Iyer and Sarin 1992), can be adjusted to provide buffering capacity within the pH range of 3.4 to 5.9. Phosphate buffer has also been used in some studies (Florence 1992; Einax and Kunze 1996) and can be adjusted to provide buffering capacity within the pH range from 5.8 to 8.0. The overlap in pH ranges of the two buffers allowed comparisons to be made between the buffers at their common pH.

To determine whether the type of buffer influenced labile metal concentrations in Pieman water, a Pieman River water sample was spiked with ionic Zn, Cd, Pb and Cu and allowed to equilibrate for several weeks at room temperature. The Zn, Cd, Pb and
Cu ASV stripping peak currents \( (i_p) \) were measured using acetate buffer in three sub-samples and using phosphate buffer in another three sub-samples. Both buffers were prepared to maintain a pH of 5.80 ± 0.02. Three standard addition curves were constructed in MQ water for both buffers. The average sensitivity \( (S_{avg}) \) of the system for each buffer was then calculated. ASV-labile metal concentrations were determined by dividing \( i_p \) measured in the sub-sample of river water, by \( S_{avg} \). Statistical analysis using Students t tests for the difference between two means showed that the ionic metal fractions measured using acetate buffer did not differ significantly from the concentrations measured using phosphate buffer (Table 3.2; \( p > 0.05 \)). These results indicate that choice of buffer did not influence the measurement of ASV-labile Cu, Zn, Pb or Cd in Pieman River water at pH 5.80.

**Table 3.2:** Effect of acetate and phosphate buffers on ionic metal concentrations measured by ASV in Pieman River water.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>[Zn] (µg/L)</th>
<th>[Cd] (µg/L)</th>
<th>[Pb] (µg/L)</th>
<th>[Cu] (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>21.8</td>
<td>8.2</td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Stdev</td>
<td>0.8</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Phosphate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>20.7</td>
<td>8.6</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Stdev</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td><em>p-value</em></td>
<td>0.08</td>
<td>0.18</td>
<td>0.12</td>
<td>0.41</td>
</tr>
</tbody>
</table>
3.7 Influence of ionic strength on peak current

The minimum ionic strength required for stable, reproducible measurement of ionic Cu was investigated in MQ water. Portions (50 - 1000 µL) of sodium acetate buffer (ionic strength = 0.55; pH 4.8) were added to a spiked MQ water sample (5 µg/L in Cu$^{2+}$). The sample was then purged with high-purity nitrogen for 10 minutes, followed by a rest period of 30 seconds. Analyses were performed by DPASV using a Metrohm 633 VA stand and the Polarecord E506 processor. Cu was deposited at the HMDE using a deposition potential of -0.25 V for 2 minutes after which time the metal was re-oxidised by scanning to 0.20 V. Replicate analyses were performed at each buffer concentration (Figure 3.5). Peak current was found to be relatively stable following additions totalling 200 µL of buffer corresponding to a contributed ionic strength of 0.0055.

![Figure 3.5: Influence of ionic strength on Cu peak current by additions of acetate buffer (pH 4.8).](image)

A second experiment was run to determine the effect of increasing additions of phosphate buffer (pH 5.8) on stability of Zn, Cd, Pb and Cu peak heights. A MQ water sample was spiked to 5 µg/L in each of Zn, Cd, Pb and Cu. The ionic strength of the
concentrated phosphate buffer was 1.097. Small portions of this buffer (25 - 400 µL) were added to the sample and replicate analyses were performed at each buffer concentration (Figure 3.6).

![Figure 3.6: Influence of ionic strength on Zn, Cd, Pb and Cu peak current by additions of phosphate buffer (pH 5.8).](image)

For each metal analysed during this experiment, peak current was found to be relatively stable when ≥ ~ 100 µL of phosphate buffer was added to the sample. This volume of buffer contributed an ionic strength of 0.0055 when added to 20 mL of sample.

These results showed that an ionic strength ≥ 0.0055 produced relatively stable and consistent peak heights using either the acetate or phosphate buffers. The reason for the different Cu responses observed at low ionic strength is not known (Figures 3.5 and 3.6). In order to minimise peak current variation due to small variations in buffer concentration in subsequent speciation studies, the volume of acetate or phosphate buffer added to samples was therefore calculated to give a minimum sample ionic strength of 0.01.
3.8 Influence of buffer concentration on speciation measurements

As speciation and complexation experiments were a major part of this study, it was necessary to determine the effect of the selected buffers on metal-ligand complexes and thus on metal speciation.

An experiment was designed to determine the effect of sodium acetate buffer (pH 4.8) on metal speciation in Pieman water. By monitoring the ASV-labile fractions of Zn, Cd, Pb and Cu as buffer strength was increased, whilst keeping ionic strength constant for all experiments by the addition of KNO₃, buffer effects could be isolated from ionic strength effects.

A non-acidified Pieman water sample was spiked to 10 µg/L in Zn, Cd, Pb and Cu. The sample was stored in an acid-washed HDPE bottle and equilibrated for several weeks at room temperature. A 1.1 M sodium acetate buffer (pH 4.8) and a 1.1 M KNO₃ solution were prepared in MQ water and stored in Sterilin bottles. The ASV-labile metal fractions in the sample were then analysed using various proportions of buffer and KNO₃ (Table 3.3).

After duplicate measurements of Zn, Cd, Pb and Cu peak heights in the sample, four 10 µl additions of a standard metal solution, containing 10 mg/L Zn, Cd, Pb and Cu, were added to the sample to determine sensitivity of the instrument for each metal. Duplicate analyses were performed after each spike in all cases. Samples were purged with nitrogen for 1 minute between replicate runs and for 3 minutes after a standard addition was made. For each set of experimental conditions (Table 3.3), a standard addition curve was constructed in MQ water using identical experimental conditions.
Table 3.3: Volumes of sodium acetate buffer and KNO solution added to 20 mL of Pieman sample prior to determination of ASV-labile metal fractions.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer volume(μL)</td>
<td>2000</td>
<td>1000</td>
<td>500</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>KNO₃ volume (μL)</td>
<td>0</td>
<td>1000</td>
<td>1500</td>
<td>1750</td>
<td>1900</td>
</tr>
<tr>
<td>Ionic strength</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Variations in the gradients of the standard curves produced in MQ water were independent of the buffer volume when experimental uncertainties are considered (Figure 3.7). Thus complexation of metals by the acetate buffer was not significant for the experimental conditions and reaction time described.

Figure 3.7: Effect of buffer volume on gradients of standard additions curves measured by DPASV in MQ water (● = Zn; ♦ = Cd; ■ = Cu; ▲ = Pb).

Before determining the ASV-labile fraction measured in the Pieman water, peak currents for the measurement of Zn were adjusted to account for contamination in the buffer. Zn contamination in the buffer was apparent by the significant (p < 0.01) change in calibration curve intercepts in MQ water as buffer volume increased (Figure 3.8).
Figure 3.8: Effect of buffer on the intercept of standard additions curves measured by DPASV in MQ water (● = Zn; ● = Cd; ■ = Cu; ▲ = Pb).

The ASV-labile metal fraction in Pieman water was calculated using the peak height measured in the Pieman sample for each metal and the sensitivity determined from standard ionic metal additions in MQ water (Figure 3.9).

No significant differences were observed in ASV-labile metal concentration for any metal over the buffer range and experimental conditions studied. The slope of the line representing the variation in the Zn concentration (Figure 3.9) did not differ from zero (p > 0.05), Therefore, acetate buffer was found not to alter the measured labile metal concentration, over the range of experimental conditions described.
3.9 Influence of directly reducible complexes

It is well known that AHS have a range of metal binding sites of differing stability and hence metal lability. As a result, the percentage of bound metal that is labile is a function of both the metal-ligand ratio and the time-scale of the experimental measurement (Hawke et al. 1996). An underlying assumption of the quantitation of labile metals by ASV is that metal-ligand complexes, ML, are not directly reducible. However, the direct reduction of some complexes has been found to occur and the presence of such complexes within a sample can be detected from the effect of ASV deposition potential on peak current by psuedo-polarography (Florence 1986). If such substances are present, the peak current will increase continuously with an increase in \( E_D \) instead of increasing from zero to a limiting value over a small range of \( E_D \).

Pseudo-polarograms, produced by measuring peak current following deposition at a range of potentials, should have a typical polarographic wave shape (Florence 1986). When peak height increases continuously with deposition potential, metal complexes are present which are being directly reduced at the electrode surface without first dissociating in the diffusion layer to metal ion and ligand (Florence 1986).
A pseudo-polarogram for Cu was constructed in Pieman water to determine the effect of deposition potential on the Cu peak current. The analysis was performed in 20 mL of Pieman water spiked to a concentration of ~50 µg/L in Cu and allowed to equilibrate for 6 days at room temperature and natural light conditions. In later experiments, $C_L$ for Cu in Pieman water was measured as 53 and 66 µg/L (Table 5.6) and so it is expected that a significant proportion of the total Cu concentration (50 µg/L) in the spiked sample would be complexed. This concentration was therefore considered suitable for investigating the significance of directly reducible complexes. Following deposition, ionic Cu was stripped from the HMDE using a potential scan from -0.60 V to 0.20 V (Figure 3.10).

![Figure 3.10: Effect of deposition potential on Cu peak current (Potential scan -0.60 V to 0.20 V; $CU_T$ 50 µg/L).](image)

This pseudo-polarogram produced in the Pieman water sample showed the classical polarographic shape indicating that direct reduction of Cu-ligand complexes was not occurring at the electrode and therefore not contributing to the ASV-labile measurement. Florence (1986) suggested that the deposition potential selected for analyses of natural water samples should be just sufficiently negative to produce the maximum peak current for the free metal ion, to minimise the chance of directly-
reducible complexes contributing to the ASV-labile fraction. The potential of -0.60 V was therefore selected for the deposition of Cu for all subsequent speciation studies in Pieman water, during this study.

3.10 Influence of adsorption processes at the HMDE

Interference of reduction and oxidation processes by humic material at the electrode during deposition and stripping stages of ASV has been reported (Morrison et al. 1990; Florence et al. 1992; Florence 1992; Scarano and Bramanti 1993; Muller 1996). Several methods have been described to reduce the influence of adsorbed humic material on oxidation processes at the HMDE. Muller et al. (1996) have used a method developed by Scarano and Bramanti (1993) which incorporates a cathodic scan to -1.40 V to remove adsorbed organic material from the electrode before an anodic scan is initiated to oxidise the metals of interest.

Morrison et al. (1990), Florence et al. (1992) and Florence (1992) have incorporated an acidification step and analysed the sample at both the natural pH and under acidic conditions using a method first described by Gregor and Powell (1988). Alternatively, medium exchange, where the test solution is replaced after electrodeposition by an electrolyte solution such as acetate buffer, before the oxidation of deposited metals, has also been used (Florence and Mann 1987).

The acidification and cathodic scan methods were investigated in Pieman River water but appeared to offer no advantage over the method described in Section 2.5.1. Methods incorporating a cathodic scan to remove humic material from the HMDE had no influence on sensitivity of the analytical technique when compared to the adopted method. The double-acidification method also offered no advantages and appeared to reduce sensitivity. A further disadvantage of this method is that replicate measurements cannot be performed on a single sample. Results therefore suggest that interference of reduction and oxidation processes by humic material at the electrode surface were negligible when using the adopted method as described in Section 2.5.1.
3.11 Rate of Cu ion complexation

The rate of equilibration of ionic Cu spikes in Pieman River water was investigated. Five replicate portions (200 mL) of river water were buffered to pH 5.90 ± 0.02 with sodium acetate buffer (ionic strength = 0.01). These solutions were spiked with a standard Cu solution (250 µM) to give initial Cu concentrations ranging from 250 nM to 3000 nM.

Labile Cu in each bottle was monitored at various times over the following 30 hours. A 20 mL aliquot of each sample was transferred to a glass Metrohm cell and the remaining unbound Cu was measured using ASV. In each set of analyses the most dilute sample was analysed first, proceeding to the most concentrated sample. Thus the cell was not rinsed between samples but was drained for several minutes before the next sample was added. Measurements were performed in duplicate in all cases, with a 1 minute purge between duplicate runs. Sensitivity (S) of the instrument to changes in ionic Cu concentration was determined by adding a further 4 standard additions to the sample containing the highest Cu concentration. The labile Cu remaining in solution over time was calculated by dividing peak current (i_p) by S. Results for solutions containing initial [Cu] of 1000, 2000 and 3000 nM are given in Figure 3.11.

![Figure 3.11: Change in labile Cu in Pieman River water over time (♦ = Initial [Cu_T] = 3000 nM; ■ = Initial [Cu_T] = 2000 nM; ● = Initial [Cu_T] = 1000 nM).](image-url)
The ASV-labile Cu concentration decreased rapidly initially and remained stable after approximately 5.5 hours equilibration time (Figure 3.11). This behaviour was also observed in river water initially spiked at lower concentrations. The first set of duplicate measurements in each trial was made within 15 minutes of the sample preparation. Because of the relatively fast rate of equilibration, the initial concentration of labile Cu was calculated from the spike rather than from a direct measurement.

Based on these results, a period of 18-24 hours was considered a suitable and convenient equilibration time for all subsequent complexation experiments.

### 3.12 Adsorption of ionic Cu onto HDPE

The adsorption of ionic Cu onto HDPE sample bottles was investigated concurrently with the previously described experiment to ensure that the decrease in ionic Cu observed was due to complexation with ligands in the sample and not caused by adsorption onto the bottles. This experiment was performed in the absence of NOM, as metal complexation by NOM would potentially mask adsorption effects.

Two portions (200 mL) of MQ water contained in HIDPE bottles (250 mL) were buffered to pH 5.90 ± 0.02 with sodium acetate buffer (ionic strength 0.01). They were then spiked with a standard Cu solution (250 µM) to give an initial Cu concentration of 247 nM (15.7 µg/L). Aliquots were decanted from the HDPE bottles at various times over the following 28 hours in which duplicate measurements of labile Cu were performed. Standard addition curves were constructed (At time = 15 mins, 5 hrs and 24 hrs) in aliquots of the sample to determine instrumental sensitivity. Instrumental response determined by regression analyses for the three standard curves were 0.403, 0.407 and 0.403 nA/nM. Data from these curves were used to determine [Cu$^{2+}$]. The ASV-labile Cu concentration monitored over time was compared with the initial [Cu$^{2+}$] added to the MQ water (Figure 3.12).
Any adsorption of ionic Cu to the HDPE bottles was less than 19 nM (1.2 µg/L) compared to a detection limit of 0.5 µg/L. Thus, the change observed in ionic Cu concentration in Pieman water, over time, cannot be attributed to adsorption of the metal onto the sample bottles. This experiment was performed in the absence of NOM as organic complexation may have masked adsorption effects.
3.13 Ligand stability during sample storage

To determine the potential for sample storage to alter the measured complexation parameters over time, complexation titrations were performed at various intervals in aliquots decanted from a river water sample (PAB27397) that was stored in the dark at room temperature in a HDPE 20 L carboy. $C_L$ values measured in the same sample over an 18 month period are shown in Figure 3.13.

![Figure 3.13: Change in CL measured at various intervals in a Pieman River water sample stored in the dark at room temperature (215°C; ■ = pH 5.9, solid line represents mean concentration (610 nM); ● = pH 4.8, solid line represents mean concentration (320 nM); error bars represent 95% confidence limits).](image)

The Cu-binding ligand concentration ($C_L$) of the Pieman River water sample was initially measured as 340 nM with a log $K'$ of 6.96 at pH 4.80 (Figure 3.13). When the sample was re-analysed after 2 months storage $C_L$ was determined as 330 nM with a log $K$ of 7.00.

In a longer term study in which titrations were performed at pH 5.90, the Cu-binding ligand concentration ($C_L$) of the Pieman River water sample was initially measured as 590 nM with a log $K'$ of 6.91 (Figure 3.13). When the sample was re-analysed after 2 months storage $C_L$ was determined as 590 nM with a log $K$ of 6.85. The variation in $C_L$ observed in aliquots of this sample over an 18 month period was within the analytical uncertainty of the method (Mean 95% confidence interval ± 11%). Regression
analysis of the line in Figure 3.13 showed that the slope did not differ significantly from zero using a 95% confidence limit.

These results provide evidence that given a set of Cu complexation titrations are performed within a reasonable time period (i.e. several months), complexation behaviour of the sample will not change significantly, for a constant set of experimental variables.

### 3.14 Measurement of Zn complexation

Titrations of unfiltered Pieman River water (PAB23298) with Zn ions, performed at pH 4.8, 7.2 and 8.0 using the method described in Section 2.6.3, did not produce any evidence of Zn complexation.

The total Zn concentration in the samples analysed was 22 ± 2 µg/L which may have saturated all available Zn-binding ligands. An experiment was therefore devised to determine whether natural Zn-binding ligands were saturated and whether the selected method (i.e. DPASV) provided a suitable detection window for determination of Zn ion complexation in these waters in situations where ligands are not expected to be saturated.

Chelex resin was added to two 500 mL aliquots of unfiltered Pieman River water (PAB23298) to remove metal ions and free up ligands. A MQ water sample was treated in the same way as a control sample. These samples were incubated in the dark, at room temperature for several weeks and shaken gently every second day to allow complexation and removal of metal contamination.

Prior to performing Zn ion complexation titrations in these purified samples, they were shaken then allowed to settle for 2-3 hours before filtering an aliquot through 0.2 µm membranes. This aliquot was taken from the top of each bottle to avoid uptake of Chelex resin particles, which would interfere with titration performance. Total Zn was measured in the unfiltered and filtered aliquots of the river water samples and the filtered MQ water sample (Table 3.4).
Table 3.4: Total Zn measured in Pieman River and MQ water before and after incubation with Chelex resin (mean ± 95 % confidence interval).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$[\text{Zn}^+]$ (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water (unfiltered)</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>River water + resin (unfiltered)</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>River water + resin (filtered)$^a$</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>River water + resin (filtered)$^b$</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>MQ + resin (filtered)</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

(a,b = samples replicates)

Zn concentrations were substantially reduced in the purified river water (Table 3.4). Zn ion complexation titrations performed in these samples did not show any evidence of complexation occurring. These results suggest that organic complexation of Zn is not significant in these waters or that Zn complexes are only weakly bound.

### 3.15 Conclusion

Results obtained from experimental work described in this chapter demonstrate that methods adopted for investigation of complexation and speciation during this thesis were valid and relevant. The methods used did not appear to change the parameters being measured. Thus, observed changes within samples can be confidently attributed to changes to the variable being investigated rather than to artefacts of the analytical technique.

Complexation of Cu ions by natural ligands in Pieman River water reached equilibrium after approximately 5 hours using the experimental conditions described. An incubation period $> 6$ hours would therefore have been sufficient for titration experiments, however the longer equilibration time of 18-24 hours was adopted for convenience.

Voltammetric response of Cu at the HMDE was found to be linear with time, for the range of 2- 20 minutes deposition time, in a sample containing 5 µg/L Cu. Two minutes deposition time was therefore adopted for all subsequent analyses with a stirring rate of 2500 revs/min.
Acetate and phosphate buffers were found to be appropriate for use in this study, providing buffering capacity in the range of pH 4.00 to 8.50. Addition of buffer to provide a minimum ionic strength of 0.01 was adopted for all analyses. Responses observed for ASV-labile Cu measurements performed in phosphate buffer (pH 5.8 ± 0.1) showed similar responses to measurements performed in acetate buffer of the same pH.

Zn ion complexation was not detected in natural Pieman River waters using the conditions employed for Cu ion complexation determinations.
Influence of environmental parameters on metal ion complexation

4.1 Introduction

The ability of natural waters to complex heavy metals, thus rendering them non-toxic depends on both the concentration of the ligands (commonly summarised as the complexation capacity) and the stability constants of the complexes formed (Iyer and Sarin 1992; Einax and Kunze 1996; Turoczy and Sherwood 1997).

To understand the environmental significance of a single reported complexation capacity value for waters receiving industrial or mining effluent or AMD, the influence of variation in pH and other water quality parameters (e.g. temperature and ionic strength) of the receiving waters must be investigated. Although the effect of pH on complexation has been investigated by several research groups (Shuman and Woodward Jr. 1977; Kerndorff and Schnitzer 1980; Campbell and Tessier 1987; Allen and Hansen 1996) few, if any studies have examined the effect of temperature on complexation of heavy metal ions by organic matter in natural water samples. Most speciation studies in which the complexation capacity of aquatic samples is investigated have involved titrations at a single pH and temperature (Baccini and Suter 1979; Srna et al. 1980; Hart and Davies 1981; Apte et al. 1995; Muller 1996; Wu et al. 1997).

Previous studies have shown elevated concentrations of both Cu and Zn in some mine-affected tributaries in the Pieman catchment (Koehnken 1992). Cu is a common heavy metal pollutant that is highly toxic to aquatic organisms (Florence 1986; Brealt et al. 1996). It is known to form strong complexes with natural ligands, successfully competing for binding sites with other cations (van den Berg and Dharmvanij 1984; Allen and Hansen 1996; Brealt et al. 1996). Cu is commonly selected for investigations of metal ion complexation for these reasons and so there are substantial Cu
complexation data in the literature. Less is known however about Zn complexation, particularly in the organic-rich, low ionic strength waters of western Tasmania.

The objective of this study was to investigate the influence of various environmental variables on metal complexation and hence speciation in Pieman River waters. To achieve this objective, the following experimental design was adopted:

- Firstly, Cu complexation was measured in a range of water samples to investigate spatial and temporal variability within the catchment.

- Secondly, filtration and UV-irradiation were used to investigate the nature of complexing ligands and their distribution between “dissolved” and particulate phases.

- Finally, natural water samples were manipulated in laboratory experiments to explore the effect of environmentally relevant parameters (i.e. pH, temperature, ionic strength and salinity) on metal speciation in Pieman River waters.

