Diffusive Gradients in Thin-films (DGT)

A Technique for Determining Bioavailable Metal Concentrations

March 2002

Part I

DGT Theory and Application Literature Survey

INAP
International Network for Acid Prevent

Executive Summary

Executive Summary

Trace metals exist in a variety of inorganic and organic forms in aquatic systems, ranging from simple hydrated molecules to large organic complexes. The biological availability, and hence toxicity, of metals in aquatic systems is strongly dependent on the nature of the metal species present. Accordingly, determining the chemical form, or speciation, of metals in the environment is fundamental to predicting impacts to aquatic biota.

The presently accepted Free-Ion Activity Model (FIAM) stipulates that the biological response of organisms to trace metals in waters is proportional to the free-ion activity of the metals and not their total or dissolved concentrations. This model is predicated on laboratory experiments with synthetic ligands and not natural organic matter and should therefore be applied with caution. The model nevertheless properly reflects the fact that the transport of metals across the biological membrane is the rate-limiting step in the overall toxic response — free metal ions (*e.g.,* Cu^{2+}) are readily taken up by aquatic organisms, whereas particulate and strongly complexed metals are not. Complexation of metals in aquatic systems may occur via reaction with soluble inorganic (*e.g.,* F^-, Cl^-, HCO_3^-, SO_4^{2-}, HPO_4^{2-}, *etc*.) and organic (*e.g.,* humic substances) ligands. In most cases, complexation with organic ligands reduces metal bioavailability, because most organic-metal complexes are not readily transported across cell membranes. Inorganic-metal complexes (*e.g.,* carbonates), however, typically dissociate rapidly to the free metal form. Thus, while the bioavailable fraction of metals includes both free metal ions and kinetically–labile metal complexes (*i.e.,* those with rapid dissociation kinetics), the biological response is proportional to the free-metal concentration only.

A variety of metal species have been identified in natural waters, including both inorganic and organic species. The relative proportions of the various species depend on the concentrations of the metals present, the specific characteristics of the water (*i.e.,* ionic strength and pH), and on the concentrations and strengths of the various ligands present. In addition, the metallurgical processing of base metal and gold ores involves the addition of various reagents (which may act as ligands) to enhance the recovery of desirable constituents. As a result, ligands present in mining-related discharges may contrast greatly from those present in natural waters, which in turn could have a profound effect on metal speciation and toxicity in the receiving environment.

Recognizing the limitations of conventional methods for estimating water quality, which are typically restricted to partitioning metals between total and "dissolved" phases, a variety of analytical and test methods have been used to quantify metal

toxicity and speciation in natural waters and effluents. Virtually all of the techniques suffer inadequacies that limit their general use. For example, most existing speciation and toxicity techniques must be carried out in a laboratory, rather than *in situ,* resulting in the potential for changes in metal speciation and therefore toxicity. Further, electrochemical *in situ* speciation techniques require expensive instrumentation that must be used by highly trained operators, making such techniques onerous and non-routine.

DGT represents a relatively new approach for *in situ* determinations of labile metal-species in aquatic systems. The DGT device passively accumulates labile species from solution while deployed *in situ* and therefore contamination problems associated with conventional water collection and filtration procedures are eliminated. Since DGT affords an operationally defined measure of the labile, or "bioavailable" fraction, inferences can be made with respect to metal toxicity.

The theory behind DGT is based on the diffusional characteristics of metals in a hydrogel and on the ion exchange properties of a metal-binding resin. Specifically, the technique utilizes a hydrogel layer to control the diffusive transport of metals in solution to a cation-exchange resin. In addition, since the resin used in DGT (Chelex) is selective for free or weakly complexed species, it provides a proxy for the labile fraction of metals in solution.

DGT utilizes a three-layer system consisting of: 1) a resin-impregnated hydrogel layer; 2) a hydrogel diffusion-layer; and 3) a filter membrane. The innermost two gel layers are fabricated from a polyacrylamide hydrogel. The filter membrane isolates the polyacrylamide surface from particles in the water. Labile metal ions in solution diffuse across the filter and gel layers and are pre-concentrated on the resin. Based on the laws of diffusion and the established characteristics of the diffusive path in the DGT sampler, the concentration of labile metals in solution may be calculated using the measured metal ion inventory on the resin, the sampler exposure time and the temperature-corrected molecular diffusion coefficient for the metal of interest. A qualification of the method is that the calculated labile-metal concentration depends on the diffusion coefficient D adopted for the hydrogel. Ionic strength, pH, and solution composition can influence the rate of diffusion, for example by affecting the behaviour of functional groups on the polyacrylamide, which may become ionized. Although some workers have assumed that diffusion coefficients in the gels are similar to those in water, this overstates the case because tortuosity and permeability effects lower the rate of diffusion. Such effects become more pronounced as the gel becomes tighter. These effects have been compensated for through the direct measurement of diffusion coefficients within the hydrogel.

Waters with very low cation concentrations (<2 x 10^{-4} M) also pose a potential challenge. In such settings, the sodium form of polyacrylamide used in the gel samplers can establish negative concentration gradients of Na^+ from the gel to the sample water. To preserve electro neutrality, the charge imbalance is compensated by steepened concentration gradients of cations in the opposite direction. These enhance the flux of cations to the gel, causing their "labile" concentrations to be overestimated. In most mining-impacted regions, this is not of importance because surface waters typically have higher ionic strengths. But in pristine natural waters with low ionic strength, for example, this co-diffusion effect may be important. The three-layered DGT configuration is held within a plastic holder (4 cm diameter disc). Hydrated DGT samplers are stored refrigerated in sealed plastic bags until immediately before deployment. Deployment typically involves suspension of the device *in situ* or in a stirred solution in the laboratory for a period typically ranging between one and twenty-four hours (depending on metal concentrations in the test solution). Deployment duration and temperature are recorded during deployment. Upon retrieval, the DGT units are rinsed with distilled deionized water and refrigerated in sealed plastic bags to avoid dehydration and potential contamination. The samplers are disassembled and the resin layer analyzed for metal content, by either analysis of an acid extract (*e.g.*, by ICP-MS) or through direct analysis of the resin beads by techniques such as proton induced X-ray emission (PIXE) or laser ablation.

An important consideration with respect to the interpretation of DGT data is that the methodology integrates the concentration of labile metals in solution over the deployment period. Under conditions where variability in water quality is low, DGT data are comparable to more conventional sampling approaches, which are more instantaneous in nature. If, however, an aquatic system hosts some degree of variability (such as is often the case where effluents are discharged into a natural water course), then grab samples for water quality assessment may not be directly comparable to the time-integrated metal signature determined by DGT.

Between any solid and a liquid there is a zone of laminar flow in which the process of molecular diffusion dominates solute transport. This zone (which typically ranges in thickness between 0.1 and 1 mm, depending on the interfacial flow velocity) is referred to as the diffusive boundary layer (DBL). The presence of the DBL between the DGT sampler and the bulk solution serves to increase the diffusive path-length over which ions must travel in order to be fixed by the DGT sampler. The thickness of the DBL under conditions of flow is sufficiently small such that impacts on metal uptake are negligible. However, as flow diminishes, the DBL thickens and multiple samplers of differing gel-layer thicknesses must be deployed in order to derive a DBL correction factor.

A limitation of the DGT technique is the reduced performance of Chelex-resin at both high and low pH ranges. While Chelex is a robust and tolerant resin, it functions best for most appropriate metals in the pH range of 5 to 9. Below pH 5, the adsorptive capacity of the resin diminishes and at pH values greater than 9, the resin is prone to swelling, affecting its physical characteristics. For copper, the resin operates effectively over a broader range of ~2-11.

Since both the mechanism of metal assimilation in aquatic organisms and the mode of metal uptake by DGT are governed by labile metal concentrations in solution, a correlation between DGT metal concentrations and toxicological affects would be expected. The limited DGT-bioassay correlation experiments conducted to date demonstrate clearly that DGT provides a more representative measure of metal bioavailability, and hence toxicity, than conventional water quality parameters (*i.e.,* total and/or dissolved metal concentrations). Specifically, the results indicate that metal uptake by DGT and aquatic biota (*e.g.*, *Daphnia magna* and rainbow trout) is reduced in the presence of metal-complexing ligands. In other words, metal-ligand complexes that are unavailable to aquatic biota are also undetected by DGT.