4.2 Methodology

Bulk surface water samples were collected in April 1996, March 1997 and February 1998 from an upstream site in Lake Pieman approximately 0.5 km below Bastyan Dam (Figure 1.3) and the Bastyan Power Station (Figure 5.1). This site, known as Pieman above Bobodil (PAB) receives water from many tributaries within the catchment and is relatively unaffected by mining discharges. The 1996 sample was used in initial experiments to optimise the complexation titration technique (Chapter 3). The 1997 and 1998 samples were manipulated in laboratory experiments to determine the influence of environmental factors on metal speciation. Samples from this site were also collected periodically between March 1997 and February 1998 for temporal studies.

Nine sites within the Pieman catchment were sampled in July 1997 for spatial variation studies. All samples were collected from a depth of approximately 0.1 to 0.3 m into 25 L polyethylene carboys or 2 L HDPE bottles and stored in darkness at room
temperature (21 °C) until analysed. Eight unpolluted tributary sites and one lake site (Figure 1.3; Table 2.2) were sampled in July 1997. Water at all sites was well mixed and well aerated at the time of sampling (dissolved oxygen = 77 - 100 % saturation).

Complexation titration experiments were generally performed using unfiltered water samples. For filtration studies, sample aliquots were filtered using 47 mm, track-etched polycarbonate membrane filters of pore sizes 0.4 µm or 0.2 µm (Poretics Corporation).

4.3 Temporal variation of Cu ion complexation

To investigate temporal variability in Cu complexation parameters at a given site, Lake Pieman was sampled on seven dates at one site (PAB) over a 2-year period. Cu ion complexation titrations were performed at 21.5 °C and pH 5.9 ± 0.1. pH was adjusted using sodium acetate buffer which was added to contribute an ionic strength of 0.01. Water quality data are shown in Table 4.1. $C_L$ and $K'$ results for all titrations are shown in Figure 4.1 & 4.3.

Table 4.1: Water quality data for PAB samples collected over a 2 year period.

<table>
<thead>
<tr>
<th>Sample date</th>
<th>$\text{in situ}$ pH</th>
<th>$\text{in situ}$ conductivity ($\mu$S/cm)</th>
<th>TOC (mg/L)</th>
<th>$[\text{Cu}]$ (µg/L)</th>
<th>$[\text{Zn}]$ (µg/L)</th>
<th>$[\text{Pb}]$ (µg/L)</th>
<th>$[\text{Fe}]$ (µg/L)</th>
<th>$[\text{Mn}]$ (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21/02/96</td>
<td>5.90</td>
<td>31.2</td>
<td>13.5</td>
<td>0.5</td>
<td>16</td>
<td>1.2</td>
<td>790</td>
<td>32</td>
</tr>
<tr>
<td>27/03/97</td>
<td>5.90</td>
<td>37.0</td>
<td>9.0</td>
<td>0.6</td>
<td>14</td>
<td>1.3</td>
<td>390</td>
<td>26</td>
</tr>
<tr>
<td>11/07/97</td>
<td>5.95</td>
<td>37.5</td>
<td>11.1</td>
<td>1.0</td>
<td>13</td>
<td>1.4</td>
<td>440</td>
<td>32</td>
</tr>
<tr>
<td>20/08/97</td>
<td>5.66</td>
<td>28.8</td>
<td>8.1</td>
<td>1.0</td>
<td>15</td>
<td>1.7</td>
<td>470</td>
<td>21</td>
</tr>
<tr>
<td>21/10/97</td>
<td>6.15</td>
<td>30.2</td>
<td>6.3</td>
<td>1.0</td>
<td>12</td>
<td>1.6</td>
<td>300</td>
<td>14</td>
</tr>
<tr>
<td>09/12/97</td>
<td>5.85</td>
<td>29.3</td>
<td>9.2</td>
<td>1.1</td>
<td>9</td>
<td>1.4</td>
<td>310</td>
<td>20</td>
</tr>
<tr>
<td>23/02/98</td>
<td>5.43</td>
<td>29.8</td>
<td>6.9</td>
<td>0.6</td>
<td>22</td>
<td>1.0</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

(na = not assayed).

The $C_L$ for Cu ion complexation ranged between 610 nM and 800 nM. (Mean $C_L$ ± standard deviation = 690 ± 70 nM). In an earlier experiment (Table 2.7), $C_L$ measured five times on one sample (PAB27397) over an 18 month period showed a variation of 5 % (Mean ± standard deviation = 610 ± 30 nM). The range of $C_L$ (~10 %) measured in samples from PAB collected temporally was greater than that measured in the single sample. The variation in these results over time is surprisingly low however, considering that the concentration of TOC varied almost by a factor of 2 in these samples (Table 4.1).
Figure 4.1: Temporal variation in $C_L$ for Cu measured in water samples collected from PAB over 2 years (pH = 5.90; error bars represent 95 % confidence intervals).

Complexation capacity is generally thought to be closely linked to the concentration of TOC however Figure 4.2 demonstrates a lack of any clear relationship between measured $C_L$ for Cu and TOC concentrations in the water samples from this site ($r = -0.13$). Correlations between $C_L$ and [FeT] ($r = 0.14$) and between $C_L$ and [MnT] ($r = 0.06$) were also very poor.

Figure 4.2: Relationship between $C_L$ for Cu and TOO in PAB water samples (error bars represent 95 % confidence intervals).
Variability of $K'$ was not observed at one site over time (Figure 4.3). The distribution of binding sites within a sample will have a significant influence on their ability to bind metals and the stability of the metal-ligand complexes formed. Both $C_L$ and $K'$ will therefore be dependant on the source of the ligands, the degree of humification of organic matter and the relative amounts and binding strengths of various ligands.

Observations from this study suggest there are at least two pools of organic matter in the samples. One pool may be relatively constant in concentration and composition with most of the complexing ability (ie. old stable humic materials), and the other may be variable with limited complexing capacity, possibly derived from more recent organic matter such as seasonal input of material from vegetation.

![Figure 4.3: Temporal variation in log $K'$ for Cu measured in water samples collected from PAB over 2 years (pH 5.90; error bars represent 95 % confidence intervals).](image)
4.4 Spatial variation in Cu ion complexation

To determine the variation in Cu ion complexation spatially across the catchment at one point in time, Cu complexation parameters were investigated at nine sites of differing water quality (Table 4.3). Sampling sites were selected to cover a range of catchment types, for ease of access and to coincide with sampling locations used in the Pieman River Monitoring Program (Koehnken 1992).

As pH is known to have a significant influence on Cu complexation (Section 4.7) titrations were performed in buffered solutions (pH 5.9 ± 0.1) to eliminate pH effects. Measured Cu-binding $C_L$ ranged from 720 nM to 1120 nM in these samples. Estimated values for $K'$ were within the range of $3 \times 10^6$ to $7 \times 10^6$. Concentration of $C_L$ and $K'$ measured in Pieman River water samples are within the range of values reported for other freshwater samples (Table 4.2) but direct comparison is not possible because of the different pH conditions used.

Table 4.2: Cu complexation parameters measured in freshwater samples using ASV.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>$C_L$ (nM)</th>
<th>log $K'$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pieman River, TAS, Aust.</td>
<td>5.9</td>
<td>720 – 1120</td>
<td>6.5 – 6.8</td>
<td>This study</td>
</tr>
<tr>
<td>Fly River, PNG</td>
<td>7.6</td>
<td>380 – 720</td>
<td>na</td>
<td>Apte et al. 1995</td>
</tr>
<tr>
<td>Magela Ck, NT, Aust.</td>
<td>6.0</td>
<td>70 – 480</td>
<td>7.5 – 8.3</td>
<td>Hart and Davies 1981</td>
</tr>
<tr>
<td>Lake Val Sereno, California</td>
<td>4.8</td>
<td>520</td>
<td>6.7</td>
<td>Sma et al. 1980</td>
</tr>
<tr>
<td>Swiss Lakes, Switzerland</td>
<td>8.8</td>
<td>2700</td>
<td>10.9</td>
<td>Baccini and Suter 1979</td>
</tr>
</tbody>
</table>

na = not available
Table 4.3: Water chemistry for tributary and lake sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>$C_a$ (nM)</th>
<th>$K'$ ($10^6$)</th>
<th>TOC (mg/L)</th>
<th>pH</th>
<th>g440 (m$^{-1}$)</th>
<th>Cond (µS/cm)</th>
<th>SS (mg/L)</th>
<th>Ca (mg/L)</th>
<th>Mg (mg/L)</th>
<th>Fe (mg/L)</th>
<th>Mn (µg/L)</th>
<th>Zn (µg/L)</th>
<th>Pb (µg/L)</th>
<th>Cu (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Ck.</td>
<td>930</td>
<td>6.8</td>
<td>17.6</td>
<td>5.24</td>
<td>12.6</td>
<td>36.3</td>
<td>4.4</td>
<td>0.87</td>
<td>0.78</td>
<td>0.68</td>
<td>12</td>
<td>3.3</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Farm Ck.</td>
<td>1090</td>
<td>3.9</td>
<td>22.6</td>
<td>4.82</td>
<td>19.5</td>
<td>42.5</td>
<td>1.6</td>
<td>0.26</td>
<td>0.92</td>
<td>0.48</td>
<td>10</td>
<td>8.8</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Hatfield R.</td>
<td>1040</td>
<td>3.2</td>
<td>6.3</td>
<td>6.34</td>
<td>16.0</td>
<td>34.8</td>
<td>4.0</td>
<td>0.77</td>
<td>1.35</td>
<td>0.42</td>
<td>2.0</td>
<td>4.0</td>
<td>2.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Marionoak R.</td>
<td>1120</td>
<td>5.5</td>
<td>25.4</td>
<td>5.19</td>
<td>26.8</td>
<td>45.0</td>
<td>6.4</td>
<td>0.48</td>
<td>1.18</td>
<td>0.80</td>
<td>26</td>
<td>24</td>
<td>3.2</td>
<td>1.1</td>
</tr>
<tr>
<td>PAB11797</td>
<td>960</td>
<td>5.6</td>
<td>11.1</td>
<td>5.95</td>
<td>10.9</td>
<td>37.5</td>
<td>1.2</td>
<td>1.54</td>
<td>0.87</td>
<td>0.44</td>
<td>32</td>
<td>13</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Stanley R.</td>
<td>980</td>
<td>6.2</td>
<td>16.3</td>
<td>4.70</td>
<td>13.0</td>
<td>40.0</td>
<td>0.0</td>
<td>0.58</td>
<td>0.73</td>
<td>0.25</td>
<td>1.0</td>
<td>2.3</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Sterling R.</td>
<td>1080</td>
<td>3.5</td>
<td>15.1</td>
<td>6.02</td>
<td>12.1</td>
<td>30.7</td>
<td>0.8</td>
<td>0.59</td>
<td>0.74</td>
<td>0.28</td>
<td>9.0</td>
<td>11</td>
<td>2.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Vale R.</td>
<td>720</td>
<td>5.2</td>
<td>6.2</td>
<td>7.70</td>
<td>7.1</td>
<td>174.0</td>
<td>2.0</td>
<td>31.8</td>
<td>1.6</td>
<td>0.04</td>
<td>4.0</td>
<td>2.6</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Wilson R.</td>
<td>1040</td>
<td>3.4</td>
<td>12.9</td>
<td>6.40</td>
<td>12.2</td>
<td>46.5</td>
<td>2.8</td>
<td>0.48</td>
<td>2.08</td>
<td>0.41</td>
<td>1.0</td>
<td>5.4</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

a: complexation titrations performed at pH 5.9 ± 0.1
b: natural pH measured in situ
Table 4.4: Pearson Correlation Matrix (r) for spatial variability in Pitman River catchment water quality parameters.

<table>
<thead>
<tr>
<th></th>
<th>Cl</th>
<th>TOC</th>
<th>pH</th>
<th>g440</th>
<th>Cond</th>
<th>SS</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Pb</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>1</td>
<td>-0.61*</td>
<td>-0.80**</td>
<td>-0.58</td>
<td>-0.43</td>
<td>1</td>
<td>-0.07</td>
<td>1</td>
<td>-0.11</td>
<td>1</td>
<td>0.37</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TOC</td>
<td>1</td>
<td>0.62*</td>
<td>0.76**</td>
<td>-0.72*</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>pH</td>
<td>1</td>
<td>0.83**</td>
<td>0.76**</td>
<td>-0.44</td>
<td>-0.51</td>
<td>-0.51</td>
<td>1</td>
<td>-0.38</td>
<td>-0.12</td>
<td>0.43</td>
<td>0.28</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>g440</td>
<td>1</td>
<td>0.76**</td>
<td>0.66*</td>
<td>-0.50</td>
<td>-0.23</td>
<td>-0.23</td>
<td>1</td>
<td>-0.39</td>
<td>-0.12</td>
<td>0.43</td>
<td>0.28</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>Cond</td>
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<td>0.76**</td>
<td>0.76**</td>
<td>-0.50</td>
<td>-0.23</td>
<td>-0.23</td>
<td>1</td>
<td>-0.39</td>
<td>-0.12</td>
<td>0.43</td>
<td>0.28</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>SS</td>
<td>1</td>
<td>0.66*</td>
<td>0.76**</td>
<td>-0.50</td>
<td>-0.23</td>
<td>-0.23</td>
<td>1</td>
<td>-0.39</td>
<td>-0.12</td>
<td>0.43</td>
<td>0.28</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>Ca</td>
<td>1</td>
<td>-0.58</td>
<td>-0.44</td>
<td>-0.50</td>
<td>-0.23</td>
<td>-0.23</td>
<td>1</td>
<td>-0.39</td>
<td>-0.12</td>
<td>0.43</td>
<td>0.28</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>Mg</td>
<td>1</td>
<td>-0.43</td>
<td>-0.72*</td>
<td>-0.50</td>
<td>-0.23</td>
<td>-0.23</td>
<td>1</td>
<td>-0.39</td>
<td>-0.12</td>
<td>0.43</td>
<td>0.28</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>Fe</td>
<td>1</td>
<td>-0.07</td>
<td>-0.11</td>
<td>-0.12</td>
<td>-0.23</td>
<td>-0.23</td>
<td>1</td>
<td>-0.39</td>
<td>-0.12</td>
<td>0.43</td>
<td>0.28</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>Mn</td>
<td>1</td>
<td>-0.11</td>
<td>-0.37</td>
<td>-0.12</td>
<td>-0.23</td>
<td>-0.23</td>
<td>1</td>
<td>-0.39</td>
<td>-0.12</td>
<td>0.43</td>
<td>0.28</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>Zn</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pb</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cu</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

- *: correlation significant at the 95% confidence level
- **: correlation highly significant at the 95% confidence level

a: complexation titrations performed at pH 5.9 ± 0.1
b: natural pH measured in situ
A Pearson Correlation Matrix was constructed to investigate relationships between various water quality parameters in Pieman River samples (Table 4.4). A significant positive correlation was found between TOC and CL (r = 0.61) and Fe and $C_L$ (r = 0.62) for these nine sites. These relationships do not necessarily imply causality, as this effect was not found in an earlier study (Section 4.3) and the two variables may in fact be influenced by the nature of the ligands rather than their total concentration.

Ca and conductivity were highly correlated (r = 0.99). Dissolution of limestone deposits in some tributaries contributes $Ca^{2+}$ ions, $CO_3^{2-}/HCO_3^-$ ions and conductivity. From this data, a significant negative correlation (α = 0.01) was observed between $C_L$ and both Ca (r = -0.86) and conductivity (r = -0.83). Thus as conductivity or [Ca] increases, the complexation of ionic Cu decreases possibly due to competition for binding sites by Ca ions.

Low pH values are observed when Ca and conductivity are low. Where pH is high, Ca and thus conductivity are also high as limestone contributes both Ca and alkalinity to the water. Not surprisingly, positive correlation’s were observed between pH and Ca (r = 0.75) and pH and conductivity (r = 0.72). Where TOC is high, pH is low (r = -0.80) as natural organic acids contribute to the acidity of the water. pH is also low (i.e. high TOC) when conductivity is low (r = 0.72) and carbonate I bicarbonate buffering capacity is minimal.

TOC, Fe, Zn and suspended solids (SS) correlated linearly with watercolour, when measured as g440. The contribution by SS is probably mainly associated with Fe. Fe was the only variable which showed any significant correlation with SS (r = 0.75). A positive correlation was observed between Mn and Zn (r = 0.79) and between Pb and Cu (r = 0.78).

4.5 Influence of particle size on Cu ion complexation

Because metal ions are typically sorbed to particles, many of which are colloidal in size and are often smaller than 0.1 µm, membrane filtration usually does not permit a complete analytical differentiation between truly dissolved and particulate concentrations (Morgan and Stumm 1991). Furthermore, the commonly used distinction
between inorganic and organic particles also has little environmental significance as inorganic particles are often stabilised by coatings of adsorbed organic material (Filella et al. 1995).

To investigate the nature of complexing ligands and their distribution between “dissolved” and particulate phases, filtrates of Pieman River water were titrated with an ionic Cu standard solution.

No significant variation was detected in $C_L$ or TOC (ANOVA; $p > 0.05$) in any of the filtrates investigated (Table 4.5). Filtration of samples did not reduce TOC significantly indicating that all organic material is “dissolved”. In contrast to TOC, Fe and Pb concentrations were reduced in the filtered fractions. A decrease in Mn concentration occurred in the filtered fractions however Zn concentration were unchanged indicating that all Zn was present in dissolved and/or colloidal forms. The concentrations of Cu and Cd measured are below analytical detection limits.

Complexation titrations were performed in unfiltered water samples to minimise disturbance of sample integrity. Data for samples in Table 4.5 indicate that filtration had no apparent effect on Cu binding capacity.

Table 4.5: Variation in $C_L$, TOC and metal concentrations measured in various filtrates of Lake Pieman water.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unfiltered</th>
<th>0.4 $\mu$m filtered</th>
<th>0.2 $\mu$m filtered</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC (mg/L)</td>
<td>9.0</td>
<td>8.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Stddev</td>
<td>0.6</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>$C_L$ (nM)</td>
<td>310</td>
<td>300</td>
<td>316</td>
</tr>
<tr>
<td>95% C.I.</td>
<td>11%</td>
<td>12%</td>
<td>13%</td>
</tr>
<tr>
<td>N</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Fe ($\mu$g/L)</td>
<td>390</td>
<td>260</td>
<td>130</td>
</tr>
<tr>
<td>Mn ($\mu$g/L)</td>
<td>26</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>Zn ($\mu$g/L)</td>
<td>14</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Cu ($\mu$g/L)</td>
<td>0.6</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Pb ($\mu$g/L)</td>
<td>1.3</td>
<td>0.9</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Cd ($\mu$g/L)</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>
4.6 Influence of UV irradiation on Cu ion complexation

Binding of trace metal ions by natural organic matter is believed to play a significant role in controlling their chemical speciation (Apte et al. 1988; Iyer and Sarin 1992; Breault et al. 1996). In an earlier study (Section 4.4) a positive relationship was found between organic carbon concentrations and Cu complexation capacity \((r = 0.61)\) in natural river and lake water samples collected from the Pieman catchment. This effect was not however observed in a temporal study of one lake site (Section 4.3).

When an unfiltered Lake Pieman water sample (PAB27397; pH 6.65) was UV irradiated to destroy organic matter and then titrated with an ionic Cu solution, a linear titration curve was obtained (Figure 4.4). The reduction in metal ion complexation compared with the original titration curve in Figure 4.4 supports earlier results and shows that organic ligands in Pieman River water play a significant role in complexation and hence regulation of Cu speciation. If inorganic binding of Cu ions by Fe or Mn oxyhydroxide colloids were significant in this water sample, they would still be present after UV irradiation (Florence 1986). A linear relationship after UV irradiation therefore demonstrates negligible inorganic binding by oxyhydroxide materials.

![Figure 4.4: Cu ion titration curves in natural and UV irradiated Lake Pieman water (PAB27397; ○ = UV irradiated water; ● = natural water).](image)
4.7 Influence of reaction pH on Cu ion complexation

Introduction of AMID to the relatively acidic and poorly buffered waters of western Tasmania has the potential to alter metal speciation. Knowledge of the effect of pH on binding behaviour of natural ligands is therefore important when considering metal speciation and toxicity in the Pieman River catchment.

The influence of pH on Cu binding ability of natural ligands was investigated in water collected from Lake Pieman (PAB) on two separate occasions. Replicate titrations were performed in aliquots taken from the bulk water samples, at a range of pH’s which were controlled by the addition of sodium acetate or phosphate buffer (final ionic strength added = 0.01).

Figure 4.5: Influence of pH on Cu-binding ligand concentration in Lake Pieman water collected in March 1997 and February 1998 (● = PAB27397; ♦ = PAB23298; error bars represent 95 % confidence limits).

Cu complexation capacity in Lake Pieman water was found to be highly dependant on pH (Figure 4.5). As the reaction pH of Cu complexation titrations in unfiltered Pieman water (PAB27397) increased from pH 3.9 to 8.0, the measured ligand concentration
calculated using the van den Berg / Ruzic linearisation method, increased from 120 nM (8 µg Cu/L) to 940 nM (60 µg Cu/L). In the second sample investigated (PAB23298), the Cu $C_L$ increased from 240 nM (15 µg Cu/L) at pH 4.3 to 1380 nM (88 µg Cu/L) at pH 7.6.

pH influences metal complexation with organic ligands as protons compete with the metal ions for binding sites on carboxylic acids and other organic functional groups (Allen and Hansen 1996). Introduction of acidic mine waste into aquatic environments such as the Pieman River may therefore influence ecosystems in two ways:

- by changes in the metal speciation and bioavailability, and by
- direct increases in the hydrogen ion activity (Campbell and Tessier 1987).

At lower pH, less metal is bound to organic ligands thereby increasing free ion concentrations (and potential toxicity) of waters.

Conditional stability constants varied non-systematically between $3.8 \times 10^6$ and $4.4 \times 10^7$ in PAB27397 and between $1.4 \times 10^6$ and $8.5 \times 10^6$ for PAB23298 over the pH ranges investigated.
4.8 Influence of temperature on Cu ion complexation

Water temperatures in the Pieman catchment have been reported in the range of approximately 4°C to 22°C (Koehnken 1992). Although Lu *et al.* (1997) have investigated the influence of high temperature (i.e. 20 - 100°C) on reactions of AHS with Cr, most studies of metal-AHS interactions in natural waters have been carried out at a single temperature.

The influence of water temperature on the Cu-binding ligand concentration was investigated in two Lake Pieman water samples. Replicate titrations were performed at a range of environmentally significant temperatures in aliquots taken from bulk water samples (PAB) collected in March 1997 and February 1998 (Figure 4.6). Regression analysis showed that the slope of the lines fitted to both sets of data did not differ significantly from zero ($\alpha = 0.05$). Results therefore demonstrate that Cu complexation by natural ligands in Lake Pieman water was not temperature dependent over this range.

\[\text{Figure 4.6: Influence of water temperature on Cu-binding ligand concentration in Lake Pieman water collected in March 1997 and February 1998 (○ = PAB27397; ● = PAB23298; error bars represent 95 % confidence intervals).}\]
4.9 Influence of salinity on water quality and speciation

In an earlier study (Section 4.6) organic complexation was found to be a significant regulating mechanism of Cu speciation within the freshwater environments of the Pieman River catchment. This result is expected for Cu, which is known to bind strongly with natural organic ligands in a wide variety of aquatic environments (Section 4.1). As river water mixes with seawater in estuaries creating a salinity gradient, dynamic chemical reactions (such as flocculation and adsorption) may influence both the abundance of natural ligands and the affinity of ligands for ionic Cu complexation.

To investigate the influence of salinity on Cu complexation in Pieman River water, a series of artificially mixed estuarine samples were prepared from water collected from a Pieman River estuary site (E3; Figure 6.1) in February 1998. Surface water (2 m depth) was pumped into a pre-cleaned HDPE carboy. Saline bottom water was pumped from a depth of 13 m into another HDPE carboy. The deep water (salinity 29.7) was then diluted with the surface water (salinity 1.9) in 2L HDPE Nalgene bottles by mixing the two homogenous samples in various proportions (Table 4.6).

Table 4.6: Water chemistry of mixed estuarine samples.

<table>
<thead>
<tr>
<th>Dilution (2m:13m water)</th>
<th>Salinity</th>
<th>DOC (mg/L)</th>
<th>pH</th>
<th>[Cuₒ] (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>1.9</td>
<td>6.6</td>
<td>6.90</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>90:10</td>
<td>5.8</td>
<td>6.1</td>
<td>7.29</td>
<td>1.0 ± 0.8</td>
</tr>
<tr>
<td>75:25</td>
<td>10.2</td>
<td>5.2</td>
<td>7.44</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>67:33</td>
<td>11.6</td>
<td>4.3</td>
<td>7.53</td>
<td>0.7 ± 0.5</td>
</tr>
<tr>
<td>50:50</td>
<td>17.6</td>
<td>4.8</td>
<td>7.71</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>33:67</td>
<td>21.6</td>
<td>3.9</td>
<td>7.75</td>
<td>1.2 ± 0.8</td>
</tr>
<tr>
<td>25:75</td>
<td>22.5</td>
<td>3.5</td>
<td>7.88</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>10:90</td>
<td>26.6</td>
<td>2.9</td>
<td>7.81</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>0:100</td>
<td>29.7</td>
<td>2.1</td>
<td>7.78</td>
<td>0.7 ± 0.5</td>
</tr>
</tbody>
</table>

The mixed samples were filtered under vacuum through acid washed GF/C filter papers held in a Millipore Aseptic filtration unit within 12 hours of collection to remove large particulate or planktonic material. They were then allowed to equilibrate for several weeks in the dark at 4°C. The samples were re-filtered (0.2 µm) prior to measurement of Cu ion complexation capacity, DOC and “dissolved” metal concentrations. “Dissolved”
Cu concentrations were close to or below the analytical detection limit. Samples were also analysed for ASV-labile Zn and bioavailable Zn as discussed in Section 4.12.

4.9.1. Influence of salinity on DOC

DOC behaved conservatively when surface water was mixed with more saline deep water (Figure 4.7). The fact that it behaved conservatively following incubation and filtration through 0.2 µm filters shows that flocculation of colloidal or “dissolved” organic matter as a result of increased ionic strength, was not significant.

“Dissolved” organic matter in river waters can play an important role in estuarine chemical processes (Sholkovitz 1976). In laboratory studies, Sholkovitz (1976) demonstrated that rapid flocculation of Fe, Mn, Al, P, organic carbon and humic substances occurred when river water was mixed with seawater. The amount of flocculated material increased as salinity increased from 0 to 15 - 20 but above this salinity, little additional removal of material occurred. The extent of flocculation due to de-stabilisation of river-introduced colloidal humic substances during the mixing with seawater was found to be very salinity-dependent (Sholkovitz 1976).
Studies by Fox (1983) showed that salt-induced flocculation of dissolved humic acid was not however common to all estuaries. This may reflect the differences in the composition of dissolved humic acids from different estuaries.

4.9.2 Influence of salinity on “dissolved” Fe
Flocculation and the consequential removal of Fe by filtration was significant as ionic strength increased in the mixed estuarine samples (Figure 4.8). Flocculation of Fe oxyhydroxide, which occurs in many estuaries (Teasdale et al. 1996), is the most likely cause of removal of “dissolved” Fe as a result of estuarine mixing (Yan et al. 1991).

![Figure 4.8: Influence of salinity on [Fe₉] in estuarine dilution samples.](image)

4.9.3 Influence of salinity on Cu ion complexation
Cu ion complexation titrations were performed in each of the prepared samples. The non-conservative behaviour shown in Figure 4.9 indicates that reduction in effective Cu-binding $C_L$ cannot be attributed solely to dilution of organic matter. “Dissolved” organic carbon was diluted conservatively in these samples (Figure 4.7). Thus, removal of flocculated organic material by filtration prior to titrations can be eliminated as a cause of the non-conservative behaviour of $C_L$. 
Complexing ability is clearly influenced by salinity in the Pieman River water. These results are in agreement with data reported by Hoxey (1994) who investigated complexation of Cu and Cd ions by AHS (extracted using 0.5 M NaOH) in seawater / deionised water mixtures of varying salinity. Hoxey (1994) observed a non-conservative decrease in complexation capacity for both metals with an increase in salinity. This non-conservative behaviour is in direct contrast to effects observed in the Severn Estuary where both “dissolved” Cu and Cu complexing ligands behaved conservatively (Apte et al. 1990). Both conservative and non-conservative behaviour has been observed in various estuarine studies of Cu behaviour (Teasdale et al. 1996).

The mechanism by which salinity alters complexation by natural ligands is not clearly demonstrated by these results. Similarly, the relationship between DOC (which is conservative) and $C_L$ (which is non-conservative and closely mimics the behaviour of Fe) is not obvious and several possible mechanisms are considered below.
Table 4.7: Possible mechanisms controlling Cu complexation in mixed estuarine samples.

<table>
<thead>
<tr>
<th>Possible ligand</th>
<th>Binding mechanism</th>
<th>Possible effect of seawater addition</th>
<th>Expected mixing behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>Organic complexation</td>
<td>Dilution</td>
<td>DOC = Conservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$C_L = $ Conservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fe = NDE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conformational changes</td>
<td>DOC = Conservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>from ionic strength changes</td>
<td>$C_L = $ Conservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fe = NDE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Competitive complexation</td>
<td>DOC = Conservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>by major cations (e.g. Ca)</td>
<td>$C_L = $ Non conservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fe = NDE</td>
</tr>
<tr>
<td>Fe / Mn Oxyhydroxides</td>
<td>Adsorption</td>
<td>Flocculation /settling</td>
<td>DOC = NDE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$C_L = $ Non conservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fe = Non conservative</td>
</tr>
<tr>
<td>Organic coatings on Fe/Mn flocs</td>
<td>Organic complexation</td>
<td>Flocculation /settling</td>
<td>DOC = Non conservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$C_L = $ Non conservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fe = Non conservative</td>
</tr>
</tbody>
</table>

NDE = No direct effect

1: It may be possible that a small amount of very high complexing DOC is removed with Fe but is not detected in the DOC vs salinity plot (B. Hart, pers. comm.).

Considering the expected behaviour resulting from possible mechanisms outlined in Table 4.7, dilution of riverine TOC does not solely account for the observed non-conservative behaviour of $C_L$. The ability of natural ligands to bind Cu ions may be influenced by conformational changes of the organic matter due to ionic strength or by competition for binding sites by major ions in seawater such as $Ca^{2+}$ or $Mg^{2+}$ ions. Both mechanisms can explain the conservative behaviour of TOC and non-conservative behaviour of $C_L$ (Table 4.7), and further work was undertaken to distinguish which of two mechanisms is occurring in Pieman water.

Earlier studies in fresh Pieman River water showed that binding of Cu by Fe or Mn oxyhydroxides was minimal (Section 4.6) however this mechanism may play a more significant role under estuarine conditions. Teasdale et al. (1996) have found that Cu has high adsorptive affinity for hydrous Fe oxides. Reduction in Cu ion complexation with removal of Fe is therefore not surprising and could explain at least part of the non-conservative behaviour displayed by $C_L$. 

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4.10 Influence of ionic strength on Cu ion complexation

To determine if the non-conservative effect seen in Cu-ion complexation in the mixed estuarine dilution samples was due to ionic strength alone, a series of titrations were performed in aliquots of Lake Pieman water (PAB23298) diluted using a 0.7 M KC1 solution as the diluent. The KC1 concentration was selected to give an ionic strength comparable to seawater. The ionic strength of the Lake Pieman water samples was therefore altered without the addition of other major ions present in seawater (e.g. Ca or Mg). The prepared solutions were allowed to equilibrate at room temperature (21.5 °C) in the dark for approximately 2 weeks before Cu ion titrations were performed. The incubated samples were not filtered before analysis.

The ionic strength of each mixture was determined from its measured conductivity and a calibration graph of ionic strength against conductivity for standard KC1 solutions. Contribution of Cu by the KC1 solution to the total titration concentrations was accounted for in titration calculations.

Cu ion complexation decreased conservatively as Lake Pieman water was diluted with the KC1 solution (Figure 4.10). From this experiment, where sample composition was not altered by filtration, ionic strength was shown to have little if any effect on the complexation capacity of the natural ligands for Cu ions. Steric effects caused by conformational changes to the ligands by ionic strength are therefore negligible.
A possible explanation for the non-conservative effect observed in the mixed estuarine samples in the previous section (Section 4.9.3) is direct competition for binding sites by major ions in seawater. This hypothesis would support earlier results where Ca concentrations were found to be negatively correlated with the $C_L$ for Cu ($r = -0.86$, $\alpha = 0.01$) in freshwater samples (Section 4.4).

Competition for metal-binding sites by major ions has been investigated by several authors (Hering and Morel 1988; van den Hoop et al. 1994). Van den Hoop et al. (1995) concluded that a simple model describing the equilibrium $M + CaL \rightleftharpoons ML + Ca$ could be used to describe the competition of Zn (II) and Cd (II) ions for binding sites occupied by alkaline earth metals in natural waters. In contrast, Hering and Morel (1988) did not find competitive effects between Cu and Ca and concluded that different binding sites were involved in binding of these metals or that a non-discrete ligand binding mechanism was operative. This hypothesis was tested using the WHAM modelling package (Section 4.11).
4.11 Prediction of Cu ion complexation behaviour by computer modelling

To investigate the effect of major cations (Ca and Mg) on the Cu-ion binding capacity of natural ligands in Pieman River water under estuarine conditions, the WHAM computer program was used to predict speciation by simulating the mixing of model river water with seawater.

Estuarine mixing was simulated using the ionic composition of Savage River water (Table 5.1) but with all trace metals other than Cu removed. The ionic composition for seawater was obtained from Stumm and Morgan (1996). Two scenarios were considered. Speciation was initially investigated assuming that the total Cu concentration (830 nM, 53 µg/L) remained constant throughout the estuary. The second scenario assumed that the total Cu concentration behaved conservatively and that [Cu] in seawater was negligible compared to the river water concentration.

The following assumptions formed an integral part of both simulations:

- DOC is half the DOM by mass and that [FA] = [DOC] in g/L
- HA / FA concentrations are negligible in pure seawater
- FA behaves conservatively on mixing
- pH 8.0 is maintained throughout the estuary
- metal bound to FA is “non-labile”. All other forms including metal in the diffusion layer are “labile”. In fact, it is likely that some of the fulvic and humic bound metal in Pieman samples may be only weakly bound.
Figure 4.11: Predicted metal behaviour during simulated mixing of model river water and seawater solutions ([CuT] = 834 nM; ● = bound-Cu; ▲ = labile-Cu; ♦ = bound-Ca; ■ = bound-Mg).

When [CuT] was held constant, bound forms of the major ions (Ca and Mg) were predicted to behave conservatively above an ionic strength of 0.06 (Figure 4.11). In contrast, the bound Cu concentration behaved non-conservatively and decreased non-linearly as salinity increased.

Holding [CuT] constant but varying [FA] and major ions conservatively had a marked effect on Cu speciation, supporting the hypothesis that competition by Mg and Ca for binding sites influences Cu speciation. The change in [FA] had little effect on overall Mg / Ca speciation because of the high concentrations of these metals in estuarine and marine waters.
Figure 4.12: Predicted metal behaviour during simulated mixing of model river water and seawater solutions (■ = [CuT] diluted conservatively; ● = bound-Cu; ▲ = labile-Cu).

When [CuT] and major ions were altered conservatively (Figure 4.12), predicted concentrations of bound Mg and Ca changed only slightly from the previous scenario (Figure 4.11) and so they have not been replotted here.

FA-bound Cu (i.e. assumed to be non-labile) remained as a high proportion of total Cu but showed a slight negative deviation from the conservative mixing line. Inorganic Cu showed a complex pattern — reaching a maximum at ~ 75 % seawater (salinity = 26). Nevertheless inorganic Cu increased over most of the salinity range investigated (from salinity 3.5 to 26).

Predictions from both scenarios support laboratory findings where $C_L$ for Cu in river water was found to behave non-conservatively when diluted with seawater. Computer speciation modelling and laboratory studies support the hypothesis that competition for binding sites by major ions in seawater (Ca and Mg) is a significant regulating factor of Cu speciation in estuarine waters.
4.12 Influence of salinity on Zn speciation and toxicity

The influence of salinity on Zn speciation and toxicity was investigated in the series of mixed samples of Pieman River estuarine water as previously described in Section 4.9. This study was also designed to assess the potential of ASV as a surrogate indicator of bioavailable Zn, measured using the algal enzyme (β-D-galactosidase) bioassay (described in Section 2.6.6).

4.12.1 Bioassay sensitivity

The influence of ionic Zn on the activity of the enzyme β-D-galactosidase in the marine green alga Dunaliella tertiolecta at salinity 20 and 34 is shown in Figure 4.13.

![Graph showing the effect of ionic Zn on enzyme activity of β-D-galactosidase in Dunaliella tertiolecta at salinity 20 and 34.]

Figure 4.13: The effect of ionic Zn on enzyme activity of β-D-galactosidase in Dunaliella tertiolecta at salinity 20 and 34.

Toxicity response curves produced at two salinities are shown in Figure 4.13. Bioavailable Zn in test solutions was estimated from the reduction in enzyme activity relative to a control using the toxicity response calibration curves (Figure 4.13). Dunnett’s Multiple Comparison Test was used to determine which concentrations were significantly different to controls at a significance level of $\alpha = 0.05$. This allowed
estimation of the NOEC and LOEC (Table 4.8) which were similar at both salinities tested. The enzyme bioassay was sensitive enough to detect significant toxic effects at \([\text{Zn}] = 3 \, \mu\text{g/L}\). Results of three calibration curves (salinity 34 performed in duplicate) show that response to Zn was not significantly different at these two salinities.

Table 4.8: Zn toxicity endpoints estimated using Dunnetts Multiple Comparison Test.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>EC(_{50}) ((\mu\text{g/L Zn}))</th>
<th>95% confidence limits for EC(_{50})</th>
<th>NOEC ((\mu\text{g/L Zn}))</th>
<th>LOEC ((\mu\text{g/L Zn}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>29</td>
<td>24 - 35</td>
<td>&lt; 3</td>
<td>3</td>
</tr>
<tr>
<td>34</td>
<td>30</td>
<td>26 - 35</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>24</td>
<td>21 - 27</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

4.12.2 Zn speciation and toxicity in Pieman River water samples

“Dissolved” Zn, was found to behave conservatively in the mixed estuarine samples (Figure 4.14). The lack of curvature of the line indicates that removal of flocculated Zn by filtration was not significant and that dilution was the major regulating factor for “dissolved” Zn.

Because of the scatter in the data, it is not possible to determine if the ASV-labile Zn demonstrated conservative behaviour in these samples. The fact that the ASV-labile fraction was comparable in concentration to the “dissolved” fraction at all salinities investigated indicates that Zn was not significantly strongly-complexed.
Figure 4.14: Zn speciation measurements in estuarine dilution samples (■ = “dissolved” Zn; ● = bioavailable Zn; ▲ = ASV-labile Zn).

The relationship between ASV-labile Zn and bioavailable Zn from the experimental data is shown in Figure 4.15. The 1:1 line shown in black in Figure 4.15 represents the hypothesis tested in this experiment, whereby ASV-labile measurements are equal to bioavailable Zn measurements. If the methods agreed exactly, all data would fall on this line. Statistically the experimental relationship does not differ from the theoretical line using a 95 % confidence interval for the slopes of the two lines. The $r^2$ value shows however, that only 56 % of the variation in the bioavailable Zn data can be predicted by the ASV-labile Zn measurements.
Figure 4.15: The relationship between ASV-labile Zn and bioavailable Zn estimated using an algal enzyme inhibition bioassay. (The black line represents the theoretical 1:1 relationship; the blue line represents the line of best fit to the experimental data).

It is impossible to believe that bioavailable Zn really follows the scattered pattern around the line indicated by the enzyme method (Figure 4. 15). As a result of this scatter it is not possible to conclude whether Zn conforms to the FIAM (i.e. if the labile metal ion concentration is equivalent to the bioavailable fraction). Zn toxicity to the green alga Dunaliella tertiolecta was detected in these waters indicating that at current concentrations, Zn has the potential to interfere with phytoplankton ecology and so alter the estuarine ecosystem.

4.13 Summary and conclusions

Complexation of Cu ions in river water was shown to be predominantly associated with the “dissolved” organic fraction. Inorganic binding of Cu ions by Fe or Mn oxyhydroxide colloids was shown to be negligible in Lake Pieman water. A significant correlation was found between $C_L$ and TOC ($r = 0.61$) for nine fresh water samples however this relationship was not observed in another temporal study at one lake site. Results suggest that TOC does not exclusively account for complexation capacity in fresh Pieman River water and $C_L$ may be depend on both the concentration and characteristics of the DOM.
Complexation of Cu ions by natural ligands in Lake Pieman water was found to be independent of temperature over the environmentally relevant range of 4 - 28°C. It was however found to be highly dependent on pH. At lower pH, less Cu is complexed thereby increasing the free ion concentrations. AMID may therefore influence the Pieman River ecosystems by changes in the metal speciation and by direct increases in the hydrogen ion activity.

Although organic carbon behaved conservatively in estuarine water samples, $C_L$ for Cu was found to behave non-conservatively in estuarine waters. These laboratory results were supported by predictions using the WHAM model. Dilution of riverine TOC did not account for the observed non-conservative behaviour of $C_L$. Ionic strength was also shown to have little effect on $C_L$ for Cu and so steric effects caused by conformational changes to the ligands by changes in ionic strength are considered negligible. Speciation modelling and laboratory studies supported the hypothesis that competition for binding sites by major ions in seawater (i.e. Ca$^{2+}$ and Mg$^{2+}$) may account for some of the non-conservative effects demonstrated by $C_L$ for Cu in Pieman River estuarine waters.

“Dissolved” Zn, was found to behave conservatively in artificially-mixed estuarine samples indicating that dilution was the major regulating factor for “dissolved” Zn. Because of the scatter in the data, it was not possible to determine if the ASV-labile Zn demonstrated similar behaviour. The “dissolved” Zn was found to be predominantly ASV-labile at all salinities investigated indicating that Zn was not significantly strongly-complexed in these samples.

Zn toxicity to the green alga *Dunaliella tertiolecta* was detected in these waters suggesting that at current concentrations, Zn has the potential to alter the phytoplankton ecology. As a result of the scatter in the bioassay data it is not possible to conclude whether Zn conforms to the FIAM in these waters. Results have shown that the enzyme method lacked the sensitivity necessary to clearly define behaviour of bioavailable Zn in this estuary.
CHAPTER 5

Metal speciation in freshwater environments of the Pieman River catchment

5.1 Introduction

Discharge and drainage from both past and current mining operations enter the Pieman rivers and lakes (Koehnken 1992). Tributaries of the Pieman River receiving mining effluent from waste discharge or acid mine drainage (AMD) have been found to contain total metal concentrations considerably above background levels (Koehnken 1992) and are of regulatory concern. Little is known however, about the speciation or bioavailability of the various metals found in these waters.

This chapter describes trace metal speciation measurements performed in various freshwater environments within the catchment. Initially, speciation is discussed by considering total, “dissolved” and ASV-labile metal concentrations (Section 5.4). The utility of DGT is then examined in situations where steady-state river conditions exist and in other situations where water quality is highly variable. Speciation measurements made by in situ application of DGT have been compared with ASV laboratory measurements and the influence of strong complexation on these measurements is discussed (Section 5.5). The ability of WHAM to predict metal speciation in these waters is also assessed. Finally, the implications of the speciation measurements for freshwater ecosystems are considered.

5.2 Methodology

5.2.1 Tributaries

Metal speciation was investigated in the Ring, Que, Still and Savage Rivers, which are mine-affected tributaries of the Pieman River (Figures 1.3 & 5.1). These streams were selected as previous studies had shown they had elevated metal concentrations (Koehnken 1992).
Fourteen DGT assemblies were deployed in the Savage River just above its junction with the Pieman River estuary. Replicates (3 or 4) were collected at various intervals over the following 72 hours (4 collected after 12 hours deployment; 3 after 24 hours; 4 after 48 hours and 3 after 72 hours). Water quality parameters were measured at this site throughout the deployment period. Four DGT units were deployed in each of the Ring, Que and Stitt Rivers for 29, 23.5 and 23.5 hours respectively. Water quality parameters were measured at these locations at the start and end of the deployment period.