Studies of complexation kinetics (chemical reaction rates) suggest that DGT affords a powerful tool for assessing the formation rates of metal-ligand complexes. The results also indicate that speciation (and hence toxicity) cannot be predicted from chemical equilibrium considerations alone and that the kinetics of metal-ligand complexation reactions play an important role in metal bioavailability. The use of DGT for assessing the kinetics of formation of metal-ligand complexes has potential applications to mining-related environmental issues. Specifically, at mine sites that discharge water (*e.g.,* tailings pond overflow, treated effluents, *etc*.) to the receiving environment, an understanding of how rapidly-introduced metals interact with natural ligands could aid in predicting spatial and temporal water quality impacts to the receiving environment. This would in turn facilitate development of site-specific discharge limits appropriate to the assimilative capacity of the receiving waters.

Research to date also suggests DGT is an effective method for assessing metal complexation-capacity. Such estimates can be used to constrain better the assimilative capacity of receiving waters, and thus aid in determining site-specific discharge criteria. DGT can be used in this capacity by providing insight into the concentration of metal-ligand binding sites and the strength of metal-ligand complexes.

In summary, DGT provides an *in situ* measure of labile (free and/or kinetically-labile) metal species in solution. DGT can be applied to various aspects of environmental chemistry including metal speciation, metal toxicity/bioavailability, metal-ligand

complexation kinetics and metal complexation-capacity. Given its range of application, ease of use and relatively low cost, DGT has the potential to become a routine water quality monitoring tool in studies of mining-impacted systems. In particular, DGT-inferred values may compliment standard metal measurements (*e.g.*, total and dissolved metals), while affording site-specific information with respect to metal toxicity.

The advantages, limitations and considerations for the application of DGT are summarized in Table 1. In general, the advantages include *in situ* deployment, speciation capabilities, sensitivity, time-integrated signal and low-risk of contamination. In addition, the DGT device can be deployed and retrieved by minimally trained personnel at relatively low cost. The primary limitations of the technique include the limited functional pH range (5 to 9 for most metals), the limited application to certain metals/metalloids, unsuitability in waters of very low cation concentration ($<2 \times 10^{-4}$ M), and uncertainties in the magnitudes of diffusion coefficients used to calculate labile-metal concentrations. In addition, since the link between DGT and metal uptake by aquatic biota is related to the FIAM, DGT is also subject to some of the limitations of the FIAM. For example, the FIAM has limited utility for the prediction of metal toxicity for certain modes of metal uptake by biota, including those for non-polar neutrally-charged species (*e.g.*, $HgCl_2$), siderophore-metal complexes, and low-molecular weight ligands (*e.g.*, citrate). Slow uptake through membranes will not obey the FIAM but may be described more closely by DGT assays.

Although research to date demonstrates that DGT techniques can be applied to various aspects of environmental chemistry, further research is necessary to rigorously defend the precise nature of DGT measurements in varying environments. More work, for example, is required to assess the spectrum of species measured by DGT in various aquatic environments and to determine the rates at which such species will diffuse through the hydrogel under different physical and chemical conditions, including varying thickness of the gel layer. In addition, the relationship between DGT values and toxicity to aquatic biota requires further study. Finally, since mining-related effluents present complex solutions hosting a spectrum of organic and inorganic agents (*e.g.*, CN, xanthates, frothers, *etc.*) which have the potential to complex trace metals, thereby influencing metal bioavailability and toxicity, further work is needed to assess the utility of DGT in systems influenced by mining-related discharges, and to determine how DGT values vary in response to the presence of different ligands.

Table 1:
Summary of the advantages, limitations and considerations associated with the use of DGT in aquatic systems

Advantages	Limitations/Considerations
Speciation: provides an *in situ* measure of labile metal species in solution. Allows inference of metal bioavailability and toxicity.	pH: limited pH range of Chelex resin (pH 5 to 9, but ~2-11 for Cu) Accuracy of assayed concentrations depends on knowledge of diffusion coefficients in hydrogel. These may not be rigourously known. Co diffusion can be a constraint in waters of very low cation concentrations ($<2 \times 10^{-4}$ M)
Sensitivity: pre-concentration of metals allows for ultra trace-level determinations.	Capabilities have not been rigourously defined for some metals/metalloids (*e.g.*, Mo, As, Se)
Contamination: the potential for contamination associated with water collection and filtration is eliminated.	FIAM: subject to some of the limitations of the free-ion activity model.
Analytical Interferences: obviates matrix effects associated with conventional analyses of high ionic-strength solutions.	Metal Species Detected: requires more precise determination of the spectrum of species detected (*e.g.*, kinetically-labile).
Time Integration: provides a time-integrated measure of labile-metal species over desired deployment interval.	Mining-Impacted Systems: more work is required to validate the use of DGT in mining-impacted environments.
Multitude of Applications: • Metal speciation • Toxicity testing • Complexation kinetics of metal-ligand complexes • Complexation Capacity • Development of site-specific discharge criteria and assimilative capacity	DGT vs. toxicity: additional study is required assess the relationship between DGT values and toxicity to aquatic biota.
User Friendly: deployment and retrieval do not require specialized equipment or highly trained personnel.	
Costs: costs are compatible with standard water quality analyses.	

Table of Contents

Table of Contents

LIST OF FIGURES

LIST OF TABLES

1 Introduction

1. Introduction

The technique of diffusive gradients in thin-film (DGT) has been developed over the past ten years by academic researchers in order to provide a method for *in situ* determinations of "labile" metal species in natural waters. Since it is the labile fraction of the total metal inventory that is considered most bioavailable to aquatic organisms, DGT has the potential to become an important tool in toxicological and environmental assessments. The technique is particularly germane to the needs of the mining industry, given the lack of appropriate and routine *in situ* methods for assessing the site-specific toxicity of metal-contaminated waters.

The International Network for Acid Prevention (INAP) commissioned Lorax Environmental Services Ltd. (Lorax) to conduct a two-phase study to establish the current state-of-knowledge of DGT technology (Phase-1) and to conduct further validation work on DGT (Phase-2). More specifically, phase-one of the study, which is the subject of this section, consists of a comprehensive literature survey, which reviews the latest developments in DGT techniques and assesses the applicability of DGT to mining-related environmental studies. To this end, over 250 papers were reviewed and approximately 200 are cited in Part I of this report.

Under the present regulatory framework, water quality criteria for mine sites are typically based on total metal concentrations in the receiving environment and/or laboratory bioassays conducted on mine effluents. The principal weaknesses of these approaches relate to the fact that total metal concentrations are not a good proxy for metal bioavailability (*i.e.*, toxicity) and that the handling, transport and processing of water samples associated with bioassays may result in significant changes in metal speciation, and therefore toxicity. The limitations of these approaches are described in detail in Chapters 2 and 3.

DGT represents a relatively new approach to water sampling, which essentially obviates many of the limitations outlined above. In general, DGT samples labile metal species in aquatic systems and therefore affords *in situ* assays of metal speciation. Accordingly, the technique allows inferences to bioavailability and toxicity. The DGT approach also obviates contamination problems associated with water collection and filtration. To date, DGT has been successfully used to estimate concentrations of labile species of iron, manganese, cadmium, cobalt, copper, nickel, lead and zinc. However, additional validation/verification work is required to advance the DGT sampler as a reliable *in situ* toxicity indicator for routine use by the mining industry.

The inclusion of metal toxicity (Chapter 2) and speciation (Chapter 3) in this review was necessary to define the mechanisms of metal assimilation by aquatic biota, and to relate these processes to metal uptake via DGT. A comprehensive review of the DGT technique is presented in Chapter 4. This includes a detailed description of the theory and design of the DGT water sampler, a discussion of DGT applications, and a summary of the current considerations and limitations. Academic researchers have also developed a DGT pore water sampler that has considerable potential as a tool for assessing the subaqueous reactivity of tailings and contaminated sediments, as well as for providing a measure of sediment toxicity. Although a full assessment of the DGT pore water sampler or "sediment probe" is outside the scope of the present INAP study, the details of its function and application are provided for completeness (Chapter 5).

2 Metal Toxicity

2. Metal Toxicity

2.1 Introduction

Many trace metals are micronutrients and represent essential dietary components of aquatic organisms. Such "nutrient" metals include Fe, Cu, Zn, Ni, Mn, Co, Cd, Mo, Se, Sn, and V (Florence, 1982). In natural marine environments and freshwaters, most trace elements are typically present in trace quantities (<10 nM) and are passively and/or actively assimilated by organisms to satisfy physiological requirements. In metal-contaminated systems, however, metals can accumulate within the cells and tissues of organisms, which could result in effects deleterious to cellular function. Indeed, varying "toxic" responses, ranging from impaired metabolism to death have been observed for most metals, including the micronutrient elements outlined above.

In the following sections, metal toxicity is first described with respect to the nature of metal-induced physiological impairment (Section 2.2). Subsequent to a discussion of the processes governing the metal uptake by cells (Section 2.3), factors which ameliorate metal toxicity are described (Section 2.3). The background outlined in the latter sections forms the foundation for discussion of the Free-Ion Activity Model (FIAM) (Section 2.5). The FIAM is based on the positive relationship between bioavailability (*i.e.*, toxicity) and the concentration of the free metal-ion, and provides the link between toxicity and DGT-derived metal concentrations. The chapter concludes with a brief review of the current methodologies for toxicity testing (Section 2.6).

2.2 Nature of Toxicity

The bioavailability and toxicity of metals to aquatic biota have been examined extensively using a variety of test organisms including phytoplankton (Sunda and Guillard, 1976; Anderson and Morel, 1979; Fisher, 1986; Phinney and Bruland, 1997; Errécalde *et al*., 1998; Sunda and Huntzman, 1992; Errécalde and Campbell, 2000; Franklin *et al.*, 2000), bivales (Zamuda *et al.*, 1985; Tessier *et al*., 1993; Absil *et al*., 1994; Wang and Fisher, 1996), crustaceans (LaPorte *et al*., 1997; Barata *et al*., 1998; Reinfelder *et al*., 1998; Baillieul and Blust, 1999; Carvalho *et al*., 1999; Wang and Fisher, 1999a; Soegianto *et al*., 1999; Rainbow *et al*., 2000), fish (Spry and Winer, 1991; Wilkenson *et al*., 1993; Roy and Campbell, 1995; Hollis *et al.,* 1996; Playle, 1998; Meyer *et al*., 1999) and aquatic insects (Craig *et al.*, 1999; Bervoets and Blust, 1999, 2000). The effects of metal toxicity are variable, but are generally expressed as mortality, decreased growth rate, decreased fecundity and decreased metabolic activity. Metals are toxic because in sufficient concentrations they are able to compete for intracellular sites

normally occupied by functional metabolites, thereby interfering with normal cell functions. Metal coordination sites are rarely entirely specific for a single metal, and therefore surface sites designed to bind nutrient metals will also bind non-essential (and potentially toxic) metals with similar ionic radii and coordination geometry. Cu, Zn and Cd, for example, have been shown to compete for Mn uptake sites (Sunda and Huntzman, 1998). Once inside the cell, competing metals can bind to nutrient-metal coordination sites such as metabolic sites on metalloproteins, resulting in a loss of metabolic function and inhibition of cellular function.

2.3 Uptake Mechanisms

The accumulation of metals in organisms can occur via uptake of metals in food sources and/or exposure to metals in the surrounding medium (Hare and Tessier, 1996; Wang and Fisher, 1999a, b). Sources of food-bound metals may include phytoplankton, detritus, and inorganic particles (*e.g.*, suspended solids and sediments). Indeed, for many aquatic invertebrates, the trophic transfer of metals from prey to predator accounts for a major portion of the total metal accumulation (Reinfelder *et al.*, 1998). In general, the nature of metal assimilation from food particles is complex, being influenced by a number of abiotic (*e.g.*, temperature, pH) and biological (*e.g.*, ingestion rate, gut volume/gut passage time, digestive enzyme activity and metal partitioning) factors. A more detailed description of sediment toxicity is provided in Chapter 5.

Metals may also be assimilated via contact/ingestion of water and sediment pore waters. For example, waterborne metals can be biologically assimilated via binding to the gills of fish, bivalves and crustaceans (*e.g.*, Wilkinson, *et al*., 1993; Hollis *et al.*, 1996; Playle, 1998; Soegianto, *et al.*, 1999), across digestive membranes (Reinfelder *et al.*, 1998) and via direct uptake across the cell membranes of unicellular organisms (Anderson and Morel, 1979).

The regulation of materials into and out of a cell is facilitated by the cell membrane, which simply termed, is composed of phospholipids and large protein molecules. Phospholipid molecules are arranged in a bilayer in which nonpolar (*i.e*., hydrophobic) fatty acids are sandwiched between polar (hydrophilic) phosphate groups. Globular proteins (integral proteins) are dispersed throughout the lipid bilayer, and act as conduits for the transport of materials to the cell interior.

Most metal species are extremely hydrophilic, and as a result, their passage through the hydrophobic lipid membrane is restricted. Dissolved metals are typically incorporated passively into the cells of organisms via specialized pumps, channels and carriers which operate across the membrane surface. Pump and channel metal-transporters comprise

membrane proteins that facilitate metal transport across the cell membrane (Figure 2-1). Metal ions bind to receptor sites on the protein, and are subsequently transported across the membrane and released into the cytoplasm of the cell. Conversely, carriers represent lipid-soluble molecules that bind metals on the cell exterior, diffuse across the membrane and transfer the metal complex to the cell interior.

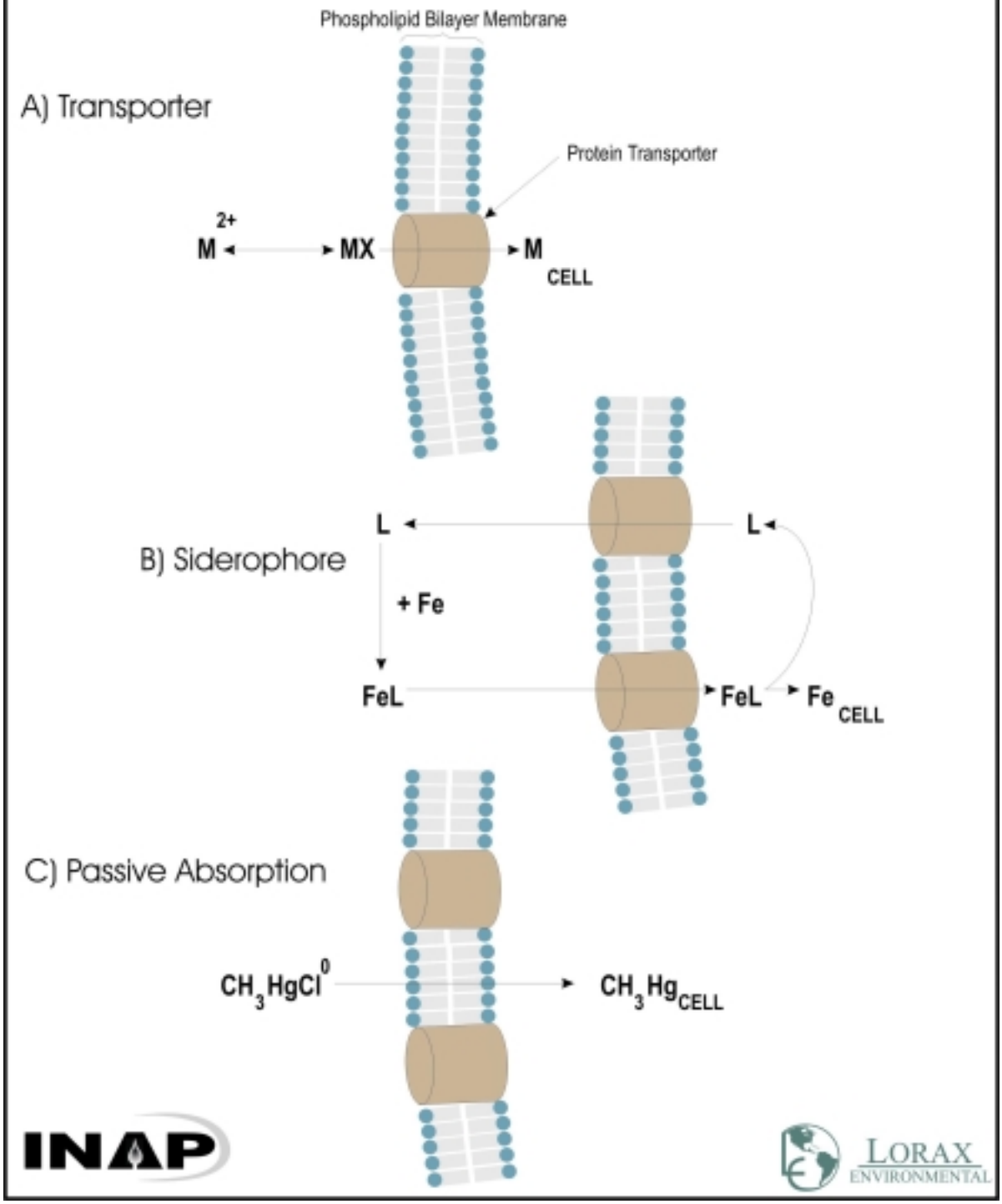
Metal transport across cellular membranes may also be facilitated via extracellular chelators. Aquatic prokaryotes, for example, release high-affinity Fe chelators, or siderophores, into the environment when they become iron-limited (Wilhelm and Trick, 1994). Siderophores complex Fe at the cell surface, with the resulting Fe-chelator complex being transported by membrane receptors into the cell cytoplasm (Figure 2-1). The Fe is subsequently remobilized and incorporated into the cell. A detailed discussion of siderophore structure and function is presented in Chapter 3. Similar strategies have been demonstrated for phytoplanktons which have been observed to produce extracellular Cu complexing ligands in respond to Cu limitation (Croot *et al*., 2000). The passive adsorption of neutral, non-polar metal complexes into cells has also been shown to be an important process for a few metal complexes (Figure 2-1). For example, $HgCl_2$ and CH_3HgCl can diffuse directly across bilayer membranes due to their lipid solubility (Gutknecht, 1981). Other works have also demonstrated that lipophilic organic-chelates can diffuse through cell membranes, thereby bypassing cellular barriers (Phinney and Bruland, 1997).