At the time of DGT deployment at each tributary site, water samples were collected by hand for the determination of TOC, g440, alkalinity and total, “dissolved” and ASV-labile metal concentrations. A second set of samples was collected from the Savage River when the final set of DGT units was retrieved.

Sampling was performed in late February 1998. Heavy rainfall on the previous day produced high streamflow, high particulate load and high turbidity in the Savage and Que Rivers. At the end of the 24 hour deployment period, streamflow had subsided in both streams. Water clarity had also improved markedly in the Que River at this time.

5.2.2 Lake sites
Vertical profiles of metal speciation were investigated at two sites within Lake Pieman. The site known as Pieman below Huskisson (PBH; Figure 5.1) is situated downstream of inputs from the Ring, Argent and Stitt Rivers and just downstream of the Huskisson River junction. The Ring River is affected by AMD arising from the closed Hercules Mine and also receives runoff from the Renison Bell tin mine (Koehnken 1992). It typically has high Zn, Fe and sulphate levels and low alkalinity. The Huskisson River contributes ~ 15 % of the total water input to Lake Pieman introducing effluent from the Hellyer and Que River mines (Koehnken 1992). Previous studies have shown that the water column is not homogenous at this site. Although concentrations of chemical constituents generally increase with depth, various layers within the water column are often found to be enriched with chemicals associated with mining effluent (Koehnken 1992). The influence of physical mixing mechanisms on inputs at this site, under various climatic conditions, has been discussed by Koehnken (1992).
The Pieman at Reece Darn (PRD) site (Figure 5.1) is situated at the downstream end of Lake Pieman. This site is also influenced by seasonal stratification, however the distinct chemical layers that are observed at the PBH site tend to be more diffuse at downstream sites. The water column in Reece Dam has been shown to become thermally stratified during the February-March period (Koehnken 1992) with turnover occurring during the colder winter months. Water is released at the Reece Dam Power station into the lower Pieman River estuary.

**Figure 5.1:** Schematic representation of sampling locations for speciation studies in Lake Pieman (sites marked in red; not to scale).

DGT units were deployed for approximately 48 hours at PBH and PRD at a series of depths determined from in situ water quality measurements (i.e. pH, conductivity, temperature, DO, turbidity). Water samples were collected for the determination of total, “dissolved” and ASV-labile metal concentrations and various water quality parameters (i.e. alkalinity, g440, TOC and DOC) at the start of the deployment period.
5.3 General water quality

5.3.1 Tributaries

The Savage River discharges into the lower Pieman River within the boundary of the State Reserve. Historically, elevated Cu, Mn and sulphate concentrations have been found in this stream as a result of discharge of waste from the open cut Savage River Iron mine, approximately 25 km upstream of the sampling site (Koehnken 1992). Previous studies of this tributary have also shown that Cd and Zn concentrations do not differ significantly from those found in streams unaffected by mining discharges (Koehnken 1992). Although Cd and Zn measurements by DGT were performed in the Savage River during this study (Section 5.5.1), total and “dissolved” concentrations of these metals were so low that further consideration of their speciation in this tributary was not undertaken.

The Ring River is a significant source of heavy metal pollutants to Lake Pieman (Koehnken 1992). The Renison Bell Tin mine currently discharges waste runoff directly into the Argent River with smaller discharges into the Ring River. The Ring River site sampled during this study however, was located above the influence of the Renison Bell mine lease. Heavy metal concentrations measured in the Ring River during this study are a product of AMD and runoff (via Bakers Creek) from the non-functioning zinc-lead-silver Hercules Mine.

Although the Pasminco Rosebury lead-zinc-silver mine currently discharges directly into Lake Pieman via a system of retention ponds, leachate from older tailings ponds and the mining lease drain into the Stitt River before discharge into Lake Pieman (Koehnken 1992).

Runoff from the Hellyer Mine and the non-functioning Que River Mine, both zinc-lead-silver deposits, enter the Que River which discharges into Lake Pieman via the Huskisson River.

General water quality data for the Pieman River tributary sites are given in Table 5.1.
Table 5.1: Water quality data for Pieman River tributary sites based on average measurements recorded at the time of DGT deployment and retrieval (23 - 27 Feb 1998) or obtained from the Pieman River Monitoring database.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ring River</th>
<th>Stitt River</th>
<th>Que River</th>
<th>Savage River</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.91</td>
<td>5.37</td>
<td>4.96</td>
<td>7.31 ± 0.14</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>97.0</td>
<td>61.8</td>
<td>828.5</td>
<td>444 ± 150</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>9.0</td>
<td>10.6</td>
<td>12.0</td>
<td>13.6 ± 1.1</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>3.3</td>
<td>0.0</td>
<td>4.7</td>
<td>30.8</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>5.3</td>
<td>8.2</td>
<td>8.6</td>
<td>7.3</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>4.2</td>
<td>7.3</td>
<td>7.5</td>
<td>7.2</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO₃)</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>41.2</td>
</tr>
<tr>
<td>aCa (mg/L)</td>
<td>3.1</td>
<td>2.4</td>
<td>91.3</td>
<td>11.3</td>
</tr>
<tr>
<td>aMg (mg/L)</td>
<td>2.4</td>
<td>1.1</td>
<td>4.3</td>
<td>14.8</td>
</tr>
<tr>
<td>aK (mg/L)</td>
<td>na</td>
<td>na</td>
<td>6.1</td>
<td>1.3</td>
</tr>
<tr>
<td>aNa (mg/L)</td>
<td>6.1</td>
<td>5.3</td>
<td>20.2</td>
<td>10.5</td>
</tr>
<tr>
<td>aCl (mg/L)</td>
<td>11.8</td>
<td>8.3</td>
<td>15.8</td>
<td>18.1</td>
</tr>
<tr>
<td>aSO₄₂⁻ (mg/L)</td>
<td>23</td>
<td>9</td>
<td>499</td>
<td>82</td>
</tr>
</tbody>
</table>

* Average data from 1990-1997, Pieman River Monitoring database

* Based on average measurements ± 1/2 range at 0, 12,24,48 and 72 hours from this study

na: not available

All streams had similar TOC and DOC concentrations. Measured conductivity values are consistent with mean major ion concentrations for each stream on the Pieman River Monitoring database. These ion concentrations were used for modelling of Cu speciation with WHAM.

The pH of the Stitt, Que and Savage Rivers were within the operational range of the Chelex 100 resin used in the DGT assemblies (pH 5 - 8.3; Zhang and Davison 1995) (Table 5.1). The pH of the Ring River (pH 4.91) was just outside the optimum operational range of the resin however this does not preclude the use of this technique (Zhang and Davison 1995). Recoveries have been found to decrease when the pH of a solution is below 5, with significantly lower recoveries (< 15 %) achieved at pH 2 - 3. In a solution at pH 4 however, over 90 % of the total Cd concentration measured by AAS, was measured by DGT (Zhang and Davison 1995).

In another Pieman tributary stream (Baker Creek; Table 2.2) which was severely affected by acid-mine drainage, a pH of 3.3 was measured. DGT assemblies were therefore not deployed at this site. Development of alternative resins for use in DGT could extend the range of natural waters for which this technique is applicable.
5.3.2 Lake Pieman below Huskisson (PBH)

The lake was thermally stratified at this site during the sampling period (Figure 5.2). Temperature dropped from 15°C to 10°C across the thermocline between 23 - 26 m. The thermocline and oxycline coincided at this depth resulting in a warm (16 - 17°C), high DO (8 mg/L) water layer overlying a cooler (10°C), low DO layer. DO was measured as low as 0.7 mg/L at 30 m depth but anoxia was not detected at this site.

A distinct band of mine affected water was detected immediately above the thermocline. The chemical characteristics of this layer, identified by an increase in conductivity, alkalinity and pH, are indicative of the Huskisson River as the source (Koehnken 1992). The Huskisson River is the only tributary feeding Lake Pieman with significant alkalinity (Koehnken 1992).

Organic carbon concentrations remained relatively constant (6 - 8 mg/L) in surface waters at this site. A decrease in TOC was detected between 20 m and 26 m, which coincided with the layer of mine affected water. The concentration then increased below this layer to 10 mg/L at 30 m depth.
Figure 5.2: Water quality profiles measured at PBH (21 Feb 1998)
5.3.3 Reece Dam (PRD)
Temperature dropped from 15°C to 7°C across the thermocline between 10 - 30 m at PRD (Figure 5.3). A weak oxycline was detected between 5 and 10 m depth where DO decreased from 7.5 mg/L in surface water to 5.0 mg/L at 10 m depth. A corresponding change in pH was observed decreasing from 5.6 in surface waters to 5.4 in deeper water. A small conductivity maximum of 50 µS/cm was detected at 30 m depth. Alkalinity and organic carbon concentrations remained relatively constant throughout the water column with TOC generally ranging from 6 mg/L at the surface to 8 mg/L at 50 m depth.

![Figure 5.3: Water quality profiles for PRD (20 Feb 1998).](image-url)
5.4 Metal speciation measurements

5.4.1 Tributaries

Total, “dissolved” and ASV-labile metal measurements performed in water samples collected from tributary sites, coinciding with the start of the DGT deployment period, are shown in Figure 5.4 to 5.7 and summarised in Table 5.2. In the Savage River, metal concentrations were also measured in water samples collected at the end of the deployment period (Section 5.5.1.a (v)).

Figure 5.4: Speciation of Cu, Fe and Mn in the Savage River (24 Feb 1998; TM total metal; DM = dissolved metal; ASV = ASV-labile M).
Figure 5.5: Metal speciation measured in the Ring River (23 Feb 1998; TM = total metal; DM = dissolved metal; ASV = ASV-labile metal).
Figure 5.6: Metal speciation measured in the Stitt River (23 Feb 1998; TM total metal; DM = dissolved metal; ASV = ASV-labile metal).
Figure 5.7: Metal speciation measured in the Que River (23 Feb 1998; TM total metal; DM = dissolved metal; ASV = ASV-labile metal).

Table 5.2: Summary of metal speciation measurements for tributary sites (23 - 24 Feb 1998).

<table>
<thead>
<tr>
<th>River</th>
<th>([M_D]/[M_I]) %</th>
<th>([\text{ASV-labile}]/[M_O]) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
<td>Mn</td>
</tr>
<tr>
<td>Savage</td>
<td>22-44</td>
<td>90</td>
</tr>
<tr>
<td>Ring</td>
<td>70</td>
<td>97</td>
</tr>
<tr>
<td>Stitt</td>
<td>65</td>
<td>97</td>
</tr>
<tr>
<td>Que</td>
<td>58</td>
<td>100</td>
</tr>
</tbody>
</table>

na : not available
In each of the three tributaries where Zn speciation measurements were performed, nearly all Zn (> 97 %) and Mn (> 90 %) was found to be “dissolved” (i.e. < 0.4 µm; Table 5.2). The ASV-labile Zn fractions dominated the “dissolved” fractions indicating that strong complexation of Zn was not occurring or that the complexation capacity for Zn ions was exceeded in all streams.

In contrast to Mn and Zn, “dissolved” fractions of Fe, Pb and Cu varied between streams. With the exception of the Ring River, ASV-labile Cu concentrations showed that Cu speciation was significantly regulated by strong complexation. In the Ring River, the high concentration of Cu, combined with a relatively low “dissolved” organic carbon content (4.2 mg/L) and low pH may have minimised the proportion of this metal in the complexed form.

The “dissolved” fractions of Cd showed more variation between streams than Zn and Mn but this fraction still dominated the total Cd concentration. ASV-labile measurements suggest that a significant proportion of the “dissolved” Cd is bioavailable in these streams. Both the “dissolved” and ASV-labile fractions of Pb showed considerable variation in behaviour between streams.

These results clearly demonstrate that metal speciation is highly variable within the Pieman River catchment. Tributary sites show considerable variability in both metal concentration and speciation patterns, even between tributaries that have similar pH (e.g. Ring and Que Rivers). It is therefore not possible to make catchment-wide assumptions about the bioavailability of these metals.

5.4.2 Lake Pieman below Huskisson (PBH)

Metal speciation measurements performed on discrete water samples collected at PBH are shown in Figure 5.8 & 5.9. The plume of mine affected water is clearly visible in metal profiles. Elevated concentrations are found from 10 to 15 m due to mixing of surface and plume waters. Cu, Zn and Mn were predominantly present as “dissolved” forms. “Dissolved” Pb concentrations were not determined at this site. Total Cd concentrations were so low that the samples filtered for “dissolved” Cd were not analysed. The ASV-labile Cd fraction was slightly higher than the total concentrations measured except in water samples collected from the deeper sites. A small amount of
contamination may have increased the labile fraction into the measurable range and so these measurements have not been presented.

Both Fe and Mn total concentrations increased in the plume (Figure 5.9). A significant proportion of the total Fe was present as particulate species. In contrast to Fe, all the Mn was present in “dissolved” forms throughout the water column, which is consistent with results observed in the four tributary sites (Section 5.4.1).

ASV4able Cu measurements showed that a significant proportion (typically > 90 %) of the total Cu was complexed and therefore not bioavailable. The concentration of ASV-labile Cu increased in the plume when the total and “dissolved” Cu concentration increased.

Measurements by ASV did not detect any labile Pb at any depth. This result suggests that all the Pb present was in the form of strong complexes. Measurements by ASV also showed that a significant proportion of total Zn was complexed and non-labile (Figure 5.8). On average, about 57 % of the total Zn concentration was present as ASV-labile species, calculated using data from depths where \([Zn_T]\) exceeded the analytical detection limit (i.e. 20 µg/L).

Total and “dissolved” Zn increased significantly in the plume. The ASV-labile Zn also increased however the concentration of bound Zn (calculated by difference between the “dissolved” and labile fractions) was higher than expected (up to 135 µgZn/L; 2065 nM at 28 m depth) based on measured Cu complexation capacities. Given that the \(C_L\) for Cu in these waters was found to be approximately 840 nM to 1040 nM (Table 5.6) and Cu is known to be strongly bound in situations where Zn is not (e.g. Que and Stitt Rivers), the high proportion of bound Zn is more likely to be attributed to additives in the mine waste (e.g. chemicals used in ore flotation), rather than to complexation by natural organic matter. Adsorption by colloidal Mn and Fe oxyhydroxide materials may also be a significant regulating mechanism for Zn in these waters.
Figure 5.8: Cu, Cd and Zn speciation measurements at PBH (21 Feb 1998; = total metal; = ASV-labile metal; = dissolved metal).
Figure 5.9: Fe, Mn and Pb speciation measurements at PBH (21 Feb 1998; • = total metal; • = dissolved metal; ▲ = ASV-labile metal).
5.4.3 Reece Dam (PRD)
Speciation measurements performed in PRD are presented in Figure 5.10 & 5.11. Total Cu ranged from 1.5 to 2.0 µg/L. Because of the low total Cu concentrations, the “dissolved” fraction and the ASV-labile Cu were not analysed. Pb and Cd were also present in very low concentrations. Total Cd was below the typical detection limit by GF-AAS (i.e. < 0.1 µg/L). Further consideration of its speciation at this site was therefore not undertaken.

Total Cu and Fe were found to increase with depth. Increases in total Cu, Fe and Pb concentrations at 15 m and 50 m suggest some stratification of the water column with respect to these metals. This effect may reflect different mixing histories for various inputs into the lake and/or contributions from bottom sediments. The upper water column (to 10 m depth) is above the oxycline and thermocline (Figure 5.3). There is no change in other physico-chemical properties below 50 m that might explain the increase in concentration of these metals at this depth (Figure 5.3). Stratification may therefore result from bottom waters being poorly mixed with overlying waters and from remnant upstream plumes.

Surface concentrations of total metals also changed in different ways between PBH and PRD (Table 5.3). From PBH to PRD, total Zn increased in concentration whilst Fe and Mn decreased in surface waters. This conflicting behaviour probably reflects different sources and sinks for those metals (including different concentrations in tributary streams flowing into the lake).

**Table 5.3:** Total metal concentrations in surface waters at PBH and PRD.

<table>
<thead>
<tr>
<th>Metal (µg/L)</th>
<th>PBH</th>
<th>PRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>17</td>
<td>47</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Pb</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Cu</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Mn</td>
<td>66</td>
<td>53</td>
</tr>
<tr>
<td>Fe</td>
<td>410</td>
<td>320</td>
</tr>
</tbody>
</table>
Fe was present in predominantly “dissolved” forms throughout the water column to a depth of 55 m ([FeD]/[FeT] \% = 83 ± 12 \%). Similar results were seen for Mn (Figure 5.10) and Zn (Figure 5.11) where the “dissolved” fractions dominated their respective total concentrations and were constant to a depth of at least 55 m despite stratification of the water column at 10 m depth (Figure 5.3).

**Figure 5.10:** Fe and Mn concentrations at PRD (20 Feb 1998; ● = total metal; ♦ = dissolved metal).
Figure 5.11: Cu, Zn and Pb concentrations at PRD (20 Feb 1998; = total metal; = dissolved metal).
5.5 Assessment of utility of DGT

To allow valid comparisons between ASV measurements performed on discrete water samples with time-integrated in situ DGT measurements, it is necessary to establish that steady state conditions existed over the DGT deployment period. This criterion can be satisfied by analysis of discrete samples, collected at least at the start and end of the deployment period, supported by in situ water quality measurements throughout the deployment period. Where this has not been performed, non-steady state conditions can be detected by the failure of the following conditions:

- DGT-labile metal concentrations ≤ “dissolved” metal concentrations
- DGT-labile metal concentrations ≥ ASV-labile metal concentrations (based on the measurement time scales of the two techniques).

5.5.1 Applications under steady-state conditions

a) Savage River

The fourteen DGT assemblies deployed in the Savage River and retrieved at various times over the following 72-hour period were analysed for Cu, Cd, Mn, Fe, Zn and Pb. The utility of DGT has been established in a well-constrained system in earlier laboratory experiments (Table 2.6). The sampling protocol followed for this tributary was designed to determine the utility of DGT for in situ measurement of these metals in a natural river system and to investigate metal speciation in this stream.

Water quality parameters (pH and temperature) measured during the deployment period (0, 12, 24, 48, 72 hours) showed little variation during the DGT measurement period (Table 5.1).

i) Accumulation of Cu, Cd and Mn by DGT

A linear increase in metal accumulated by DGT is predicted from theory in cases where the metal concentration in surrounding water is constant (i.e. steady state conditions). Results for Cu, Cd and Mn in the Savage River show this was true for the 72 hours of DGT deployment (Figure 5.12). Deployment of DGT in coastal oceanic waters for 1 to 6 hours has also demonstrated linear accumulation rates of Zn, Mn and Fe (Zhang and Davison, 1995) in a situation where steady state concentrations might also be expected.
to occur. These field studies confirm findings of laboratory studies (Zhang and Davison, 1995) concerning the reliability of DGT as a metal accumulation tool for environmental studies.

The linearity of accumulation with time seen in Figure 5.12 also suggests that the performance of the DGT units was not affected by biofouling during the deployment times utilised. Results from these experiments suggest that if non-linear accumulation of these metals were measured in future studies of river waters, it would most likely be due to temporal variability in their concentrations (i.e. non-steady state conditions).

Figure 5.12: Measured mass of Cu, Cd and Mn accumulated by DGT assemblies deployed for various times in the Savage River.
ii) **Precision of DGT**

Based on the measured mass of metals accumulated by the resin gels, the time-averaged concentrations of Cu, Cd and Mn measured in the bulk solution of the Savage River during the deployment period are given in Table 5.4. Results from this study produced a relative standard deviation (rsd) of 11 % for Cu and 9 % for Mn measured in the Savage River compared to an analytical precision of 5 % or better. Analysis of these replicate DGT deployments is thus consistent with earlier laboratory studies (Section 2.6.4) and work by Zhang and Davison (1995) which gives a precision of 10 % for Cu and Mn.

**Table 5.4:** Calculated *in situ* concentrations of DGT-labile metals in the Savage River after ≥ 24 hours deployment.

<table>
<thead>
<tr>
<th>Metal</th>
<th>N</th>
<th>Concentration* (µg/L)</th>
<th>rsd %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>10</td>
<td>0.045 ± 0.007</td>
<td>16</td>
</tr>
<tr>
<td>Cu</td>
<td>10</td>
<td>7.3 ± 0.8</td>
<td>11</td>
</tr>
<tr>
<td>Mn</td>
<td>9</td>
<td>460 ± 40</td>
<td>9</td>
</tr>
</tbody>
</table>

Mean ± standard deviation

iii) **Preconcentration by DGT**

Uncertainties calculated for Cd concentrations are larger than for Cu and Mn (Table 5.4). The total Cd concentration in the Savage River was below analytical detection limits (typical detection limit for Cd by GF-AAS ≤ 0.1 µg/L based on 3 times the standard deviation of a blank solution). DGT produced a measurable concentration because of its in-built pre-concentration step. Concentrations in the eluent were still low however (0.12 - 1.14 µg/L), resulting in the analytical precision of ± 16 %, not unreasonable given the low free Cd concentration (45 ng/L).
iv)  Accumulation of Fe, Zn and Pb by DGT

Figure 5.13: Measured mass of Fe, Zn and Pb accumulated by DGT assemblies deployed for various times in the Savage River.

Interpretation of the accumulation curves for Zn, Fe and Pb (Figure 5.13) is more complicated than for Cu, Cd and Mn. Total Zn measured in the Savage River was less than the analytical detection limit of the flame-AAS (typical detection limit 20 µg/L). Extracts analysed for DGT-labile Zn were also close to the limit of detection of flame-AAS. This combined with the possibility of contamination by Zn in the field laboratory at Corinna may have produced significant scatter in the data. The slope of the line
representing the accumulation of Zn by DGT over time was however found to be greater than zero ($\alpha = 0.05$) despite the noise in the data.

Total and “dissolved” Fe concentrations measured in the Savage River were well within the measurable range of the analytical techniques. Chelex accumulated Fe would be expected to be present in much lower concentrations than the “dissolved” fraction which includes thermodynamically stable oxyhydroxides and colloidal Fe. Scatter in the data at such low concentrations makes it difficult to interpret the linearity of the accumulation curve. The slope of the line representing the accumulation of Fe by DGT over time (Figure 5.13) was also found to be greater than zero ($\alpha = 0.05$) despite the noise in the data.

Total Pb concentrations measured in the Savage River were also below the analytical detection limit for the GF-AAS (typical detection limit = 0.5 µg/L). Accumulation of labile Pb showed linear behaviour initially but then decreased as deployment time increased. The reason for this behaviour is not known. Like Cd, concentrations of Pb were very low in this tributary. DGT produced a measurable concentration because of its in-built pre-concentration step but results indicate that DGT cannot be used until the nature of the loss from the resin gel layer is understood.

v) Metal speciation

Figure 5.12 provides strong evidence for stability with respect to metal concentrations during the measurement period and strongly supports the assumption of steady state river conditions in this stream. Thus valid comparisons can be made between ASV laboratory measurements in temporally discrete water samples and the in situ time-averaged DGT measurements.

Total, “dissolved” and labile metal concentrations measured in the Savage River are shown on Figure 5.14 to 5.16. A line connects the labile metal concentrations measured by DGT because they represent the average concentration of DGT-labile metal at the sampling location, during the measurement period.

Results suggest that the Cu concentration and speciation was relatively constant during this sampling period (Figure 5.14). Labile Cu measurements by both ASV and DGT,
which respectively represent 10 % and 25 % of the “dissolved” Cu concentration, both indicate that most of the Cu was strongly complexed in the Savage River. DGT measured higher concentrations than ASV for Cu in this system. This result is consistent with the measurement time scales of the two techniques (Section 1.2.4) with the longer diffusion times of DGT allowing for a greater proportion of labile metals to be accumulated by the resin layer. In addition to this, the different measurement principles of the two techniques would be expected to have a major impact on what is measured. Accumulation by chelex involves a competitive complexation reaction whilst ASV relies (for organic complexes) on the reduction of Cu from complexes at the electrode.

Figure 5.14: Speciation of Cu in the Savage River (23 - 27 Feb 1998; ■ = total metal; = dissolved metal; = ASV metal; = DGT-labile metal).

Speciation measurements for Mn in this stream are shown in Figure 5.15. Given the well-aerated condition of the river at the time of sampling, Mn would be expected to be in its oxidised form of MnO2 (s). Labile Mn measurements by DGT were however equivalent to the initial “dissolved” fraction. Over 75 % of the total Mn measured at the start and end of the deployment period was found to be DGT-labile. This is surprising, as Mn2+ ions are expected to be measurable by DGT but MnO2(s) is not. These results therefore suggest that Mn was not yet oxidised in this tributary due to the slow oxidation rate of Mn2+ (Laxen, Davison and Woof 1984; Johnson and Chiswell 1996). The DGT-labile Mn may also represent some organically complexed Mn2+ ions, Mn (II)
has been found to interact with carboxylate anionic groups in AHS forming weak (log $K' \approx 2$) Mn (II) - humate complexes (Lu et al. 1997).

The difference between the total and “dissolved” Fe measurements shown in Figure 5.16 is indicative of a high particulate load that was evident by the turbidity of the river water at the time of sampling. The labile Fe concentrations measured by DGT were significantly lower than the “dissolved” fraction that includes thermodynamically stable oxyhydroxides and colloidal Fe. The dominant oxidation state expected for Fe in these well-aerated tributaries is Fe(III) (Teasdale 1996). Fe$^{2+}$ ions would therefore not be expected to exist at measurable levels and the chelex accumulated Fe measured in this study may also include adsorbed Fe colloids or organic complexes. Fe(II) has however been previously measured in oxic fresh and marine waters and organic complexation has recently been shown to be significant in coastal and oceanic waters (Gledhill and van den Berg 1995). Gledhill and van den Berg (1995) measured Fe(II) in surface waters of the North Sea to a depth of 20 m and also at 70 m depth. The presence of Fe(II) was attributed to photochemical reduction in surface waters and to breakdown of organic matter in waters below the photic zone.
b) Ring River

At the time of DGT deployment in the Ring River, water flow was high due to high rainfall over the preceding days. When the DGT units were collected, water level had dropped by approximately 15 cm from the pre-deployment level. The DGT units were still fully submersed, despite this change in water level. At the time of deployment a pH of 4.68 and conductivity of 86.5 µS/cm were measured. At the end of the deployment period a pH of 5.14 and conductivity 107.6 µS/cm were measured. Average water quality data are reported in Table 5.1.

Speciation measurements in the Ring River (Figure 5.17) indicate that with the exception of Fe, the DGT-labile fraction was equivalent to the “dissolved” fraction. For Cu, Cd and Zn in this stream, ASV and DGT measurements were equivalent to the “dissolved” fractions supporting the hypothesis (Section 2.6.4) that under steady-state conditions, ASV and DGT measure the same concentration in the absence of strong complexation and the absence of colloidal material.

Measurements therefore suggest that Cu, Cd, Zn and Mn were not strongly complexed in this tributary and that steady state conditions existed in this stream over the DGT deployment period.
Figure 5.17: Metal speciation measured in the Ring River (23 Feb 1998; TM = total metal; DM = dissolved metal; ASV = ASV-labile metal).
5.5.2 Applications under non conditions

Under non-steady state conditions, comparison of DGT-labile metal concentrations to forms measured in discrete samples is not possible unless sufficient samples have been collected over the DGT deployment period to characterise the time-averaged concentration of other species (e.g. total, “dissolved”).

Deployment under such conditions still provides the mean DGT-labile metal concentration during the deployment period. This is important information for assessment of the exposure of aquatic organisms to metals. Comparison of DGT-labile concentrations to those measured in discrete samples may only provide information on the variability of metal concentrations over time. Results from DGT units deployed in the Stitt and Que Rivers and Lake Pieman (i.e. PBH and PRD) show that non-steady state conditions persisted in these sites during deployment.

a) Stitt River

As with the other tributaries sampled during this study, water flow receded during the DGT deployment period in the Stitt River. At the time of DGT deployment, pH was initially 4.98 and conductivity was 55.6 µS/cm. When the DGT units were retrieved, a pH of 5.76 and conductivity of 68.1 µS/cm were measured. Average water quality data are reported in Table 5.1. Speciation measurements performed in the Stitt River are shown in Figure 5.18.
Concentrations of DGT-labile Zn and Mn exceed the concentration of “dissolved” Zn and Mn in samples collected at the start of deployment. This indicates that water flowing past the DGT units was subsequently richer in these metals. DGT-labile Cu, Cd and Fe concentrations are greater than ASV-labile concentrations (Cu, Cd) but less than “dissolved” metal concentrations (Cu, Cd, and Fe) as would be expected for steady state conditions. Given the results for Zn and Mn however, it is not possible to draw conclusions on speciation, other than to conclude that the average DGT-labile concentrations may be elevated compared to those at the start of deployment.
b) Que River

Figure 5.19: Metal speciation measured in the Que River (23 Feb 1998; TM = total metal; DM = dissolved metal; ASV = ASV-labile metal).

When the DGT assemblies were deployed in the Que River, the water was fast flowing with poor clarity as a result of heavy rainfall on previous days. The pH was initially 4.72 and the conductivity was 567 µS/cm. When the DGT units were retrieved, a pH of 5.20 and conductivity of 1090 µS/cm were measured. Water quality data are reported in Table 5.1.

DGT units were deployed at the edge of the stream, north of the bridge on the Murchison Highway. Water samples were collected at this time for analysis of total,
“dissolved” and ASV-labile metals. A small weir located several metres downstream of the deployment anchor point maintained water level.

For all metals, the DGT-labile concentration is less than the “dissolved” metal concentration as it should be for steady state conditions. Non-steady state conditions are however indicated in this stream because ASV-labile concentrations measured at the start of deployment exceed the time averaged DGT-labile metal concentrations for Zn and Cd. The results suggest that labile metal concentrations decreased over the deployment period. This may be due to variation in metal concentration with flow, an increase in ligand concentration or formation of different complexes.

C) Lake Pieman (PBH)

DGT speciation measurements performed at PBH showed maximum metal concentrations coincided with the increase in conductivity, alkalinity and pH discussed in Section 5.4.2. The DGT-labile fractions of Cu, Cd and Zn (Figure 5.20) were significantly higher than the total or dissolved metal concentration measured in discrete samples collected at the start of the DGT deployment period. These results show that the water column was therefore not in a steady state with respect to these metals during the measurement period and that water with significantly higher Cu, Cd and Zn concentrations entered Lake Pieman in the deep water plume over this time (Figure 5.20).

DGT-labile Fe concentrations were low with respect to the total and “dissolved” fraction probably because most of the Fe is in the form of insoluble oxyhydroxides of Fe $^{3+}$ in these well-oxygenated waters. An increase in DGT-labile Fe was however observed at a depth of 22 m coinciding with maxima observed for other metals (Figure 5.21).

DGT-labile Mn concentration did not show substantial increase over the total Mn concentration measured in the initial “spot” samples (Figure 5.21). Reasons for this are not clear.
Figure 5.20: Measurements of Cu, Cd and Zn at PBH (21 – 23 Feb 1998; • = total metal; ◀ = dissolved metal; ■ = DGT-labile metal).
Figure 5.21: Measurements of Fe and Mn at PBH (21 - 23 Feb 1998; ♦ = dissolved metal; ■ = DGT-labile metal).

d) Reece Dam (PRD)
Speciation measurements performed at PRD are presented in Figure 5.22 & 5.23. During the period of DGT deployment, very strong winds affected the dam site generating significant turbulence. Deployment of DGT units for 48 hours gave results showing little variation in DGT-labile Cu concentration with depth (Table 5.5). The proportion of DGT-labile Cu decreased from about 24 % of the total Cu concentration in surface waters to 15 % of the total Cu concentration in deeper waters. The proportion of DGT-labile Fe also decreased with depth as the total Fe concentration increased. The proportion of DGT-labile Fe decreased from 18 % of the total Fe concentration in surface waters to about 2 % of the total Fe concentration in deeper waters.
Table 5.5: DGT-labile Cu and Fe concentrations at PRD.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0.4 ± 0.1</td>
<td>14</td>
<td>15 - 24</td>
<td>23</td>
</tr>
<tr>
<td>Fe</td>
<td>31 ± 13</td>
<td>14</td>
<td>2 - 18</td>
<td>6</td>
</tr>
</tbody>
</table>

*[M]* measured at time of deployment (see Figure 5.22).

On average, DGT-labile Cu represented approximately 23 % of the total Cu concentration showing that a significant proportion of the Cu present was strongly complexed throughout the water column at this site. Cu ion complexation titrations performed in water collected from 5 m and 40 m depth at the Reece Dam confirmed this hypothesis and showed that $C_L$ for ionic Cu was approximately 53 - 66 µg Cu/L in these waters (Table 5.6).

Table 5.6: Complexation parameters measured in water samples collected from PRD.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>$C_L$ (nM)</th>
<th>95 % C.I. (nM)</th>
<th>$C_L$ (µg Cu/L)</th>
<th>95 % C.I. (µg Cu/L)</th>
<th>log $K'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1040</td>
<td>80</td>
<td>66</td>
<td>5.3</td>
<td>6.7</td>
</tr>
<tr>
<td>40</td>
<td>840</td>
<td>50</td>
<td>53</td>
<td>3.3</td>
<td>6.9</td>
</tr>
</tbody>
</table>

(pH 5.9, 21.5 °C).
Figure 5.22: Cu and Fe speciation measurements at PRD (20 - 22 Feb 1998; ● = total metal; ♦ = dissolved metal; ■ = DGT-labile metal).

Two metals (Mn and Zn) gave results indicating non-steady state conditions during deployment (Figure 5.23). The DGT-labile Mn concentration significantly exceeded the total Mn concentration (measured at the start of deployment) down to a depth of 30 m. Concentrations below 30 m are 20 - 50 % higher than the total Mn concentration measured. These results suggest that the original manual sampling was performed when the Mn concentration was low. Elevation of the DGT-labile Mn concentration in surface waters is considered to be due to remobilisation of sediments from dam walls and release of Mn$^{2+}$ ions as a result of wind generated turbulence. This effect should be greatest in surface waters (i.e. to 30 m depth) and negligible at deeper sites.

DGT-labile Zn concentrations showed considerable scatter and were 2 - 4 times higher than total Zn concentrations over the entire water column. The Zn results are probably caused by contamination from a galvanised chain used to secure the marker buoy near the dam wall. The DGT units were deployed within 5 m of this chain. Wave action on
the buoy would have generated turbulence throughout the water column - creating a cylindrical envelope of Zn enriched water around the chain.

Because of weather conditions and the nature of Reece Dam (with depths of up to 90 m and significant water flow over the dam wall), the chain and buoy assembly provided the only suitable anchor point for the boat, from which sampling was performed, and for securing the DGT units. Contamination by Zn was therefore unavoidable under these conditions.

![Graph showing Mn and Zn speciation measurements at PRD (20 - 22 Feb 1998)](image)

**Figure 5.23:** Mn and Zn speciation measurements at PRD (20 - 22 Feb 1998; ● = total metal; ♦ = dissolved metal; ■ = DGT-labile metal).

### 5.5.3 Conclusions about DGT

DGT is a useful addition to the current suite of speciation tools already available to environmental managers. Although the application of DGT is more complicated and time-consuming than that required for the collection of a set of discrete samples for analysis by ASV, DGT offers some advantages over other techniques in situations where *in situ* speciation measurements are desirable. DGT is a simple tool that is relatively cheap and easy to use in the field. The technique has an in-built pre-
concentration procedure that is applicable to many metals and can be used after minimal personnel training. In addition to these advantages, metals are delivered for analysis in a small volume of nitric acid solution (2 mL) which is free of any matrix effects with respect to the metals examined in this study and automatically preserves the sample integrity. The risk of sample contamination is low as samples are easily stored until analysis and minimal sample handling is required between deployment and analysis.

DGT produces an *in situ* speciation measurement integrated over time (12 - 72 hours in our studies), which is potentially useful for monitoring in streams of highly variable water quality, or for long-term ecotoxicity studies. It does not however detect concentration maxima and minima, which are also important for assessing toxicity of aquatic environments. The chance of detecting maxima and minima in “spot” samples is also unlikely however due to the dynamic nature of such systems, unless samples are collected at an appropriately high frequency or if measurements were performed continuously. The usefulness of a time integrated concentration may be questioned for some circumstances, however such a measurement provides additional speciation information by determining an “average” labile concentration which may not be provided by the analysis of a set of “spot” samples. This has been demonstrated by speciation measurements performed at PBH.

This study has shown that DGT is a useful tool for providing a time-averaged labile metal concentration. Under steady state conditions however, measurements by DGT can also be compared directly with ASV measurements. Information generated by DGT under various situations is summarised in Table 5.7 and discussed below.

**Table 5.7:** Summary of information provided by DGT measurements.
In the absence of strong complexation or where the complexation capacity has been exceeded DGT produced time-averaged measurements, which were equivalent to ASV measurements in cases where steady-state river conditions existed (e.g. Zn, Cu and Cd in Ring River).

When strong complexation was occurring under steady-state river conditions, the DGT measurements were greater than the ASV-labile fraction. These results are consistent with the measurement time scales of the two techniques, however in some cases additional speciation information was revealed when both speciation measurements were examined simultaneously (e.g. Cu in Savage River).

Where strong complexation was occurring but river conditions were variable, DGT produced a time-averaged concentration but did not provide speciation information directly comparable to the total, “dissolved” and ASV-labile concentrations measured in “spot” samples (e.g. Cu, Cd, Pb in Stitt River; Cu in Que River; Cu, Cd, Zn in deep sites at PBH).

In the absence of strong complexation or where the complexation capacity has been exceeded DGT produced time-averaged concentrations in cases where variable non-steady-state river conditions existed. In such cases, DGT does not provide directly comparable speciation information with respect to total, “dissolved” and ASV-labile fractions measured in “spot” samples (e.g. Mn in PRD, Zn in Stitt and Que Rivers).

### 5.6 Assessment of WHAM for prediction of Cu speciation

WHAM is designed to calculate equilibrium chemical speciation in surface and ground waters, sediments and soils. The model is especially suitable for problems where chemical speciation is dominated by organic matter (Tipping 1994). The utility of WHAM for predicting speciation in Pieman River tributaries was investigated for Cu in the Que, Ring, Stitt and Savage Rivers.

Chemical compositions of tributary sites are given in Table 5.1. These parameters were used as input data for calculating inorganic and organic Cu species by WHAM. By default the WHAM model assumes that DOC is half the DOM by mass and that [FA] =
This assumption was initially adopted for the Pieman River modelling (Table 5.9). Predictions were also performed after altering the DOC composition, DOC concentration, relative proportions of Fe (II) to Fe (III) and the Mn concentration, which were identified as possible key parameters involved in the regulation of Cu speciation.

5.6.1 Effect of altering the DOC composition

As highlighted by Zhang and Davison (in press), it cannot be assumed that FA is the only active organic ligand in natural waters. Little information is available about the character of ABS in Pieman River water however mass fractionation has indicated that DOC in a Farm Creek sample (Pieman River catchment; Figure 1.3) was exceptionally well degraded (humified) and dominated by hydrophobic acids which are typical fulvic acids (Table 5.8). Approximately 10 % of the DOC was identified as HA (Leenheer 1995). For WHAM calculations, 90 % of the DOC was assumed to be FA.

Table 5.8: Characterisation of isolated DOC fractions from a Farm Creek sample by FTIR Spectrometry (Leenheer 1995).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mass (mg)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic neutrals</td>
<td>9.3</td>
<td>Glycolipid material</td>
</tr>
<tr>
<td>Hydrophilic neutrals</td>
<td>1.9</td>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Hydrophobic acids</td>
<td>266.8</td>
<td>Fulvic acids</td>
</tr>
<tr>
<td>Hydrophilic acids</td>
<td>30.7</td>
<td>Hydroxy acids</td>
</tr>
<tr>
<td>Bases</td>
<td>13.4</td>
<td>Primary amides</td>
</tr>
</tbody>
</table>

Excellent agreement between the measured concentration of labile-Cu by ASV and the concentration predicted by WHAM was achieved in two of the streams when a ratio of 10 % HA : 90 % FA was used in the model (Table 5.9).

Table 5.9: Comparison of ASV measurements of Cu speciation with speciation predicted by WHAM in four Pieman River tributaries.

<table>
<thead>
<tr>
<th>River</th>
<th>pH</th>
<th>Labile / [Cu	extsubscript{II}] % (ASV)</th>
<th>Labile / [Cu	extsubscript{II}] % (WHAM) HA = 0 %</th>
<th>Labile / [Cu	extsubscript{II}] % (WHAM) HA = 10 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ring</td>
<td>4.91</td>
<td>92</td>
<td>89</td>
<td>88</td>
</tr>
<tr>
<td>Stitt</td>
<td>5.37</td>
<td>13</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>Que</td>
<td>4.96</td>
<td>4.8</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>Savage</td>
<td>7.31</td>
<td>5.5</td>
<td>73</td>
<td>56</td>
</tr>
</tbody>
</table>
In the two streams with pH below 5, little effect was observed by changing the HA : FA ratio. In the Que River for example, changing the HA : FA ratio between 0 and 20 % had a negligible effect on the WHAM-calculated labile concentration which in all cases was well above the labile concentration measured by ASV (i.e. 4.8 %).

Changing the HA : FA ratio had a significant impact on estimates of speciation by WHAM where the pH was $\geq 5.37$. At 10 % HA in the Savage River for example, 56 % of total Cu was estimated by WHAM to be labile, in contrast to 73 % labile Cu estimated in the situation where HA = 0 %. The concentration of labile Cu estimated by WHAM was however still well above the 5.5 % measured by ASV.

5.6.2 Effect of altering the DOC concentration

The DOC concentration is an important parameter for speciation predictions by models such as WHAM (Zhang and Davison *in press*). Measurement of DOC can be difficult particularly in estuarine samples where the variable conductivity is difficult to cope with. An uncertainty in the measured concentration of $\pm 10 \%$ would not be considered unreasonable for some samples.

The influence of DOC was investigated by varying the input [FA] by $\pm 25 \%$ of the measured DOC value. In the Stitt River (pH 5.37) the proportion of labile-Cu estimated by WHAM decreased by $\sim 30 \%$ when the DOC value increased by $\sim 25 \%$. In contrast to this result, in the Que River (pH 4.96) changing the DOC concentration had little effect on Cu speciation.

5.6.3 Effect of altering the Fe(II) I Fe(III) ratio

WHAM predictions were initially performed using the time-averaged DGT data as the Fe (II) input and the difference between the total Fe and the DGT-labile Fe as the Fe (III) input (Table 5.9). The influence of altering the Fe (II) : Fe (III) ratio on Cu speciation predicted by WHAM was investigated in the Stitt, Que and Savage Rivers.

When Fe (III) exceeded Fe (II), the size of the ratio was not important and little change in Cu speciation was observed. When Fe(II) was altered to exceed Fe(III), the predicted bound Cu concentration increased significantly for both the Que and Stitt Rivers. Little change was observed however in the Savage River where pH was $> 7$. In these well-
5.6.4 Effect of altering the Mn concentration
Mn may be present as $\text{Mn}^{2+}$ or $\text{MnO}_2$. The former may compete with $\text{Cu}^{2+}$ for ligands. The WHAM model assumes that all Mn is present as Mn $2^+$. To simulate the condition whereby Mn $2^+$ represents only a portion of the total Mn, Mn concentrations entered into the model were altered. The influence of altering the Mn concentration on Cu speciation predicted by WHAM was investigated for the Que and Stitt Rivers. The Mn concentration was found to have very little effect on Cu speciation in either tributary even when the Mn concentration used in the model varied within the range of 0.1 to 2 times the measured total Mn concentration.