Metal uptake via a membrane transport protein can be described by the following equation:

$$ML \xleftrightarrow{k_d} M + X_{transporter} \xleftrightarrow{k_f} MX_{transporter} \xleftrightarrow{k_{in}} M_{cell}$$

in which a metal-ligand complex (ML) dissociates, and the metal (M) binds to the protein receptor ($X_{transporter}$) forming a membrane transport site complex ($MX_{transporter}$). The complex is subsequently transported across the membrane to be incorporated into cellular metabolism (M_{cell}). In this sequence, k_d is the dissociation constant for the metal complex (ML), k_f is the kinetic rate constant for the formation of the membrane transport site complex ($MX_{transporter}$), and k_{in} is the kinetic constant for transfer of the bound metal across the membrane and subsequent release into the cytoplasm. The metal complex (ML) may be present as a hydrated aquo ion (M^{z+}), an inorganic complex or an organic complex.

Figure 2-1 **Mechanisms of Intracellular Transport of Trace Metals** (from Hudson, 1998)



The rate of cellular uptake of metals can be limited by both thermodynamic and kinetic considerations (Hudson, 1998; Sunda and Huntsman, 1998). For example, if $k_d \gg k_{in}$, the concentration of bound metal ($MX_{transporter}$), and therefore the uptake rate, is related to the external free metal ion concentration. Under such conditions, the rate of uptake is not limited by the dissociation kinetics of the ML complex. The latter is an example of equilibrium control (*i.e.*, "thermodynamic control") in which the rates of formation and dissociation of the $MX_{transporter}$ species are essentially equal, and much greater than the cellular uptake rate (k_{in}). Such equilibrium considerations form the foundation of the Free-Ion Activity Model (Campbell, 1995). Conversely, for metal complexes with much slower exchange kinetics, k_{in} may greatly exceed k_d. Consequently, the rate of metal uptake is limited by the kinetics of metal binding to membrane transporter sites. In other words, the rate constant of formation (k_f) and assimilation (k_{in}) are nearly equal, and much greater than the dissociation rate constant (k_d) (Hudson, 1998).

2.4 Mitigating Factors

There are several mitigating factors, both environmental and intracellular, that can result in a decrease in the effective toxicity of metals to aquatic biota. In the following paragraphs, the environmental influences of metal speciation (*e.g.*, organic complexation), hardness and pH on metal toxicity are described. This will be followed by a discussion of detoxification mechanisms used by organisms to mitigate metal toxicity.

Trace metals exist in natural waters in a variety of chemical phases, mostly as cations complexed by inorganic and organic ligands (Florence, 1982). The chemical speciation of metals has significant influence on cellular uptake, and hence bioavailability and toxicity. For example, the bioavailability of several metals (*e.g.*, Cd, Cu, Zn) is reduced in the presence of organic chelators (Zamuda *et al.*, 1985). The topic of metal speciation is discussed in detail in Chapter 3.

Other water quality variables including hardness (Ca and/or Mg concentration), alkalinity and pH are known to influence the toxicity of metals to aquatic biota. The toxicity of metals to aquatic organisms, for example, generally decreases with increasing water hardness (Spry and Wiener, 1991; Mayer *et al.*, 1999). Two processes have been suggested to account for these observations: 1) Ca and Mg successfully compete with trace metals for membrane transport sites on cellular surfaces; and 2) the complexation of metals with carbonate (CO_3^-) decreases the free metal ion concentration and thus metal bioavailability (Barata *et al.*, 1998). The influence of pH on metal toxicity is less clear. Some works have demonstrated an increase in metal toxicity with decreasing pH, due to the increase in free metal-ion activity at lower pH (Hodson *et al.*, 1978). Conversely,

other studies have shown a decrease in metal toxicity with decreasing pH (Bervoets and Blust, 2000; Franklin *et al.*, 2000). The latter observations have been attributed to the increased competition of H^+ with trace metals at the cell membrane surface.

In addition to environmental factors which influence metal toxicity, cells have evolved metal detoxification mechanisms to aid in mitigating elevated metal levels. Aquatic organisms (*e.g.*, fish, molluscs), for example, produce metal-binding peptides in order to regulate tissue metal levels. These low molecular-mass cysteine-rich proteins, termed metallothioneins, have been shown to provide a potentially useful proxy for metal exposure (Roesijadi and Fowler, 1992; Legras *et al*., 2000). Similarly, algae and higher plants synthesize low molecular-weight, cysteine-rich polypeptides known as phytochelatins to bind toxic metals (Ahner and Morel, 1995; Lee *et al*., 1996; Ahner *et al.*, 1997). Such detoxification mechanisms have been demonstrated for Cd, Cu, Zn and Hg (Sunda and Huntsman, 1998). It has also been suggested that organisms export metal-phytochelatin complexes from the cell as a means of limiting intracellular metal accumulation (Lee *et al*., 1996). Algae have also been shown to produce extracellular metal-binding ligands in response to metal exposure (Zhou and Wangersky, 1989; Leal *et al.*, 1999). In this manner, the formation of ligand-metal complexes in the surrounding medium results in a decrease in concentration of free metal-ions, thus favouring conditions for cell growth (Moffett and Brand, 1996; Gledhill *et al.*, 1999).

2.5 Free-Ion Activity Model

It has been demonstrated that the bioavailability (and hence toxicity) of many metals in aqueous systems (*e.g.*, Cu, Zn, Fe, Mn, Cd) is proportional to their free ionic activity (M^{z+}) rather than to the total concentration (Anderson *et al.*, 1978; Campbell, 1995). According to the model, free metal ions [M^{n+}] are derived from hydrated aquo complexes or kinetically-labile inorganic complexes [ML], in which the ML complex is characterized by rapid dissociation kinetics. The latter considerations form the foundation for the Free-Ion Activity Model (FIAM) (Sunda, 1991; Campbell, 1995). The FIAM is consistent with the nature of cellular uptake mechanisms which involve protein transport sites. Specifically, the FIAM assumes: 1) the complexation reactions of metals and ligands in solution are essentially at equilibrium; 2) the binding of metal ions with membrane transport sites is close to equilibrium; and 3) the kinetics of transmembrane transport is slow in comparison to the surface complexation reaction. Therefore, under these assumptions, metal uptake is expressible as a function of the concentration of the free metal ion, irrespective of the strength or concentration of the dissolved ligands present in solution. Metal-organism interactions which conform to the FIAM have been demonstrated for a number of test species including uni-cellular algae (*e.g.*, Anderson *et*

al., 1978; bacteria (*e.g.*, Sunda and Gillespie, 1979), marine invertebrates (*e.g.*, Zamuda and Sunda, 1982) and fish (*e.g.*, Roy and Campbell, 1995).