5.6.5 Conclusion
Predictions of Cu speciation by WHAM agreed closely with field measurements by ASV in the two streams that were sampled above current mining operations. These sites (Ring and Stitt) were receiving AMD and runoff from non-1lining mines but were not receiving active mine input. In contrast, in the Que and Savage Rivers, where streams were under the influence of active mine waste, WHAM predictions were very poor. Regardless of the input parameters altered when performing simulations, WHAM always over-estimated the labile fraction and therefore under-estimated the bound fraction at these sites. One possible explanation is that flotation agents from mine processing may be binding metals more strongly than can be predicted from natural water quality parameters by computer modelling. More detailed research is required to test this hypothesis.

5.7 Ecological implications of metal speciation

5.7.1 Cu
At all four tributary sites and at PBH, measured total Cu concentrations exceeded the lower limit of the current water quality criteria range for the protection of ecosystems ($\text{[Cu}_T\text{]} = 2.0 — 5.0 \mu\text{g/L}; \text{ANZECC 1992}$). In the Que ($\text{[Cu}_T\text{]} = 60.4 \mu\text{g/L}$), Savage ($\text{[Cu}_T\text{]} = 52.4 \mu\text{g/L}$) and Stitt ($\text{[Cu}_T\text{]} = 4.08 \mu\text{g/L}$) Rivers, over 80 - 90 % of “dissolved” Cu was found to be strongly complexed and therefore not considered as bioavailable.
Labile concentrations were therefore within or below guidelines. Similar results were found for PBH in which > 90 % of total Cu ([CuT] = 5 µg/L) was strongly complexed. At PRD, [CuT] = 1.5 to 2 µg/L and the DGT-labile fraction represented about 17 - 30 % of the total Cu. In the Ring River, all Cu was found to be labile ([CuT] = 47 µg/L), easily exceeding current water quality criteria for protection of aquatic ecosystems.

5.7.2 Zn

In the three tributaries sampled where speciation measurements were possible, ASV-labile Zn concentrations were equal to the total and “dissolved” Zn concentrations suggesting that strong complexation was not a significant regulating factor of Zn speciation in these streams at the time of sampling. Zn concentrations in these streams were very high and therefore probably well in excess of the potential Zn binding capacity of these waters.

Zn concentrations exceeded current water quality criteria at all tributaries sites investigated. At PBH, significant Zn complexation was observed. Total and “dissolved” Zn concentrations exceeded 300 µg/L at some depths at this site and so even the labile fraction was still well in excess of the current water quality criteria for protection of aquatic ecosystems.

Based on current water quality guidelines ([ZnT] = 5.0 -50.0 µg/L provided Fe not present as Fe (II); ANZECC 1992), Zn poses a significant ecological threat in both tributary and lake sites.

5.7.3 Cd

In the three tributaries where speciation measurements were possible, all Cd was found to be present in the “dissolved” state. At current concentrations, Cd exceeds current water quality guidelines ([CdT] = 0.2 - 2.0 µg/L depending on hardness; ANZECC 1992) in each of the Que, Stitt and Ring Rivers even with respect to the labile fractions. Cd concentrations were too low for determination of speciation in the Savage River.

At PBH, the time averaged labile concentration was ~ 0.5 µg/L in surface waters but was as high as 3.5 µg/L at 24 m depth, easily exceeding the current guideline. At PRD,
the time averaged labile concentrations were shown to be < 1 µg/L throughout the water column, at least to a depth of 55 m.

5.7.4  Pb
In the Ring and Stitt Rivers, total Pb concentrations greatly exceeded current water quality criteria for protection of aquatic ecosystems ([Pb\text{r}] = 1.0 - 5.0 µg/L; ANZECC 1992). At PBH, total Pb concentrations were found to be within current guidelines in surface waters, however in deeper waters concentrations of > 7 µg/L were measured. At PRD, total Pb concentrations were found to be within the guidelines (1.0 - 1.5 µg/L).

5.7.5  Mn
In all four tributaries and the two lake sites, all Mn was present as “dissolved” (i.e. < 0.4 µm) species. Given the well-aerated condition of the tributary streams at the time of sampling, Mn is expected to be in its oxidised form of MnO₂(s). Labile Mn measurements by DGT were however equivalent to the “dissolved” fraction in all tributaries with the exception of the Que River. As Mn²⁺ is expected to be measurable by DGT but MnO₂(s) is not, these results suggest that Mn was not oxidised in three of the four tributaries sampled. Water quality criteria for the concentration of Mn have not been applied in the current guidelines (ANZECC 1992).

5.7.6  Fe
With the exception of the Que River, DGT-labile Fe was significantly lower than the “dissolved” fraction in each of the tributaries sampled. This is expected as Fe is known to be rapidly oxidised to Fe(III) in the presence of oxygen and appropriate microorganisms (Teasdale et al. 1996). In the Que River the DGT-labile fraction was equivalent to the “dissolved” fraction suggesting that oxidation was retarded or that Fe²⁺ ions were stabilised as organic complexes (Gledhill and van den Berg 1995) that were able to dissociate in the measurement time scale of DGT. Flocculation agents associated with current mining activities may have caused this unusual effect.

Concentrations of Fe were very high at some sites. In the Savage and Que Rivers and at PBH, the current water quality criteria were exceeded with respect to Fe with concentrations as high as 2000 µg/L detected in deep waters at PBH ([Fe] = 1000 µg/L provided Fe not present as Fe(II); ANZECC 1992).
5.8 Conclusion

This work has investigated metal speciation in several freshwater environments of the Pieman River catchment and compared ASV and DGT for measurement of labile metal concentrations in river waters.

A clear conclusion from this study is that metal ion complexation and hence speciation is highly variable within the Pieman River catchment. Results for the tributary sites show considerable variability in both metal concentration and speciation. This presents major difficulties for environmental managers, as it is therefore not possible to make catchment-wide assumptions about the bioavailability of these metals. These results emphasise the importance of site-specific sampling protocols and speciation testing.

DGT is useful in providing a time averaged measurement and may provide a useful analogy to bioaccumulation techniques. It does not however detect concentration maxima and minima, which are also important for assessing toxicity of aquatic environments. However, while discrete sampling permits more detailed speciation measurements (e.g. the particulate / “dissolved” / labile distinction), a true average concentration is not obtained.

These results therefore demonstrate the importance of combining speciation techniques to more fully understand water chemistry and its variability with time. Ideally, DGT would be combined with in-line analysis (e.g. by ASV) to capture time-averaged data, and maxima and minima data.
CHAPTER 6

Speciation in the Pieman River estuary

6.1 Introduction

After release from Reece Dam (PRD) power station, the Pieman River flows approximately 38 km through an area classified as the Pieman River State Reserve to its mouth at Pieman Heads. This region of the river is a highly dynamic salt estuary. Its flow is partially restricted by a sand bar at its mouth, effectively increasing the residence time of bottom waters. The wedge position is variable because of the intermittent and controlled nature of the discharge of water from Reece Dam. It is also influenced by the strength of tides and discharge from the Whyte, Savage and Donaldson Rivers as well as numerous smaller tributaries (Koehnken 1992). Of the total freshwater discharge at Pieman Heads (190 cumecs averaged over one year) approximately 77% is from Reece Dam, 9% is from the Whyte River and 7% is from each of the Savage and Donaldson Rivers (Koehnken 1992).

Water quality in salt wedge estuaries is influenced by one or more chemical, biological or hydrological processes at any given time. The prediction of trace element speciation and reactivity is therefore highly complex (Millward 1995). Important biological and physico-chemical processes include:

- salinity and pH changes as river water is diluted with seawater
- redox reactions where anoxia occurs
- complexation by natural organic and inorganic ligands
- competitive complexation by major ions, such as Ca$^{2+}$ and Mg$^{2+}$
- flocculation and settling of colloidal or particulate material
- sediment fluxes
- bioturbation
- microbiological activity (Millward 1995)
The most important reactions with respect to mobility of metals in aquatic environments are those that result in a transfer of metal ions between “dissolved” and particulate phases (Teasdale et al. 1996). Elemental species that are influenced only by dilution of river water by seawater or vice versa and are not affected by other reactions will display a linear relationship with salinity. These elements are said to behave conservatively (Miliward 1995; Teasdale et al. 1996). Non-conservative behaviour can manifest itself in two ways. A positive deviation from linearity on a salinity-concentration plot implies inputs into solution (e.g. dissolution of particulate matter, release from sediments or anthropogenic sources). Alternatively, a negative deviation from linearity implies removal from solution (e.g. precipitation, adsorption or flocculation of colloidal material (Miliward 1995; Teasdale et al. 1996).

The objective of this study was to investigate the influence of the catchments estuarine environment and hydrodynamics on metal speciation.

6.2 Methodology

This study was conducted during a two week sampling trip to the catchment in February 1998. Metal speciation was investigated at a total of nine sites along the estuary (Figure 6.1). At the sites upstream from and including Corinna, total, “dissolved” and ASV-labile metals were measured in water samples collected as previously described (Section 2.3.2) using a peristaltic pump and a close-interval sampler. Metal concentrations were measured using GFAAS in low salinity surface waters. In all other samples, metal concentrations were measured by ASV and the method of standard additions to avoid matrix effects due to salinity variations. Cu ion complexation titrations were also performed on non-filtered, non-acidified water samples.

DGT assemblies were deployed at various depths at four sites downstream from Corinna (Sites E1 to E4). Water samples were collected from each site for analysis of total and “dissolved” metals and ASV-labile Zn. DGT samples were analysed using AAS. DGT-labile Zn concentrations are not available as useful data was not obtained due to restrictions imposed by limited sample volume (~ 0.5 mL).

At one site (E3), water quality profiles were measured and water samples were collected three times throughout the DGT deployment period in order to determine the stability of
the position of the halocline whilst the DGT assemblies were suspended in the water column. Tidal amplitude was monitored during this period by measuring the distance to the surface of the water from an arbitrary datum point on the pier at Corinna.

6.3 Hydrodynamics of the Pieman River estuary.

6.3.1 Variation of salt-wedge incursion
During the February 1998 study, the leading edge of the salt-wedge was initially detected just upstream of the Meredith River inlet (Figure 6.1). Over the following week, the front of the wedge had been pushed downstream (~11 km) to Corinna, a small village located on the north bank of the river approximately halfway between the Reece Dam wall and the river mouth.

![Figure 6.1: Schematic representation of sampling locations (marked in red) for speciation studies in the Pieman River estuary (Not to scale; shaded region represents Pieman River State Reserve).](image)

6.3.2 Vertical water quality profiles
During the sampling period, a significant freshwater layer (~6 m deep) occurred above a sharp halocline at the four downstream sites, E1 to E4. Across the halocline, salinity increased from ~2 to 28 over a depth of 2 m (Figure 6.2). The small vertical range of the halocline, combined with its vertical migration in response to tides and discharge variability made it difficult region to sample for intermediate salinities. Similar profiles were measured at the upstream sites but are not shown here.
Figure 6.2: Temperature-salinity-depth profiles for Pieman River estuarine sites E1-E4 (26 Feb 1998, 10:15 - 15:15 hours; = salinity, = temperature. At all sites, sampling was continued to within 1 m of the bottom).
Figure 6.3: "Dissolved" oxygen-depth profiles for Pieman River estuarine sites E1-E4 (26 Feb 1998, 10:15 – 15:15 hours).
The water column was homogenous with respect to temperature at all sites (Figure 6.2) except for a slight increase in temperature in the uppermost 0.5 m. Between sites El and E4, pH varied from 6.9 to 7.8 at a depth of 2 m and in deep waters pH ranged from 7.5 to 8.1.

Surface waters were well oxygenated and DO concentrations were \(\geq 8 \text{ mg/L} \) at all four sites (Figure 6.3). At sites El and E2, a 6 m layer of well oxygenated water lay above a broad (~ 6 m) oxycline in which DO decreased to 4 mg/L and persisted at this concentration to 12 m depth. The oxycelines detected at the more downstream sites (E3 & E4) were deeper, with an obvious reduction in DO concentration occurring at ~ 8 m depth. A sharper oxycline was detected at site E3. DO concentration decreased from 8 to 4 mg/L between 8 m and 10 m. Although DO concentrations were significantly reduced in deep waters at all sites, anoxic conditions were not detected.

6.3.3 Variation in the estuarine environment during DGT deployment

Salinity and water temperature were measured three times during the DGT deployment period at site E3, to monitor the stability of the halocline. Measurements were performed at the time of the DGT immersion in the river (25 Feb), at a time during the middle of the deployment period (26 Feb) and when the DGT units were retrieved (27 Feb). Temperature-depth profiles measured at this site showed little variation. Variation in temperature was typically ± 0.1°C throughout the water column except at the water surface where variation was approximately ± 0.5°C. The profile recorded mid-deployment (Figure 6.2; 26 Feb 1998; 1215 - 1230 hours) is typical of the temperature profiles recorded.

During the 48 hours deployment period, salinity remained stable in the surface layer (0 - 5 m) and in the deep saline layer (10 - 18 m). The position of the halocline was less stable (Figure 6.4). A salinity of 10 for example, was detected over a depth range of 1.4 m (i.e. from 5.7 m to 7.1 m depth). Salinities of 15 and 20 were detected over depth ranges of 1 m each. This has the potential to impact on DGT measurements performed in the halocline region if metal concentrations and their reactivity were altered during the deployment period.
Figure 6.4: Variation in the position of the halocline during the 48 hour DGT deployment period at site E3 (25 - 27 Feb 1998).

When each water quality profile was measured, water samples were also collected at the depths of DGT deployment (2 m and 13 m depth) for analysis of total and “dissolved” metals, ASV-labile Zn and organic carbon. Except for TOC and DOC, duplicate samples were collected on each of the three occasions and analysed individually, resulting in six measurements for each analyte. For TOC and DOC, single samples were collected on each of the three occasions and analysed individually, resulting in three measurements for each. The average results from all analyses are presented in Table 6.1. Because total metal concentrations were very low in the 13 m samples, the filtered samples were not analysed.
Table 6.1: Variation in metal and organic carbon concentrations detected in water samples collected on three occasions during the 48 hour DGT deployment period at site E3.

<table>
<thead>
<tr>
<th>Depth</th>
<th>Cu</th>
<th>Cu</th>
<th>Cd</th>
<th>Cd</th>
<th>Zn</th>
<th>Zn</th>
<th>ASV-Zn</th>
<th>TOC (mg/L)</th>
<th>DOC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 m</td>
<td>3.4</td>
<td>2.4</td>
<td>0.10</td>
<td>0.10</td>
<td>41.5</td>
<td>45.0</td>
<td>26.8</td>
<td>5.8</td>
<td>6.6</td>
</tr>
<tr>
<td>Stdev</td>
<td>0.3</td>
<td>1.1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.7</td>
<td>0.8</td>
<td>0.5</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>13 m</td>
<td>3.5</td>
<td>na</td>
<td>0.03</td>
<td>na</td>
<td>11.6</td>
<td>na</td>
<td>6.9</td>
<td>2.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Stdev</td>
<td>0.5</td>
<td>na</td>
<td>0.03</td>
<td>na</td>
<td>0.6</td>
<td>na</td>
<td>1.7</td>
<td>0.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

(na = not analysed, [M] in μg/L).

The change in water level due to tidal movement and water release from the Reece Dam power station was monitored by measuring water levels at the Corinna pier (Figure 6.5). A maximum fluctuation of 0.5 m was observed during this period. Smaller water level fluctuations generally follow a diurnal tidal cycle. Because of the relatively small water level fluctuation, it is reasonable to assume that the immersed DGT assemblies remained within a layer of water of relatively constant water quality during the entire measurement interval.

Figure 6.5: Water level fluctuation measured relative to an arbitrary bench mark on the Corinna pier during the period of DGT deployment at E3 (25 - 27 Feb 1998).
6.4 Chemical composition of surface waters

6.4.1 Water quality of surface waters

Salinity data for all estuarine surface-water study sites and their location with respect to the river mouth are presented in Table 6.2.

Table 6.2: Location and salinity of Pieman River estuary surface-water sampling sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth (m)</th>
<th>Distance from mouth (km)</th>
<th>Salinity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAN</td>
<td>0.2</td>
<td>24</td>
<td>0.04</td>
</tr>
<tr>
<td>Nancy</td>
<td>0.2</td>
<td>22</td>
<td>0.13</td>
</tr>
<tr>
<td>Whyte</td>
<td>0.2</td>
<td>21</td>
<td>0.35</td>
</tr>
<tr>
<td>BAC</td>
<td>0.2</td>
<td>19.5</td>
<td>0.42</td>
</tr>
<tr>
<td>Corinna</td>
<td>0.2</td>
<td>19</td>
<td>0.73</td>
</tr>
<tr>
<td>E1</td>
<td>2.0</td>
<td>16</td>
<td>0.90</td>
</tr>
<tr>
<td>E2</td>
<td>2.0</td>
<td>11</td>
<td>1.45</td>
</tr>
<tr>
<td>E3</td>
<td>2.0</td>
<td>7</td>
<td>2.25</td>
</tr>
<tr>
<td>E4</td>
<td>2.0</td>
<td>3</td>
<td>2.55</td>
</tr>
</tbody>
</table>

* at time of sampling (24 – 26 Feb 1998)

General water quality data measured at the time of sample collection are shown in Figure 6.6. Salinity increased from 0.04 to 2.55 indicating that mixing of river water and seawater was slight along the estuary. Little variation was detected in temperature, pH, TOC or DOC with increasing salinity at these sites. The good agreement between TOC and DOC measurements show that organic matter was present in predominantly “dissolved” forms (i.e. < 0.4 µm) and that flocculation of organic matter was not a significant process in the low salinity (salinity < 2.6) region of the estuary.
Figure 6.6: Variation in water quality parameters measured in surface waters of the Pieman River estuary (24 - 26 Feb 1998; ♦ = TOC).
6.4.2 Total and “dissolved” Fe

Total Fe concentrations measured in estuarine surface waters were similar to total Fe concentrations measured in the surface waters of Reece Dam. The “dissolved” Fe measured at estuarine sites (Figure 6.7) represented between 49 to 68% of the total Fe. This fraction was comparable to but maybe slightly less than that found in fresh waters of Reece Dam where the “dissolved” fraction represented approximately 60% - 80% of the total Fe concentration. This suggests that if particle formation is occurring at the low salinity range (salinity = 0.04 to 0.73) in these waters settling of the flocculated material has not occurred and thus has not had a significant effect on the total Fe load. Laboratory studies reported earlier (Section 4.9.2) have shown however that flocculation and settling occurs at higher salinities.

![Figure 6.7: Total and “dissolved” Fe in estuarine surface waters (■ = [FeT]; ● = [FeD]; error bars represent ± standard deviation of replicate measurements by flame-AAS).](image)

6.4.3 Total and “dissolved” Mn

Total and “dissolved” Mn concentrations measured in estuarine surface waters were also similar to those measured in Reece Dam and varied between 40 and 60 µg/L (Figure 6.8). The increase in Mn concentrations observed at salinities ≥ 1.5 (coinciding with sites E2 and E3) may be attributed to inputs from the Savage River which contributes about 7% of the total river volume and was shown to contain Mn concentrations between 400 to 600 µg/L (Figure 5.15). In contrast to the behaviour of
Fe in estuaries where flocculation of a large proportion of the “dissolved” Fe occurs during mixing of river and seawater (Moore et al. 1979; Yan et al. 1991), “dissolved” Mn has been found to behave conservatively (Moore et al. 1979; Boughriet et al. 1992). In Reece Dam and other freshwater sites investigated, almost all the Mn was found to be present in “dissolved” forms. Similar results were found in estuarine samples where the 0.4 µm filtered fractions were equivalent to the total concentrations. It is possible that flocculation of colloidal Mn species is not significant at these salinities. Slow oxidation kinetics coupled with photo-reduction in surface waters may ensure a significant dissolved concentration is present in this water. In addition to this, geochemical cycling of Mn may also be regulated by complexation of Mn$^{2+}$ by “dissolved” organic ligands rather than solely by changes in oxidation state and precipitation of MnO$_2$.(s).

![Figure 6.8: Total and “dissolved” Mn concentrations in estuarine surface waters (■ = [Mn$_T$]; ● = [Mn$_D$]; error bars represent ± standard deviation of replicate measurements by flame-AAS).](image)

### 6.4.4 Total, “dissolved” and DGT-labile Cu

Total and “dissolved” Cu concentrations measured in surface waters remained relatively constant along the length of the estuary (Figure 6.9). Surface water samples were collected from depths varying between 0.2 and 2.0 m and had salinities ranging from 0.04 at the most upstream site to 2.55 at the most downstream site. The average total Cu concentration was 2.7 ± 0.6 µg/L and the average “dissolved” Cu concentration was 2.3 ± 0.7 µg/L showing that most of the Cu was present in “dissolved” forms.
Uncertainties associated with “dissolved” Cu measurements were equivalent in magnitude with those associated with total Cu but have been left off the plot for clarity. Measurements performed in surface waters at sites E1 to E4 showed that DGT-labile Cu also remained relatively constant suggesting that Cu speciation did not vary significantly within the low salinity region ranging from 0.9 to 2.55.

**Figure 6.9:** Cu concentrations in surface waters of the Pieman River estuary (■ = [Cu\textsubscript{T}] ± 95 % confidence limits; ● = [Cu\textsubscript{D}]; ▲ = [DGT–labile Cu]).

### 6.4.5 Cu ion complexation

Cu binding \( C_L \) was investigated in natural estuarine surface water samples by titration with ionic Cu. Results presented in Figure 6.10 show that the estimated ligand concentration (\( C_L \)) was relatively constant in the estuarine surface waters (salinity range of 0.04 to 0.73) but were less than the concentrations measured in Lake Pieman water. \( C_L \) ranged from 555 - 760 nM (35.3 - 48.2 µg Cu/L) in estuarine surface waters. In Lake Pieman water (PRD, salinity < 0.04; Table 5.6), which is released into the estuary, \( C_L \) was found to be 840 - 1040 nM (53 - 66 µg Cu/L). In a laboratory study reported earlier (Section 4.9.3) \( C_L \) measured in mixed estuarine samples was found to decrease significantly at salinity > 5. Thus while TOC and DOC remain constant, increasing salinity may still reduce the availability of Cu binding sites. Any change in Cu speciation would not be detected given the scatter in Figure 6.9.
6.4.6 Total and “dissolved” Pb and Cd

Total and “dissolved” Pb and Cd concentrations were measured in surface waters of the lower estuarine sites (E1 to E4) and are represented in Table 6.3.

Table 6.3: Total and “dissolved” Pb and Cd concentrations (µ ± standard deviation; µg/L) measured in lower estuary surface waters (2 m depth).

<table>
<thead>
<tr>
<th>Site</th>
<th>[Pb\textsubscript{T}]</th>
<th>[Pb\textsubscript{D}]</th>
<th>[Cd\textsubscript{T}]</th>
<th>[Cd\textsubscript{D}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>1.1 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>&lt;0.3\textsuperscript{a}</td>
<td>&lt;0.3\textsuperscript{a}</td>
</tr>
<tr>
<td>E2</td>
<td>1.4 ± 1.0</td>
<td>1.1 ± 1.0</td>
<td>&lt;0.3\textsuperscript{a}</td>
<td>&lt;0.3\textsuperscript{a}</td>
</tr>
<tr>
<td>E3</td>
<td>1.3 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>&lt;0.3\textsuperscript{a}</td>
<td>&lt;0.3\textsuperscript{a}</td>
</tr>
<tr>
<td>E4</td>
<td>1.0 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>&lt;0.3\textsuperscript{a}</td>
<td>&lt;0.3\textsuperscript{a}</td>
</tr>
</tbody>
</table>

a: detection limit for Cd by ASV = 0.3 µg/L

Total Pb measured in Reece Dam during this study ranged between 0.8 and 1.2 µg/L (Figure 5.11). Results therefore suggest that total Pb concentrations in surface waters of the estuary (Table 6.3) are fundamentally the same as in the water entering the estuary from Reece Dam. Similar results were found for Cd where the total concentration of Cd in Reece Dam was found to be < 0.1 µg/L measured by GF-AAS.
Because of the very low concentration of Pb and Cd encountered at these four sites, analysis of these metals was not performed in the upper estuarine sites. At current concentrations in surface waters, both metals are present close to the lower limit of current water quality criteria ([Pb_{T}] < 1.0 - 5.0 \, \text{µg/L}; [Cd_{T}] < 0.2 - 2.0 \, \text{µg/L} depending on hardness; ANZECC 1992).

6.4.7 Total, “dissolved” and ASV-labile Zn
Total and “dissolved” Zn concentrations measured in surface waters remained relatively constant along the length of the estuary (Figure 6.11). Total Zn concentrations ranged between 42 and 59 \, \text{µg/L}. Most of the Zn was found to be present in “dissolved” forms and the ASV-labile fraction, which constituted between 30 % and 65 % of the “dissolved” Zn, increased with salinity along the length of the estuary.

![Figure 6.11: Zn concentrations in surface waters of the Pieman River estuary (24 - 26 Feb 1998; ■ = [Zn_{T}] \pm 95 \% confidence limits; ● = [Zn_{D}]; ▲ = [ASV-labile Zn]).](image)
6.5 Influence of salinity on water quality and speciation

6.5.1 Behaviour of organic matter

TOC and DOC concentrations were measured in water samples collected from various depths at nine estuarine sites (Figure 6.12). TOC and DOC measurements usually agreed closely (i.e. within an experimental uncertainty of ± 0.4 mg/L), indicating that the organic carbon was in a predominantly “dissolved” form (i.e. < 0.4 µm).

![Figure 6.12: Influence of salinity on organic carbon concentrations in the Pieman River estuary (● = TOC; ♦ = DOC).](image)

From this study, 98% of the variation in DOC could be predicted by variation in the salinity of the estuarine water samples analysed. This indicates that riverine organic carbon is diluted conservatively as seawater mixes with the freshwater in this system. The lack of curvature of the line indicates that flocculation of colloidal organic matter was not significant and dilution is the major regulating factor for organic matter. These field results agree well with earlier laboratory studies performed on “artificially-mixed” estuarine samples (Section 4.9.1). The conservative behaviour observed in Pieman waters also agrees with results reported for DOC in other estuarine studies (Moore et al. 1979; Fox 1983; Mantoura and Woodward 1983; van den Berg et al. 1986; Yan et al. 1991).
6.5.2 Zn speciation

During studies of the freshwater environment in this catchment (Chapter 5), “dissolved” Zn concentrations as high as 400 µg/L were measured at some sites in Lake Pieman and up to an order of magnitude higher than this in some tributaries directly receiving mine waste water. Although other metals were also investigated in the estuary, Zn speciation and bioavailability is a primary concern for water quality in this catchment and its estuary and was therefore studied in most detail.

6.5.3 Influence of salinity on total and “dissolved” Zn

The behaviour of total and “dissolved” Zn concentrations in the estuary was investigated at salinities ranging from 0.04 to 30 (Figure 6.13).

![Figure 6.13: Dilution of total and “dissolved” Zn concentrations with seawater in the Pieman River estuary (February 1998) (● = [ZnT]; □ = [ZnD]).](image)

Total and “dissolved” Zn both showed conservative behaviour indicating that flocculation of colloidal Zn is not significant and dilution is the major regulating factor for total and “dissolved” Zn concentrations. These results agree with data reported by Yan (1991) who found no evidence of removal of Zn in estuarine waters of New Jersey.
Extrapolation of the regressed “mixing” line (Figure 6.13) to a salinity of 35 gave a value of 2.1 µg/L for total Zn and 1.6 µg/L for “dissolved” Zn. These concentrations are in general agreement with Zn concentrations measured in Australian coastal waters, which have been reported as 1.5 µg/L (Florence and Batley 1980). They were however up to two orders of magnitude higher than values reported for coastal waters by Apte et al. (1998) and Stumm and Morgan (1996) (see Table 6.6).

In the Pieman River, total Zn was predominantly “dissolved” indicating the absence of suspended particulate Zn species. Total and “dissolved” Zn concentrations remained relatively constant in surface waters where effects of dilution by seawater were minimal (Section 6.4.2).

6.5.4 Influence of low to mid salinity on Zn speciation
As salinity increased from 0.04 to 17 the proportion of “dissolved” Zn which was ASV-labile increased from ~ 40 - 50 % to 80 % (i.e. bound Zn decreased from 50 - 60 % to 20 %) (Figure 6.14). These results are for samples collected from surface waters to the halocline. If Zn speciation was influenced only by dilution, for example dilution of both Zn ions and of natural organic ligands by seawater, the proportion of labile Zn would be expected to remain relatively constant and the data in Figure 6. 14 would form a horizontal line (i.e. slope = 0).

The results from this field study suggest that another process is influencing speciation in this region of the water column. The proportion of labile Zn increases as salinity increases. Regression analysis of the line in Figure 6.14 showed that the 95 % confidence interval for the slope did not include zero (p < 0.001). Results show that the complexation of Zn ions is reduced as ionic strength increases. This effect may be due to competition for binding sites by major ions in seawater such as Ca$^{2+}$ and Mg$^{2+}$ ions (Millward 1995).
**Figure 6.14:** Influence of salinity (0.04 to 17) on Zn speciation (Error bars represent 95% confidence limits).

### 6.5.5 Influence of high salinity on Zn speciation

Zn speciation was also investigated in the deeper saline waters at sites E1 to E4 in the lower estuary. Figure 6.15 shows data given in Figure 6.14 but also includes data from the four deep sites sampled.

**Figure 6.15:** Influence of salinity (0.04 - 30) on Zn speciation ($\bullet$ = surface to mid salinity water; ■ = high salinity bottom water; error bars represent 95 % confidence limits).
If dilution and competitive complexation were the only influences on Zn complexation, the line in Figure 6.15 would continue to increase linearly with an increase in salinity. Results therefore suggest that one or more additional processes influence Zn speciation at deep-water sites. Little change in salinity (1 unit) occurs between sites as the bottom water consists predominantly of seawater. Salinity change is therefore unlikely to cause this effect.

Table 6.4: Water quality measured in bottom waters at sites E1 - E4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Site</th>
<th>Site</th>
<th>Site</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance upstream from mouth (km)</td>
<td>E1</td>
<td>E2</td>
<td>E3</td>
<td>E4</td>
</tr>
<tr>
<td>Salinity</td>
<td>28.3</td>
<td>28.8</td>
<td>29.3</td>
<td>28.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
<td>7.8</td>
<td>7.7</td>
<td>8.1</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>4.0</td>
<td>3.8</td>
<td>4.1</td>
<td>6.1</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>2.6</td>
<td>2.4</td>
<td>2.2</td>
<td>2.0</td>
</tr>
<tr>
<td>[Zn]$^2+$ (µg/L)</td>
<td>16.3</td>
<td>10.6</td>
<td>11.6</td>
<td>9.7</td>
</tr>
<tr>
<td>[Zn]$^{3+}$ (µg/L)</td>
<td>15.9</td>
<td>7.9</td>
<td>11.6</td>
<td>8.3</td>
</tr>
<tr>
<td>ASV-labile Zn/Zn$_T$ (%)</td>
<td>33</td>
<td>65</td>
<td>59</td>
<td>81</td>
</tr>
</tbody>
</table>

The proportion of labile Zn decreased systematically with the residence time or age of the bottom salt water as it moved in an upstream direction along the bottom of the estuary (Table 6.4). The concentration of both total and “dissolved” Zn at site E1 is nearly twice that at site E4. A small increase in TOC was also detected in the older salt water suggesting that organic complexation may be an effective controlling mechanism of Zn speciation in these waters. Natural ligands may be released from the sediment by de-flocculaton or dissolution as a result of turbulence, bioturbation or microbiological activity from interstitial waters, which are known to contain high concentration of complexing ligands (van den Berg et al. 1986). It is also possible then that Zn may be added to bottom waters as tightly bound organic complexes, reducing the proportion of labile metal.

Non-labile (i.e. bound) Zn concentrations were determined for estuary waters (Sites E1 to E4) by difference between the “dissolved” Zn concentration and the ASV-labile concentration (Figure 6.16). Bound Zn concentrations in surface waters of the Pieman River estuary decreased from 26 µg/L (399 nM) in surface waters to 5 µg/L (72 nM) at
the halocline. In deep, saline waters, bound-Zn ranged from 1 µg/L (16 nM) in the newer seawater to 11 µg/L (161 nM) in the older seawater.

Bound and labile Zn both behave non-conservatively in this estuary. The curve in Figure 6.16 demonstrates removal or reduction in bound-Zn in the low to mid-salinity range with inputs in the high salinity bottom waters.

**Figure 6.16:** Influence of ionic strength on Zn speciation measured in the Pieman River estuary (■ = [$Zn_T$]; ● = bound-Zn; ▲ = labile-Zn).

The data in Figure 6.16 are represented with respect to ionic strength rather than salinity to allow easier comparison with the predicted model data in Figure 6.17. The ionic strength of seawater (i.e. 0.64) was calculated by WHAM from the ionic composition values for seawater obtained from Stumm and Morgan (1996).

The behaviour of Zn speciation measured in the Pieman River estuary (Figure 6.16) agreed closely with the predicted behaviour estimated by WHAM in model river water / seawater mixtures (Figure 6.17). Computer simulation by WHAM in model river water / seawater mixtures was performed as described in Section 4.11, except with the removal of Cu and addition of Zn into the model (i.e. [$Zn_D$] 765 nM; 50 µg/L). [$Zn_D$], [FA and major ions concentrations were altered conservatively. In contrast to [$Zn_D$] predicted Zn species behaved non-conservatively. FA-bound Zn was predicted as greater than half
the total Zn concentration in the lower salinity water but this fraction was significantly reduced where the river water was diluted by ~ 50 % with seawater. FA-bound Zn was negligible in high salinity waters where total Zn inputs from river water are also expected to be low.

Figure 6.17: Predicted behaviour Of Zn during simulated mixing of model river water and seawater solutions (■ = [Zn\textsubscript{T}] diluted conservatively; ● = bound-Zn; ▲ = labile-Zn).

Zn concentrations up to 60 \( \mu \text{g/L} \) were measured in some estuarine surface samples. A significant proportion of the total Zn was not measurable by ASV and is therefore not expected to be bioavailable. ASV-labile Zn concentrations up to 28 \( \mu \text{g/L} \) were measured however, which lies midway within the current water quality criteria ([Zn] < 5.0 - 50.0 \( \mu \text{g/L} \) provided Fe not present as Fe(II); ANZECC 1992).

Variation in Zn speciation can essentially be explained in terms of complexation and adsorption reactions (van den Berg and Dharmvanij 1984). Processes that appear to be controlling Zn speciation in the Pieman River estuary are depicted schematically in Figure 6.18 and are discussed below:

- In addition to organic complexation, trace metals such as Zn may interact with the Fe and Mn oxides and co-precipitate.
• Near the sediment-water interface and within the sediments Fe and Mn oxides may undergo reduction and dissolution if anoxic conditions occur. High DGT-labile Mn concentrations were detected at deep sites indicating elevated levels of Mn$^{2+}$. The non-conservative behaviour demonstrated by Mn$^{2+}$ (Figure 6.20) suggests that Mn flocculation and settling from surface waters and resuspension from sediments provided significant inputs to Mn concentrations in deep waters. Kinetics of Zn adsorption on MnO$_2$ have been investigated by van den Berg and Dharmavanij (1984). Equilibration time for adsorption of Zn on MnO$_2$ was estimated as ~ 5 hours in seawater of salinity 8.7 and ~ 8 hours in seawater of salinity 31.8 (van den Berg and Dharmvanij 1984).

• Ca$^{2+}$ and Mg$^{2+}$ probably occupy adsorption sites of MnO$_2$ in seawater so cation exchange must take place (van den Berg and Dharmvanij 1984). Thus, the observed increase in the bound Zn concentration with increased residence time of the underlying seawater layer in the Pieman River estuary may be a function of adsorption rate which is controlled by the rate of displacement of competing cations as well as addition of complexed Zn from the sediments.

Figure 6.18: Model of processes influencing Zn speciation in the Pieman River estuary.
6.5.6 Behaviour of other meta’s in the estuary

Although Zn speciation and bioavailability is a primary concern for water quality in this catchment and its estuary, other metal concentrations and their behaviour were also investigated in bottom waters. Results of measurements performed in bottom waters of the four lower estuarine sites are presented in Table 6.5.

Table 6.5: Metal concentrations (µg/L) measured in bottom waters

<table>
<thead>
<tr>
<th>Metal</th>
<th>Fraction</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E1</td>
</tr>
<tr>
<td>Cu</td>
<td>Total</td>
<td>1.6</td>
</tr>
<tr>
<td>Cu</td>
<td>DGT</td>
<td>0.5</td>
</tr>
<tr>
<td>Mn</td>
<td>DGT</td>
<td>na</td>
</tr>
<tr>
<td>Fe</td>
<td>DGT</td>
<td>na</td>
</tr>
<tr>
<td>Pb</td>
<td>Total</td>
<td>0.7</td>
</tr>
<tr>
<td>Cd</td>
<td>Total</td>
<td>na</td>
</tr>
<tr>
<td>Cd</td>
<td>DGT</td>
<td>na</td>
</tr>
</tbody>
</table>

(salinity > 20; 26 Feb 1998; na = not available).

In order to investigate the fate of metals in this estuary, metal concentrations recorded in bottom waters have been compared with riverine inputs, with non-polluted oceanic metal concentrations and with current water quality criteria (Table 6.6).
Table 6.6: A comparison of metal concentrations measured in lower Pieman River estuary sites (E1 - E4) with current water quality criteria and metal concentrations determined in Australian coastal and ocean waters. Total metal concentrations are given unless otherwise indicated.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Number of sites sampled</th>
<th>Measured range in surface waters (µg/L)</th>
<th>Measured range in bottom waters (µg/L)</th>
<th>Water quality criteria (µg/L)</th>
<th>Measured range in marine waters (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>4</td>
<td>42 – 54</td>
<td>10 – 16</td>
<td>&lt; 0.022</td>
<td>0.004 – 0.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(a)</td>
<td>(b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.031</td>
<td>(a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.025 – 0.089</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.280</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(a)</td>
<td>(c)</td>
</tr>
<tr>
<td>Cu</td>
<td>4</td>
<td>1.7 – 3.4</td>
<td>0.8 – 3.5</td>
<td>2.0 – 5.0</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(a)</td>
<td>(c)</td>
</tr>
<tr>
<td>Pb</td>
<td>3</td>
<td>0.9 – 1.4</td>
<td>&lt; 0.5 – 0.7</td>
<td>1.0 – 5.0</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(a)</td>
<td>(c)</td>
</tr>
<tr>
<td>Cd</td>
<td>2</td>
<td>0.12 – 0.22</td>
<td>&lt; 0.3</td>
<td>0.2 – 2.0</td>
<td>0.003 – 0.028</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.016 – 0.220</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(b)</td>
<td>(c)</td>
</tr>
<tr>
<td>Fe</td>
<td>2</td>
<td>*15.7 – 30.6</td>
<td>*6.4 – 19.3</td>
<td>1000</td>
<td>0.003 – 0.028</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.016 – 0.220</td>
</tr>
<tr>
<td>Mn</td>
<td>2</td>
<td>*34.4 – 43.2</td>
<td>*61.7 – 78.0</td>
<td>NSC</td>
<td>0.003 – 0.028</td>
</tr>
</tbody>
</table>

* DGT-labile concentration
a: Total metals in New South Wales coastal waters (Apte et al 1998)
b: Dissolved metals in Pacific Ocean surface waters (Stumm and Morgan 1996, p 618)
c: Total metals in the Pacific Ocean near Sydney (Florence and Batley 1980)
1: Provided iron not present as Fe(II)
2: Depends upon hardness of water
NSC: No set criteria (ANZECC 1992)
#: ANZECC (1992)
a) Total, “dissolved” and DGT-labile Cu

Total and “dissolved” Cu concentrations measured in surface waters at sites El to E4 ranged between 1.7 and 3.4 µg/L (Table 6.6). Bottom water concentrations did not differ significantly from surface water concentrations and ranged between 0.8 and 3.5 µg/L. Concentrations in bottom waters were 1 to 2 orders of magnitude higher than concentrations measured in Australian coastal waters (Table 6.6) suggesting that the Cu concentration of the incoming salt water is increased substantially by river water inputs.

Cu does not appear to mimic the behaviour of Zn and showed no obvious increase in concentration with increasing residence time of the bottom waters (Table 6.5).

Total Cu concentrations were generally < 5.0 µg/L in both surface and bottom waters which complies with the upper limit of the current water quality criteria (ANZECC 1992; [CuT] < 2.0 - 5.0 µg/L depending on hardness). Concentrations up to 7.4 µg/L were measured however around the mid-halocline region of the water column at one site (E3). Speciation measurements showed that a significant proportion of the total and “dissolved” Cu was non-available and may therefore be non-bioavailable. Relative concentrations of total, “dissolved” and labile Cu measured at site E4 for example are shown in Figure 6.19.

Figure 6.19: Cu speciation at E4 (■ = total Cu; ▲ = dissolved Cu; ♦ = DGT-labile Cu).
b) **Total and “dissolved” Pb**

Total and “dissolved” Pb was measured at various depths at three of the lower estuary sites (El, E2 & E3). Measurements at these sites showed that Pb concentrations in bottom waters (< 0.5 - 0.7 µg/L) below the halocline were less than those measured in surface waters (0.9 - 1.4 µg/L).

Pb concentrations measured in Australian coastal waters have been reported as 0.009 µg/L (Apte et al. 1998) and 0.15 µg/L (Florence and Batley 1980) (Table 6.6). Pb concentrations in bottom waters of the Pieman River estuary were equivalent to those reported by Florence and Batley (1980) but were an order of magnitude higher than those reported by Apte et al. (1998). This large range means it is not possible to determine whether bottom waters are significantly enriched in lead by river water inputs. Because of the low total and “dissolved” Pb concentrations, speciation measurements were not performed.

Total Pb concentrations were < 5.0 µg/L in both surface and bottom waters which complies with the upper limit of the current water quality criteria (Table 6.6). Concentrations of up to 1.4 µg/L were measured in some surface waters therefore exceeding the lower limit of the water quality criterion range for Pb.

C) **Total and “dissolved” Cd**

Cd was found to be present in very low concentrations, with total and “dissolved” concentrations being typically < 0.5 µg/L in surface and bottom waters. DGT-labile Cd concentrations were typically < 0.1 µg/L at the sites investigated and have greater reliability because of the pre-concentration of Cd inherent in this technique.

These results mean it is not possible to determine whether the Cd composition of the incoming salt water is altered significantly by river water inputs as quantitation was restricted by the analytical detection limit.

At present however, Cd does not appear to pose an environmental threat at the sites investigated in this estuary based on current water quality criteria (Table 6.6).
d) **DGT-labile Fe**

DGT-labile Fe was investigated at two estuarine sites (E2 & E3). Fe measured by DGT (Table 6.5 & 6.6) represented a relatively small proportion of the total Fe measured in the surface water (380 to 490 µg/L). Salinity was constant from 0 to 6 m indicating a well mixed water column. If the surface water concentrations of total and “dissolved” Fe are assumed to be representative of the freshwater layer, a maximum of 7 % of total Fe and 12 % of “dissolved” Fe was measurable by DGT at 4m depth at both sites. Similar low concentrations were also detected within the region of the halocline and in bottom waters.

Oxidation of Fe, in the presence of oxygen and appropriate micro-organisms occurs rapidly. Under oxidising conditions, the dominant oxidation state is Fe (III) which has very low solubility in river water (Teasdale 1996). Given the well-oxygenated condition of the Pieman River at the time of sampling, hydrated Fe(II) ions would not be expected to exist at measurable levels. The difference between the “dissolved” Fe and the DGT-labile fraction observed in the Pieman River estuary is probably due to the presence of a colloidal, inorganic fraction (i.e. oxyhydroxide material) or organically complexed Fe (van den Berg *et al*. 1986).

DGT-labile Fe concentrations measured in bottom waters were found to be at least 3 orders of magnitude higher than reported values for Australian coastal oceanic waters (Table 6.6). These results show that the expected chemical composition of the incoming salt water is altered substantially with respect to Fe, by accumulation from river water inputs.