While metal bioavailability and toxicity tend to decrease in the presence of natural organic ligands and synthetic chelating agents (*e.g.*, EDTA), there are some noted exceptions:

- Some non-polar neutrally-charged species (*e.g.*, AgCl, $HgCl_2$) are lipophilic (lipid soluble), and as a result, are able to diffuse freely across cellular membranes. In this manner, metal uptake occurs in the absence of metal binding to extra-cellular ligands (Gutknecht, 1981; Engel et al., 1981).
- The presence of synthetic organic ligands (*e.g.*, dithiocarbamate, 8-hydroxyquinoline) has also been shown to increase the cellular uptake of several trace metals (*e.g.*, Cu and Ni) (Phinney and Bruland, 1997). Uptake is facilitated by the diffusion of the lipophilic organic complex across the cellular membrane.
- Metal bioavailability can be enhanced in the presence of low molecular-weight ligands (*e.g.*, citrate) (Errécalde *et al.*, 1998; Errécalde and Campbell, 2000).
- Metals can be incorporated into cells via the formation and transmembrane transport of siderophore-metal complexes (Wilhelm and Trick, 1994).

As will be discussed in Chapter 4, the FIAM forms the foundation for the mode of metal uptake by DGT. Specifically, the parallels between the nature of "bioavailability" and metal uptake by DGT relate to the mode of assimilation by both aquatic biota and the DGT sampler.

2.6 Assessment Techniques

Several techniques have been developed to assess the toxicity of metal-contaminated waters/effluents to aquatic biota (Table 2-1). In general, toxicity testwork is limited to a few standard tests due to their recognition by regulatory agencies, and generally involves determinations of mortality of standard test species (*e.g.*, *Daphnia magna* and rainbow trout). Such tests typically entail exposing test species to a solution at several dilutions, and measuring organism mortality. Survivorship is then related to the concentration gradient in order to determine the concentration at which 50% mortality occurs (*e.g.*, LC50). The LC50 result is typically expressed as a "percent strength" of the original effluent. Bacterial tests (*e.g.*, Microtox®) have also been shown to afford reliable measures of toxicity in a wide range of industrial wastewaters and impacted water courses (Chang *et al.*, 1981; Codina *et al.*, 1993; Hao *et al.*, 1996). A suite of less commonly used tests also exist, including assessments of mortality to marine invertebrates (*e.g.*, rotifers, Snell and Persoone, 1989) and lettuce root elongation (Miller *et al.*, 1985) (Table 2-1). In general, the different tests exhibit varying sensitivities to metal toxicants,

reflecting the varying responses of different test organisms and the nature of test conditions. Therefore, in detailed evaluations of metal toxicity, it is recommended to employ a battery of tests specific to the aquatic environment of concern, in order to best develop site-specific environmental criteria (Toussaint *et al*., 1995).

Table 2-1:
Examples of toxicity test protocols

Test	Organism	Test Duration	Parameter Measured	Test End Point
Standard Toxicity Tests				
Daphnia	*Daphnia magna*	48 h	Mortality	[1]LC50
Rainbow trout	*Salmo gairdneri*	96 h	Mortality	LC50
Ceriodaphnia	*Ceriodaphnia dubia*	48 h	Mortality	LC50
Green algae	*Selenastrum capricornutum*	96 h	Growth	[2]EC50
Mysid shrimp	*Mysidopsis bahia*	96 h	Mortality	LC50
Fathead minnow	*Pimephales promelas*	96 h	Mortality	LC50
Microtox	*Photobacterium phosphoreum*	5 min	Luminescence	EC50
Other				
Polytox	Blend of bacteria	21 min	Respiration	EC50
Rotifer	*Branchionus calyciflorus*	24 h	Mortality	LC50
Brine shrimp	*Artemia salina*	24 h	Mortality	LC50
Lettuce root	*Lactuca sativa*	96 h	Root elongation	EC50

[1]LC50 = lethal concentration at which a mortality of 50% is realized after 48 h.
[2]EC50 = effective concentration at which measured parameter is reduced by 50%

Standardized bioassay methods are required within the regulatory framework in order to provide a basis for comparison among wastewaters and receiving environments. Such methods provide an indicator of the collective toxicity (*e.g.*, synergistic effects) of all components contained within solution, and thus afford information that cannot be garnered from elemental analyses alone. However, bioassay techniques are characterized by certain limitations. A fundamental criticism of toxicity testwork is preservation of speciation. The time period between sample collection and bioassay procedures is often on the order of days to weeks, and as a result, the speciation, and hence toxicity, of the test solution may be altered during the course of transport, handling and sample processing. In addition, the adoption of bioassays with standard test organisms ignores the site-specific sensitivity of local floral and faunal assemblages. Standard bioassay procedures also rarely take into account the characteristics of the receiving environment which may affect metal toxicity (*e.g.*, complexation capacity, ligand strength, ligand concentrations, complexation kinetics, *etc.*).

3 Metal Speciation

3. Metal Speciation

Trace metals exist in a variety of inorganic and organic forms in aquatic systems, ranging from simple hydrated molecules to large organic complexes. The biological availability, and hence toxicity, of metals in aquatic systems is strongly dependent on the nature of the metal species present. Accordingly, generating an understanding of the chemical form, or "speciation", of metals in the environment is fundamental to predicting impacts to aquatic biota. In the following chapter, the notion of metal speciation is introduced (Section 3.1). A discussion of metal speciation in natural waters (Section 3.2) and mining-influenced systems (Section 3.3) is then presented. The chapter concludes with a review of current speciation techniques (Section 3.4) and a summary (Section 3.5).

3.1 Introduction

"Speciation" as defined by the International Union of Pure and Applied Chemistry (IUPAC) refers to the atomic or molecular form of an analyte (Hill, 1997). Many elements are commonly categorized according to their chemical forms, or species, in the environment. The fundamental difference in chemical behaviour of various sulphur species (SO_4^{2-}, H_2S, *etc*.) and nitrogen species (NH_4^+, NO_3^-, NO_2^-, N_2, *etc*.), for example, is routinely acknowledged. Comparable classification of metal species in water is not routinely carried out, however, largely due to a lack of user-friendly and cost-effective techniques. The conventional practice of distinguishing “dissolved” (that which passes through a 0.45 μm filter) and “total” metal concentrations (which include the particulate phase) presents the only speciation method routinely conducted. Although such dissolved/particulate separation can provide valuable information, it is widely recognized that the speciation of metals exerts the fundamental control on biological uptake, and hence toxicity. Increasing evidence suggests, for example, that the biological response of aquatic organisms in the face of dissolved metal concentrations is proportional to the activity of the free ions. Strongly complexed (thus, non-labile) and particulate metal species are less available (Campbell, 1995). Thus, the determination of metal speciation is as important as the determination of metal concentrations when assessing the impact of trace metals to aquatic biota.

3.2 Speciation in Natural Waters

Metals exist in a wide variety of forms in natural waters. The nature of metal speciation is a function of several variables, including the metal of consideration, pH, pE, salinity, competing cations and the types and concentrations of metal complexing agents present. In the following discussion, the speciation of metals in natural waters is reviewed with

respect to dissolved metal species (Section 3.2.1). This is followed by an examination of the influence of metal complexing agents (*i.e.*, ligands) on metal speciation, as well as examples of field and laboratory determinations. A brief examination of particulate metal species is provided in Section 3.2.2.

3.2.1 "Dissolved" Metals

The simplest, most common categorization of metals in water is separation into "dissolved" and "particulate" metal fractions by filtration. The fraction that passes through a 0.45 μm filter is typically defined as "dissolved", while the fraction collected by the filter is termed "particulate". "Dissolved" is operationally defined, and in a strict sense is incorrect, as small particulates (*i.e.*, <0.45 μm) will pass through the filter membrane. Rather, the term "filterable" is a more correct term. In practice, the "dissolved" component includes metal species that are truly dissolved, including inorganic species (free metal ions and inorganic metal complexes) and organically complexed metals, but also includes "colloidal" metal species. By definition, colloids are particles which range in size from <0.01 to 10 μm, and are typically represented by clays, Fe-oxyhydroxides, amorphous silica and calcium carbonate (Stumm and Morgan, 1981). In the following sections, the "dissolved" component is analyzed in further detail with respect to inorganic and organic speciation.