* e) **DGT-labile Mn**

DGT-labile Mn concentrations measured in surface waters at sites E2 and E3 ranged from 34.4 to 43.2 µg/L (Table 6.6). At deeper sites concentrations ranged from 61.7 to 78.0 µg/L showing significant increases in bottom water concentrations.

At both sites, labile Mn concentrations remained relatively constant at salinities < 15 at a depth coinciding with the mid-halocline / mid-oxycline regions (Figure 6.2 & 6.3). DGT-labile Mn concentration increased as salinity increased to ~ 20. Non-conservative
behaviour was clearly demonstrated at deeper sites (salinity $\approx 29$) showing that significant inputs of DGT-labile Mn occurred where the water column was in close proximity to sediments (Figure 6.20).

![Graph showing Mn concentration vs. salinity]

**Figure 6.20:** DGT-labile Mn measured at E2 and E3 (25 - 27 Feb 1998).

Concentrations in bottom waters were 2 to 3 orders of magnitude higher than concentrations measured in Australian coastal waters (Table 6.6) suggesting that the expected chemical composition of the incoming salt water is altered substantially with respect to Mn, by river water inputs.

With the exception of the Que River, DGT-labile Mn concentrations measured in fresh Pieman River waters constituted nearly all the “dissolved” fraction. In surface estuarine samples however, the DGT-labile fraction was significantly less than the “dissolved” Mn. This suggests that Mn oxyhydroxide formation is occurring as river water is mixed with seawater. All Mn in estuarine surface waters was filterable through 0.4 µm membranes. The oxyhydroxides formed may therefore be of a colloidal nature (i.e. filtrable through 0.4 µm membranes) rather than macroscopic particles.

Within oxic waters Mn$^{2+}$ is oxidised relatively slowly to MnO$_2$($s$) according to Eqn. (14) (Johnson *et al.* 1991; Stumm and Morgan 1996)
\[
2\text{H}_2\text{O} + \text{Mn}^{2+}{}_{(\text{aq})} \rightleftharpoons \text{MnO}_2{}_{(s)} + 4\text{H}^+ + 2\text{e}^- \quad (14)
\]

As particulate MnO_2 settles under gravity it is reduced back to Mn^{2+}{}_{(\text{aq})} if anoxic conditions occur.

During this study, the entire water column still contained dissolved oxygen. Therefore a significant proportion of the total Mn might be expected to be in the oxidised form, settling within the water column and concentrating near the bottom. Studies have shown that under aerobic conditions, at pH values > ~ 5.5 Mn oxides accumulate in sediments. If buried sediments become anoxic, particulate Mn will be reduced to soluble Mn^{2+} ions and some will persist as organic complexes (Lu et al. 1997). The Mn^{2+} that diffuses from the sediments may persist long enough to be measured by DGT.

**6.6 Conclusion**

In a salt wedge estuary, seawater is pushed over the sandbar that restricts the river flow at its mouth, and is gradually pushed upstream with each incoming tide. Turbulent mixing at the salt wedge-river water interface changes the salinity and thus changes the chemical composition of both the marine and fresh water environments. In addition to changes caused by variation in ionic composition and dilution, natural ligands and reduced forms of metals (i.e. Fe^{2+}, Mn^{2+} in sediments and interstitial water may be remobilised by turbulent water movement and bioturbation, by bacterial processes or by a change in the oxidation potential (van den Berg and Dharmvanij 1984).

In the Pieman River estuary, the salt-water wedge may extend up to 30 km upstream. The salinity of the surface water layer depends on the extent of incursion of the salt-water layer and the degree of mixing. The extent of salt-water incursion is largely controlled by the volume of water released from Reece Dam, the size of the ocean tides and the relative proportions of inputs from other tributaries feeding the estuary. In addition to these factors, the degree of mixing of the river water with seawater also relies on weather conditions (i.e. wind and wave action).

Despite this however, a layer of river water, fundamentally similar in chemical composition to inputs from Reece Dam, persisted for the entire length of the estuary.
during this study and was discharged into the Southern Ocean. This layer of constant
temperature, constant DO water was at least 2 m in depth with a salinity range of only
0.04 to 2.6. Significant changes in chemical composition were not seen in this surface
layer. Little variation with salinity (up to 2.6) was observed in organic carbon
concentrations, metal concentrations (Fe, Mn, Zn, Cu, Pb, Cd), Zn speciation or $C_L$ for
Cu in surface waters examined over almost the entire length of the estuary.

In deeper bottom waters several processes appear to influence the behaviour of trace
metals. Riverine TOC and DOC were found to behave conservatively in the Pieman
River estuary - concentrations measured at nine sites and various depths produced a
linear relationship with salinity. TOC concentrations were equivalent to DOC indicating
that almost all the organic carbon was “dissolved” (i.e. < 0.4 µm).

Total and “dissolved” Zn also behaved conservatively as river water was diluted with
seawater. “Dissolved” Zn concentrations were equal to total Zn concentrations
indicating that Zn was not associated with particulate material. “Dissolved” Zn
speciation was however, found to behave non-conservatively and appeared to be
influenced by dilution, competitive complexation by other cations (e.g. Ca$^{2+}$ and Mg$^{2+}$
and at deep sites by the residence time of the water. In addition to direct organic
complexation, Zn speciation may be also be associated with adsorption by flocculated
or resuspended colloidal MnO$_2$ and with organic ligands released from weak Mn(II)-
humate complexes. Field measurements of bound and labile Zn agreed closely with
those predicted by WHAM for model river water / seawater mixtures.
Summary and Conclusions

7.1 Key factors controlling trace metal speciation

Complexation of Cu ions generally accounted for over 80% of the total Cu concentration in Lake Pieman waters and was found to be associated predominantly with “dissolved” organic material. Inorganic binding of Cu ions by Fe or Mn oxyhydroxide colloids was negligible in Lake Pieman water. A significant correlation was found between $C_L$ and TOC for nine fresh water sites distributed across the catchment however this relationship was not observed in another temporal study at one lake site. These results suggest that the nature of organic matter as well as its total concentration is important in determining the extent of Cu complexation.

Laboratory studies of river water samples showed that Cu ion complexation was highly dependent on pH but independent of water temperature within environmentally relevant ranges. In low pH waters the proportion of labile (and so potentially bioavailable) Cu increases. As pH has a significant influence on Cu speciation, development of mechanisms to control and reduce AMD must be treated as a key environmental management issue for remediation of closed mining sites, decommissioning of current mines and planning of future mining activities.

In estuarine water samples, $C_L$ for Cu behaved non-conservatively. Laboratory studies showed that Cu ion complexation was highly dependant on salinity but was independent of ionic strength. The non-conservative behaviour demonstrated by Cu ion complexation appears to be a function of competitive complexation by major ions in seawater (i.e. $Ca^{2+}$ and $Mg^{2+}$).

In both fresh and estuarine Pieman River waters, Zn concentrations usually exceeded the potential complexation capacity and were often well above current water quality criteria even with respect to the labile fraction. Although total and “dissolved” Zn
fractions were diluted conservatively as river water mixed with seawater, labile Zn behaved non-conservatively. Zn speciation in the underlying, more saline water was dependent on the residence time of the seawater. Thus, in addition to direct organic complexation, Zn speciation may be also be associated with adsorption by flocculated or resuspended colloidal Mn and/or Fe oxyhydroxides and with organic ligands released from weak Mn(II) or Fe(II)-humate complexes.

A significant proportion of the total Mn concentration measured in freshwaters was measurable by DGT. DGT-labile Fe was also measurable (> 50% of the “dissolved” Fe in the Ring River; Figure 5.17). In well-oxygenated waters, these metals are expected to be oxidised (and therefore not measurable by DGT). The association of Mn$^{2+}$ and Fe$^{2+}$ ions with natural organic matter in this river appears to be significant for their speciation and mobility by reducing oxidation rates.

In the Pieman River estuary, the chemical composition of the surface water layer was essentially the same as at Reece Dam. This layer of constant temperature and constant DO water was at least 2 m deep during this study (February 1998). Both “dissolved” Zn and Cu concentrations were detected at levels above or within the current guideline range (ANZECC 1992) and therefore may be considered deleterious to the ecosystem.

The extent of salt water incursion in this estuary is a function of discharge from Reece Dam, strength of ocean tides and localised mixing created by in-flowing tributaries. The volume of water discharged from Reece Dam therefore has the potential to impact on the ecosystem by altering the relative proportions of seawater to river water.

A high discharge volume from Reece Dam will increase the relative proportion of river water to seawater thus decreasing dilution of trace metals that are present in high concentrations in river water (e.g. Zn and Cu).

Alternatively, a significant reduction in discharge may induce anoxia if river flow ceases or is very low. Under these circumstances, reduction of metals accumulated in the sediments from a century of mining activity may result in elevated concentrations of metal ions in the water column.
7.2 Metal speciation: compliance with relevant guidelines

During studies of the freshwater environment in this catchment, total Zn concentrations as high as 400 µg/L were measured at some sites in Lake Pieman and up to an order of magnitude higher than this in some tributaries directly receiving mine waste water. Concentrations of Cu were also high (e.g. [Cu_T] = 60.4 µg/L in the Que River). Cu is known to be highly toxic to many aquatic organisms. Although other metals have also been investigated throughout this thesis, Zn and Cu were identified as the current primary concerns for water quality in this catchment. Zn and Cu speciation and their compliance with water quality guidelines is summarised below.

7.2.1 Status of Zn speciation

In the three tributaries sampled where Zn speciation measurements were possible, ASV-labile Zn concentrations were equal to the total and “dissolved” Zn concentrations suggesting that all Zn was bioavailable at the time of sampling (Table 7.1).

In the lake environment (PBH), approximately half of the total Zn concentration was present as bound species (Table 7.1). Total and “dissolved” Zn concentrations exceeded 300 µg/L at some depths at this site and so even the labile fraction was still well in excess of the current water quality criteria for protection of aquatic ecosystems. At PRD, total Zn concentrations were found to be 42 - 58 µg/L throughout the water column. This concentration was also measured in surface waters of the estuary where the labile Zn constituted between 31 % and 65 % of the total Zn concentration.

Table 7.1: Successive downstream variation in Zn speciation (20 - 26 Feb 1998).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Freshwater</th>
<th>Estuary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rivers*</td>
<td>PBH</td>
</tr>
<tr>
<td>[Zn_T] µg/L</td>
<td>360-1200</td>
<td>&lt;30 - 330</td>
</tr>
<tr>
<td>[Zn_b]/[Zn_T] %</td>
<td>97 - 99</td>
<td>~ 100</td>
</tr>
<tr>
<td>[labile]/[Zn_T] %</td>
<td>94 - 100</td>
<td>32 - 73</td>
</tr>
</tbody>
</table>

*based on measurements in Que, Ring and StiU Rivers; na = not analysed

Based on current water quality guidelines ([Zn_T] = 5.0 -50.0 µg/L provided Fe not present as Fe (II); ANZECC 1992), Zn poses a significant ecological threat in the three tributary sites investigated. The lake sites and estuary surface waters sometimes exceed
the upper limit of the guidelines however labile concentrations are usually within the guideline range.

7.2.2 Status of Cu speciation

In all four tributary sites and PBH, total Cu concentrations exceeded the lower limit of the current specified water quality criteria range for the protection of ecosystems ([CuT] = 2.0 - 5.0 µg/L; ANZECC 1992; Table 7.2). In the Que ([CuT] = 60.4 µg/L), Savage ([CuT] = 52.4 µg/L) and Stitt ([CuT] = 4.08 µg/L) Rivers, over 80% of “dissolved” Cu was found to be strongly complexed and therefore is not considered to be bioavailable (Table 7.2). Similar results were found at PBH where > 90% of total Cu ([CuT] = 5 µg/L) was strongly complexed. Labile concentrations were therefore within or below the specified guideline range (ANZECC 1992) in these samples. In the Ring River samples, all Cu was found to be labile ([CuT] = 47 µg/L), easily exceeding current water quality criteria for protection of aquatic ecosystems.

At PRD, total Cu concentrations were found to be 1.5 to 2.0 µg/L throughout the water column (Table 7.1). $C_L$ for Cu was estimated as 53 - 66 µg Cu/L at this site. Similar total Cu concentrations were also measured in estuarine waters and $C_L$ for Cu was estimated as 34 - 46 µg Cu/L.

Table 7.2: Successive downstream variation in Cu speciation (20 - 26 Feb 1998).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Freshwater</th>
<th>Estuary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rivers*</td>
<td>PBH</td>
</tr>
<tr>
<td>[CuT] µg/L</td>
<td>4.0 - 60.4</td>
<td>0.6 - 5.0</td>
</tr>
<tr>
<td>[labile] / [CuT]</td>
<td>5 - 96</td>
<td>0 - 20</td>
</tr>
<tr>
<td>$C_L$ (µg Cu/L)</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

* based on measurements in Que, Ring, Still and Savage Rivers; na = not analysed

The Australian water quality guidelines are currently being reviewed but the new draft guidelines (ANZECC and ARMCANZ 1999) have not yet been endorsed. The new guidelines recognise that site-specific metal speciation information is necessary to more accurately determine trigger levels for management action that will provide appropriate protection for aquatic environments (ANZECC and ARMCANZ 1999). The proposed guidelines incorporate a hierarchical decision tree for assessing toxicants in ambient...
waters (Figure 3.4.2 in ANZECC and ARMCANZ 1999; see Appendix 1). Two examples of the application of the decision tree for assessment of Cu in the Ring and Savage Rivers, based on speciation measurements performed in this study are demonstrated below.

a) **Application of the hierarchical decision tree for assessment of Cu in the Ring River.**

- A sample of unfiltered water collected from the Ring River (24 Feb 1998; pH = 4.91; conductivity = 97 µS/cm; TOC = 5.3 mg/L; see Table 5.1) was acidified (pH < 2) at room temperature and analysed for total Cu by GFAAS. Water hardness was measured as 5.5 mg/L in this tributary. Bioavailability of Cu is known to be influenced by hardness. The measured hardness was used to calculate a hardness-modified guideline value for Cu using the algorithm given in Appendix 2. The measured total (acid-soluble) Cu concentration of 46.6 µg/L exceeded the hardness-modified guideline value (GV 0.08 µg/L).

- A sub-sample of the original water (unacidified) was filtered through a 0.4 µm membrane filter and then acidified (pH < 2) at room temperature. The “dissolved” Cu was analysed and found to be 44.5 µg/L, which still exceeds the guideline value (GV = 0.08 µg/L).

- Following the decision tree (Appendix 1) metal speciation is considered. Anodic stripping voltammetry (ASV) measurements of the water sample (unacidified) revealed a labile Cu concentration of 42.7 µg/L. This fraction includes inorganic Cu species and weakly-bound organic complexes. Speciation modelling by WHAM supported this estimate of labile Cu. For WHAM speciation calculations, the ionic concentrations given in Table 5.1 were used. The concentration of labile Cu still exceeds the guideline value.

- Based on the decision tree, the labile Cu concentration measured in this tributary presents a high risk to the aquatic ecosystem as all Cu is essentially bioavailable.
The hierarchical decision tree approach allows for well established background concentrations to be adopted as the site specific guideline value. This data does not exist for the Ring and Savage Rivers however Cu concentrations have been found to be as low as < 0.5 µg/L in Lake Murchison (Koehnken 1992). This quantitation of Cu has been limited by the analytical detection limit and so it is not appropriate to adopt this value as the true background concentration.

b) Application of the hierarchical decision tree for assessment of Cu in the Savage River.

- A sample of unfiltered water collected from the Savage River (24 Feb 1998; pH = 7.31; conductivity = 444 µS/cm; TOC = 7.3 mg/L; see Table 5.1) was acidified (pH < 2) at room temperature and analysed for total Cu by GFAAS. Water hardness was measured as 26.1 mg/L in this tributary. Bioavailability of Cu is known to be influenced by hardness. The measured hardness was used to calculate a hardness-modified guideline value for Cu using the algorithm given in Appendix 2. The measured total (acid-soluble) Cu concentration of 52.4 µg/L exceeded the hardness-modified guideline value (GV = 0.29 µg/L).

- A sub-sample of the original water (unacidified) was filtered through a 0.4 µm membrane filter and then acidified (pH < 2) at room temperature. The “dissolved” Cu was analysed and found to be 24.9 µg/L, which still exceeds the guideline value (GV = 0.29 µg/L).

- Following the decision tree (Appendix 1) metal speciation is considered. ASV measurements of the water sample (unacidified) revealed a labile Cu concentration of 2.9 µg/L. This fraction includes inorganic Cu species and weakly-bound organic complexes. The concentration of ASV-labile Cu still exceeds the guideline value.

- In summary, 5.5 % of the total Cu concentration measured in this water sample consisted of labile species. The concentration of the labile Cu exceeded the hardness-modified guideline. Based on the decision tree, the labile Cu concentration measured in this tributary still presents a high risk to the aquatic ecology. Direct
toxicity testing is recommended to confirm this and to determine whether synergy is likely where a mixture of metals is present.

We are a long way from a clear link between speciation and bioavailability for all classes of organisms to be found in such a system. Caution must therefore be applied when setting guideline values based on only one or a few specific organisms. The response of these organisms may or may not be relevant to the Pieman River system particularly if synergistic effects have not been considered.

7.3 Speciation techniques: ASV and DGT

This work has investigated metal speciation in several freshwater environments of the Pieman River catchment and compared the use of ASV and DGT for measurement of labile metal concentrations in river waters.

DGT is a useful addition to the current ensemble of speciation tools already available to environmental managers. Although the application of DGT is more complicated and time-consuming than that required for the collection of discrete samples for analysis by ASV, DGT offers some advantages over other techniques in situations where in situ speciation measurements are desirable.

This study has found that under steady state conditions and in the absence of strong complexation, there appear to be strong similarities in the masses of metal (ie. Cu, Zn and Cd) determined by DGT and ASV. When strong complexation was occurring however, the DGT measurements were greater than the ASV-labile fraction. This result is consistent with the measurement time scales of the two techniques.

DGT produces a time-averaged in situ speciation measurement, which is particularly useful for monitoring in streams of highly variable water quality, or for long-term ecotoxicity studies. It does not however detect concentration maxima and minima that occur during the measurement period, which are also important for assessing toxicity of aquatic environments. In dynamic systems, the probability of detecting concentration maxima in “spot” samples is also unlikely, unless samples are collected at an appropriately high frequency or if continuous in-line measurements are performed.
While discrete sampling allows more detailed speciation measurements (e.g. the particulate / “dissolved” / labile distinction), a true average concentration is not obtained. The two techniques are thus complementary to each other, each providing different speciation information.

### 7.4 Prediction of speciation by WHAM

Labile Cu concentrations predicted by the WHAM speciation code agreed closely with measurements by ASV in situations where the streams were not under the direct influence of active mining waste. Where streams were receiving input from current mining activities (i.e. runoff containing tailings and flotation agents), predictions for Cu speciation by WHAM were very poor.

Modelling of Cu and Zn speciation in river and seawater mixtures by WHAM strongly supported field observations in estuarine waters. Although the predictive capabilities of this computer program have not been frilly tested, results from this study indicate that such a model may provide a useful predictive tool, particularly when a greater understanding of the characterisation and variability of the FA/HA components of the DOC is achieved.

### 7.5 Recommendations for future research.

The following research would add to the results contained in this thesis and further develop a management protocol for the Pieman River:

- During this study, water at all river and estuarine sites sampled was oxygenated. It is possible that anoxia may develop under low flow conditions. Investigations of the behaviour of trace metals in Pieman sediments under oxic and anoxic conditions would enable environmental managers to better understand the potential of these sediments to act as a source of metal ions to the water column.

- Zn toxicity to the green alga *Dunaliella tertiolecta* was detected in Pieman River estuarine waters. Current concentrations of Zn in the estuary therefore have the potential to interfere with phytoplankton ecology. Further toxicity studies using endemic species could test this hypothesis. Toxicity of Cu and other metals could also be investigated using endemic species, as synergism may be important.
• DGT has been shown to be a useful addition to the current suite of speciation tools. Future work to determine the relationship between DGT measurements and relevant toxicity data could test the theory that DGT labile metal concentrations are equal to bioavailable metal concentrations.

• Initial applications of WHAM have shown that predicted concentrations agreed closely to field and laboratory measurements in cases where the water was not under the direct influence of active mine waste. Future work should be performed to further characterise the FA/HA component of the DOC from the catchment in order to customise the model for Pieman River waters and further investigate its predictive potential for west Tasmanian waters.

• This work has demonstrated that metal speciation is extremely variable within the catchment. It is highly recommended that speciation measurements be incorporated into routine monitoring programs, ideally combining several speciation techniques including some in situ measurements or in-line analysis.


Hoxey, A. M. (1994). The isolation and characterisation of humic acids using a variety of alkaline extractants, Master of Science, Deakin University, Warrnambool, Victoria, Australia.


**APPENDIX I:**

DECISION TREE FOR ASSESSING TOXICANTS IN AMBIENT WATERS (ANZECC AND ARMCANZ 1999).

Figure 3.4.2 Decision tree for metal speciation guidelines
APPENDIX 2:

RECOMMENDED HARDNESS-DEPENDENT ALGORITHMS DESCRIBING
GUIDELINE VALUES FOR SELECTED METALS IN FRESHWATER

(ANZECC AND ARMCANZ 1999).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Hardness-dependent algorithm^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>GV = exp(0.89(log_e hardness) − 7.37)</td>
</tr>
<tr>
<td>Chromium(III)</td>
<td>GV = exp(0.82(log_e hardness) − 0.59)</td>
</tr>
<tr>
<td>Copper</td>
<td>GV = exp(0.85(log_e hardness) − 4.00)</td>
</tr>
<tr>
<td>Lead</td>
<td>GV = exp(1.27(log_e hardness) − 4.14)</td>
</tr>
<tr>
<td>Nickel</td>
<td>GV = exp(0.85(log_e hardness) − 3.28)</td>
</tr>
<tr>
<td>Zinc</td>
<td>GV = exp(0.85(log_e hardness) − 2.02)</td>
</tr>
</tbody>
</table>

^a GV, guideline value expressed in µg/L; exp, exponential