3.2.1.1 Inorganic Species

The concentration of metal on a cell surface that is available to an organism and that influences its biological response has been shown to be proportional to the free metal-ion activity in the bulk solution (Campbell, 1995). The free metal ion concentration itself, however, often represents only a small portion of the total dissolved metal concentration. Another fraction exists as complexes with assorted inorganic ligands. Important metal-complexing inorganic ligands include CO_3^-, OH^-, Cl^- and SO_4^{2-} ions. For example, cadmium is known to form chloro complexes, whereas Co, Cu, Ni, Pb and Zn tend to form complexes with carbonate ion (*e.g.,* Tipping *et al.,* 1998). These species are often readily available to biota, because the complexes are typically weak and dissociate rapidly to the free metal ion (see Chapter 2).

3.2.1.2 Organic Species

Until fairly recently, the speciation of dissolved metals was widely believed to be dominated by inorganic metal species. In recent years, however, there has been an increasing awareness that for many metals in natural waters, organic metal species predominate with a wide variety of ligands involved (Bruland, 1989; Coale and Bruland, 1988; Kozelka and Bruland, 1998; Bruland, 1992; Nordstrom, 1996; Xue and Sigg, 1997). In most cases, complexation with organic ligands reduces metal bioavailability,

because most organic-metal complexes are not readily transported across cell membranes. Examples include complexes with fulvic and humic acids, which represent the byproducts of polymerization and condensation reactions of natural organic matter. The latter form complexes that are strong for some metals and fairly weak for others, but because they are often abundant, their presence results in the dominance of organic complexes in many natural waters.

A variety of particularly strong biologically produced organic ligands also exist in natural waters. Examples include metallothioneins and phytochelatin. These compounds represent low molecular weight, cysteine-rich metal-binding polypeptides that have been identified to play detoxification roles in animals and plants, respectively (Roesijadi, 1992; Lee *et al.,* 1996). For example, at high inorganic cadmium concentrations, the marine diatom *Thalassiosira weissfloglii* appears to export a cadmium-phytochelatin complex as a detoxification mechanism (Lee *et al.,* 1996). As additional examples, organic Cu-complexing ligands have been observed to be released by many algae, including marine diatoms (Zhou and Wangersky, 1989), marine microalgae (*Emiliana huxleyi*; Leal *et al.,* 1999) and cyanobacteria (Moffett and Brand, 1996). Recent evidence from freshwater environments also suggests that strong metal-complexing ligands are produced by phytoplankton (Xue and Sunda, 1997). Finally, anthropogenically produced ligands such as EDTA are becoming increasingly common in aquatic environments. Because of its strong affinity for many metals, EDTA-metal complexes can dominate metal speciation in some settings (*e.g.,* Sedlak *et al.,* 1998).

While in general organic ligands reduce metal bioavailability, there are some examples of ligands which result in an increase in metal uptake by biota. Siderophores, for example, are ligands with a high affinity for Fe that are released by organisms to facilitate the transport of Fe into the cell. Siderophores (*e.g.,* Figure 3-1) are diverse chemically, but generally have in common chelating oxygen-donor-type functional groups (Cotton and Wilkinson, 1980). Metals other than Fe may be passively transported into cells by such a mechanism by substituting for iron, resulting in more rapid metal transport than would otherwise be observed. However, this is predicted only to occur at low iron concentrations, when organisms synthesize siderophores in order to meet their cellular iron needs.

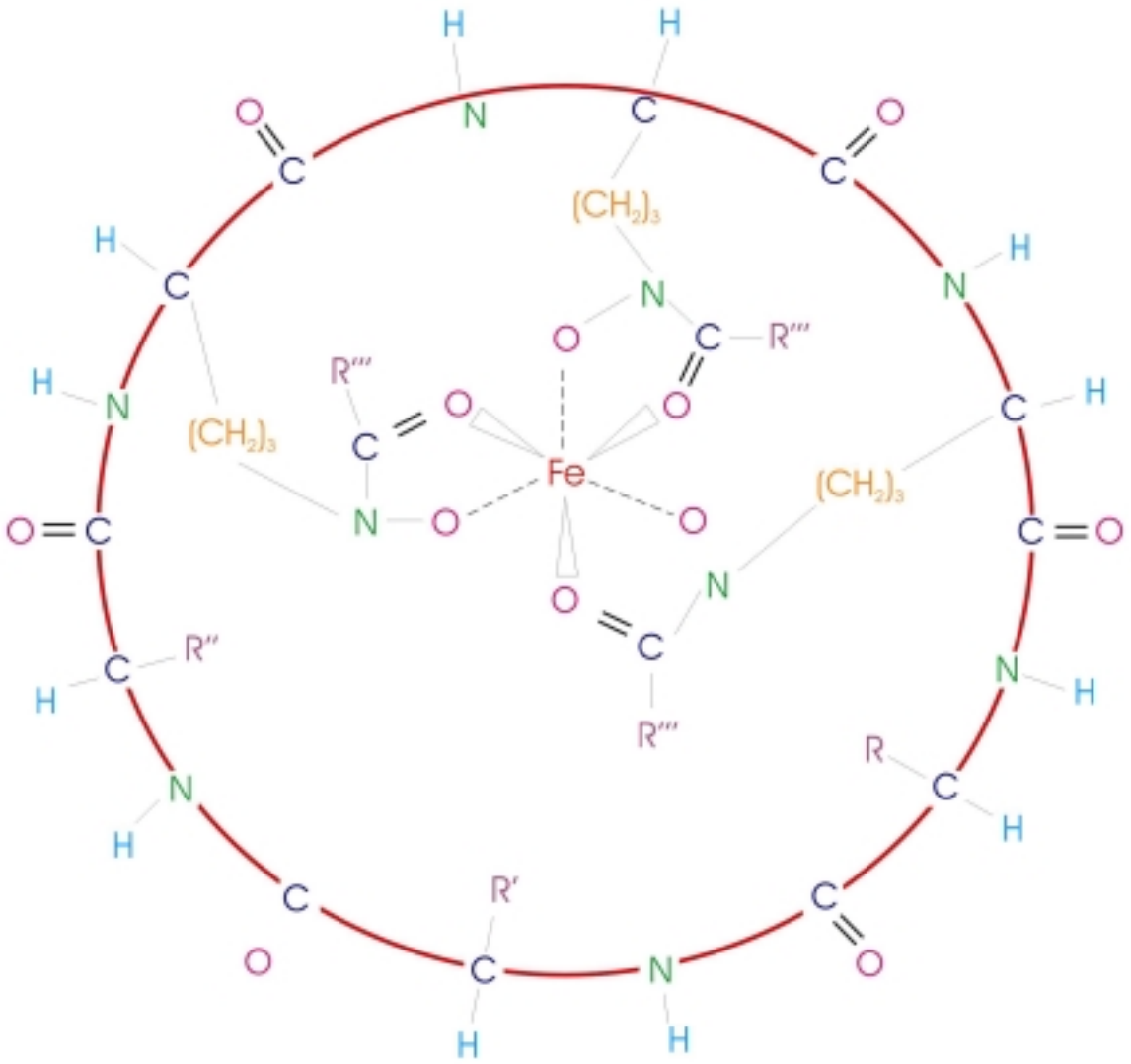
3.2.1.3 Effects of Ligand Concentration and Affinity on Speciation

Metal speciation is ultimately controlled by the combined influences of metal concentration, ionic strength, pH, competing cations, ligand concentration and ligand affinity. This can be readily demonstrated by the following calculations. The strengths

Figure 3-1

Schematic Diagram of Coordination of Iron in a Siderophere (ferrichrome)

(from Cotton and Wilkinson, 1980)



of metal complexing ligands can be expressed in terms of a stability constant, K, as follows. For the reaction between a free metal divalent cation (M^{2+}) and a ligand (L),

$$M^{2+} + L \leftrightarrow ML,$$

the equilibrium constant can be described as:

$$K = \frac{[ML]}{[M^{2+}][L]}.$$

Since $[ML] \cong [M_{diss}] - [M^{2+}]$, substituting and solving for $\frac{[M^{2+}]}{[M_{diss}]}$ yields:

$$\frac{[M^{2+}]}{[M_{diss}]} = \frac{1}{K[L]+1}.$$

What becomes apparent from this relationship is that the proportion of free metal ion depends on the product of the ligand concentration and the stability constant, K. Thus, a low-concentration ligand that has an extremely high affinity for a metal can dominate the metal speciation over a much more abundant, but weaker, metal ligand (provided both ligands are present in excess of the metal).

3.2.1.4 Case Studies of Speciation in Natural Waters

Due to limitations in existing analytical techniques, the chemical structure of the vast majority of organic ligands has not been determined. Nonetheless, electrochemical techniques are capable of quantifying the concentration and strength of all existing ligands, regardless of whether or not their precise chemical structure is known. As a consequence, numerous studies have shown that metals are complexed in many environments by organic complexes (Table 3-1). In seawater, for example, the dominant species of Cd, Cu, Pb and Zn are dissolved organic complexes, (Bruland, 1989; Coale and Bruland, 1988; Kozelka and Bruland, 1998; Wells *et al.,* 1998). These metal-organic complexes are extremely strong, being characterized by stability constants that range in strength from ~10^9- ~10^{12} (Kozelka and Bruland, 1998). Cu-complexing ligands of varying strengths have also been identified. The strongest ligands are termed L1, the second strongest L2 and the third-strongest L3 (Table 3-1). The dominance of a particular ligand will depend on their relative concentrations.

The nature of metal speciation in freshwaters has not been characterized to the same degree as in the marine environment. However, there is some information available on organic complexation in freshwaters. For example, copper speciation in Lakes Greifen and Lucerne (Switzerland) is dominated by complexation with strong ligands which are present at much lower concentrations (~20-60 nM) than a considerably more abundant class of weaker ligands (8 μM), presumably humic acids (Xue and Sunda, 1997). In a

variety of European rivers, zinc has been observed to be ~70% complexed by organic ligands with stability constants of ~10^7, resulting in a 30-50% reduction in zinc bioavailability (Jansen *et al.,* 1998). Organic species have also been shown to be dominant for lead in anoxic freshwaters (Taillefert *et al.,* 2000).

Table 3-1:
Summary of representative metal and ligand concentrations, the strength of metal-ligand complexes and the % contribution to metal speciation in a variety of freshwater and marine settings

Metal	Location	Metal Concentration (nM)	Ligand Type	Ligand Concentration (nM)	Stability Constant (log K)	% Complexed
Cu	Lakes Greifen & Lucerne[1]	~10	L1	38±19E-9	15	>90
			L2	1-3.5E-7	11-13	
			L3	8±2 E-6	8.6	
	Narragansett Bay, RI[2]	13-28	L1	16-38	>12	>99
			L2	15-40	8.8	
			L3	54-100	7.7	
	wastewater treatment near SF Bay[3]	20-200	L1 (synthetic chelates)	~1-10	7-8 11-14	5-80
			L2 (humic substances)	340-490	~7	5-50
	San Francisco Bay[4]	45	L1	13	>13.5	27
			L2	20-30	9-9.6	52-65
Cd	Narragansett Bay, RI[2]	0.29-0.8		4	8.9	73-83
Zn	Narragansett Bay, RI[2]	16-72		11-48	9	51-97
	European Rivers[5]	90-4800		260-3300	6.4-7	55-88
Pb	Narragansett Bay, RI[2]	0.13-0.32	L1	0.8	10	67-94
			L2	4-8	8.8	
Ni	wastewater treatment near SF Bay[3,6]	55-148	L1 (EDTA)	70	>12	30-100
			L2 (humic substances)	430-740	6-7	

[1] Xue and Sunda, 1997.
[2] Kozelka and Bruland, 1998.
[3] Sedlak *et al.,* 1997.
[4] Donat *et al.,* 1994
[5] Jansen *et al.,* 1998.
[6] Bedsworth and Sedlak, 1999.

3.2.2 Particulate Metal Species

Metals may be hosted in particulate phases either as part of a mineral lattice, sorbed onto particle surfaces, or as assimilated components in aquatic biota. In general, particulate

metal phases tend to be less available, and hence less toxic, to organisms in comparison to their dissolved counterparts. However, the ingestion of particles has been shown to be an important mechanism of metal accumulation in filter feeding organisms (*e.g.*, Roditi *et al.,* 2000).

Due the particle-reactive nature of many trace metals, particulate phases can play a dominant role in metal speciation and behaviour. Many trace metals (*e.g.,* Cu, Pb, Hg, Cd, Zn, Ag) have been shown to be primarily associated with suspended and sedimentary particulates in some freshwaters as opposed to dissolved phases (Davis and Leckie, 1978b; Nriagu *et al.*, 1981; Laxen, 1985; Tessier *et al.*, 1985). As a result, settling particles are an important vector for the transfer of heavy metals to lake sediments, thereby regulating the concentrations of dissolved species (Nriagu *et al*., 1981; Sigg, 1985; Jackson and Bistricki, 1995). Particulates in natural waters consist predominantly of detrital particulate and colloidal organic matter, inorganic solids such as metal oxides and hydroxides (*e.g.*, SiO_2, MnO_2, FeOOH, Al_2O_3), algal skeletal remains, carbonates and detrital aluminosilicates (*e.g.*, clay minerals, feldspars) (Tessier *et al*., 1985). In general, particulate organic matter often presents the major component of suspended material in freshwaters, accounting for up to 70% of the total particulate fraction (Nriagu *et al.*, 1982). Colloidal metal species can be quantitatively significant in many environments (*e.g.,* Wells *et al.*, 1998). In well-buffered mining- systems which receive inputs of acid rock drainage, colloids often can host the bulk of the metal inventory. In such environments, loadings of ARD can result in the precipitation of colloidal Fe-oxyhydroxides which represent preferential sorption sites for many trace elements (Jackson and Bistricki, 1995).

3.3 Metal Speciation in Mining-Impacted Systems

The metallurgical processing of base metal and gold ores involves the addition of various reagents to enhance the recovery of desirable constituents. As a result, the chemical composition of discharge waters can contrast greatly from waters in the receiving environment. Such influences can potentially have a profound effect on metal speciation. In the following section, the influence of these inputs on metal speciation is discussed.

Mining operations utilize a spectrum of chemical reagents in various stages of ore treatment, mineral recovery and effluent treatment (Table 3-2). During milling operations, for example, various inorganic (*e.g.*, activators) and organic reagents (*e.g.,* frothers and collectors) may be added to the process stream. Accordingly, variable quantities of such components inevitably report to tailings impoundments. For many operations, water balance considerations require the discharge of tailings pond overflow to receiving waters, and as a result, various metallurgical and/or treatment products are

discharged to the environment. The effect of process reagents on metal bioavailability in natural systems is poorly constrained. However, given the nature of functional groups characteristic to the suite of organic reagents utilized (*e.g.*, dithiocarbonates, carboxylic acids, esters, organo-phosphates, *etc.*) it is likely that metal speciation is strongly influenced in certain environments. In addition, the presence of high-affinity inorganic products such as cyanide compounds, chloride, and sometimes sulphate will undoubtedly have an effect on metal speciation. In particular, metal-CN complexes may have considerable importance in systems that receive loadings from gold-mining operations. The influence of such chemicals on metal speciation, bioavailability and toxicity requires further research (see Chapter 4).

Table 3-2:
Possible mining-related waste products resulting from ore processing, waste treatment and mine-site run-off

Process	Possible Waste Products
Flotation	Alkaline waste stream Sulphate Metal hydroxides Frothers (organic reagents, *e.g.*, C6-C9 alcohols, pine oil, cresol) Activators (*e.g.*, $CuSO_4$, Na_2S) Depressants (*e.g.*, lime, NaCN, $ZnSO_4$) Collectors (*e.g.*, Xanthates)
Pressure Oxidation	Ferric sulphates, arsenates , jarosites Metal hydroxides
Cyanide Destruction/Recovery	Cyanide, Cyanate, thiocyanate Ammonia Hydroxide sludges Cyanide salts Chlorine
ARD Treatment	Neutralization/Precipitation: Alkaline waste stream Hydroxide sludges Sulphates Sludge flocculaters (*e.g.*, acrylamide) Solvent Extraction: Carboxylic acids Sulphonic acids Alcohols Amines Activated Carbon: Organics
Effluent treatment before discharge	EDTA
Mine Site Run-off	ARD Petro-chemicals Blasting reagents (ammonia, nitrate) Road salt

A wide range of synthetic additives may also influence metal speciation and bioavailability in receiving waters. EDTA, for example, may be added to waste streams prior to discharge in order to minimize toxicity to biota in downstream environments. EDTA is a very strong ligand which favours complexation with many trace metals (*e.g.*, Cu, Zn, Cd, Pb, Hg). Synthetic frothing agents, representing mainly high molecular-weight alcohols (*e.g.*, methyl isobutyl carbinol), may also influence the bioavailability of metals in receiving waters.

The dynamics of mixing between effluent and the receiving waters can also affect metal speciation. As outlined above, the flocculation of ARD inputs upon contact with pH-neutral watercourses can result in the formation of colloidal Fe-oxyhydroxides. This process can result in the co-precipitation and scavenging of metals from solution. In addition, metal speciation and bioavailability can be significantly affected in systems where effluents are discharged into waters with significant quantities of metal-complexing ligands. There are two common scenarios where this can occur: 1) the discharge of mining effluent into DOC-rich waters; and 2) the discharge of mining effluent into seawater. The addition of metal-bearing effluents to DOC-rich freshwaters will result in the complexation of a certain fraction of the metal inventory, thereby potentially decreasing metal bioavailability. In this situation, the mixing dynamics becomes important in influencing the rate at which the metal comes in contact with the complexing ligands. Similarly, where relatively-fresh effluent is discharged into higher ionic strength waters (such as seawater), significant flocculation of metal-bearing colloids can occur, resulting in significantly lower concentrations of dissolved metals over short distances and time intervals (*e.g.,* Boyle *et al.,* 1976; Sholkovitz *et al.,* 1976; Karbassi and Nadjafpour, 1996).

3.4 Speciation Techniques

A number of techniques currently exist for quantifying aqueous metal speciation. Among these, three approaches have great potential as speciation tools. These include: 1) diffusive gradients in thin films (DGT); 2) voltammetry; and 3) supported liquid membrane techniques (SLM). These three approaches are described in turn below.

3.4.1 DGT

Diffusive Gradients in Thin Films (DGT) represents one of the most promising techniques for determining the concentration of labile metal species in aquatic systems (Zhang and Davison, 1995). This method is discussed in detail in Chapter 4, and therefore only a brief description is presented here. The DGT device consists of a ~4 cm-diameter disk, comprising a filter membrane underlain by a polyacrylamide gel layer,

which is in turn underlain by a trace-metal-adsorbing gel-embedded resin. Metals diffuse from solution across the filter and gel layers to the underlying resin, where metal sorption takes place. The resin only adsorbs free and kinetically-labile metal ions. This approach therefore provides a better measure of biologically-available metals than do dissolved or total metal concentrations. The principal advantages of the DGT device include the possibility of *in situ* deployment, low risk of sample contamination, sample pre-concentration and relative ease of use.

3.4.2 Voltammetry (ASV and AdCSV)

Voltammetry is a technique in which the concentrations of labile metal species are determined from a current measured in solution as a metal is taken up into, or released from, a mercury-containing electrode (Sawamoto, 1999). The instrumentation consists of a voltammetric analyzer, a three-electrode cell (working electrode, reference electrode and counter electrode) and a computer for automated measurements and data acquisition. The most commonly used working electrodes are hanging drop mercury electrodes (HDME) and rotating mercury-film electrodes (MFE) (Achterberg and Braungardt, 1999). The advantage of HDME is its reliability, while MFE offers increased sensitivity due to the concentration of metals into a smaller volume of mercury film. MFE, however, is subject to interferences as surfactants collect on the electrode surface, while HDME is not because a new mercury drop is used for each assay. One recent means of overcoming this limitation for MFE is to cover the electrode surface with a semi-permeable membrane that permits transport of ions but excludes the potential interferences (Tercier *et al.*, 1998a, 1998b). Strengths of voltammetric approaches include low detection limits (10^{-10}-10^{-12} M), multi-element capability and suitability for ship-board determinations (Achterberg and Braungardt, 1999). There are two commonly used variants of voltammetry, including anodic stripping voltammetry (ASV) and adsorptive cathodic stripping voltammetry (AdCSV).

In ASV, the metal ion of interest is deposited on the mercury electrode via the reduction of the metal to a metallic state and subsequent amalgamation with the mercury. This is followed by a voltammetric scan towards more positive potentials, during which the mercury-bound metal is oxidized and the current produced is determined. The identity of the metal is dictated by the potential of the peak, while the concentration is proportional to the height of the peak. Absolute concentrations are inferred by the standard additions method, because the sensitivity may vary between samples of different ionic strength and different concentrations of surfactants and natural organic compounds. Thus, the species measured are operationally defined to include free metal ions and species that can dissociate to free metal ions within time scales of ~10^{-3} s. ASV has been widely used, particularly in metal speciation studies in the marine environment (Bruland, 1989; Coale

and Bruland, 1988; Achterberg, *et al.*, 1994; dosSantos*, et al*., 1996; Kozelka and Bruland, 1998; Wells *et al.,* 1998).

In AdCSV, a specific ligand which is added to the sample forms a complex with the metal of interest and adsorbs onto the surface of the electrode. A voltammetric scan towards more negative potentials follows and the resulting current is measured. The current produced results from the reduction of a reducible group on the ligand or of the metal itself in the adsorbed complex. As in ASV, the identity of the metal is determined by the potential, while the concentration is determined from the size of the peak. The AdCSV approach has been widely used for metal determinations in seawater (Donat *et al.,* 1994) as well as in freshwater (Xue and Sunda, 1997).

ASV and AdCSV methods are characterized by certain disadvantages. First, both techniques are typically conducted in the laboratory, often many hours or days after sample collection. Given that changes in metal speciation can occur over these time scales, the representativeness of such data are questionable. This shortcoming, however, has recently been overcome with the development of a voltammetric *in situ* profiling (VIP) probe which allows *in situ* measurements to depths of 500 m (Tercier-Waeber *et al.,* 1999). Another significant limitation of voltammetric approaches is that the test medium requires the presence of small amounts of electrolytes in solution. Therefore, applications to low-ionic strength solutions (*e.g.*, freshwaters) are limited. Furthermore, it is difficult to carry out voltammetric determinations in solutions of poorly characterized salinity because the signal is dependent on the ionic strength. Voltammetric techniques, therefore, have most frequently been used in seawater, where the ionic strength is well characterized and essentially constant. Nevertheless, there are successful examples of voltammetric studies in freshwaters (Cheng *et al.,* 1994; Jansen *et al.,* 1998; Xue and Sunda, 1997; Vega *et al.,* 1995).

3.4.3 Supported Liquid Membrane

The supported liquid membrane (SLM) technique closely mimics the mechanism of metal uptake by biota. In this approach, labile metal species are transported across a membrane and are pre-concentrated into a solution on the opposite side of the membrane (Tercier-Waeber *et al.*, 2000; Keller *et al*., 1994; Parthasarathy and Buffle, 1994; Keller and Buffle, 2000). In detail, the aqueous sample is pumped past a porous membrane embedded with a non-water-miscible solvent in which is dissolved a hydrophobic ligand (Figure 3-2). Labile metal species are complexed by the ligand, transported across the membrane by diffusion and accumulated in a second solution on the other side that contains a ligand of greater strength than the hydrophobic ligand-transporter (Figure 3-2). By carefully controlling the ligand strengths, flow rates and solution volumes, only the

labile metal species can be transported, and pre-concentration can occur, permitting quantification of "labile" species at the low levels found in many natural waters. The strengths of SLM include:

1) the technique mimics transport across biological membranes (see Chapter 2);

2) pre-concentration permits use of the technique at very low metal concentrations; and

3) the technique can be tailored to mimic biological uptake of different metals via adjustment of the ligands used.

Disadvantages include the degree to which the approach must be optimized for each metal and the sophistication of the equipment, as well as limited prospects for *in situ* deployment in the field.

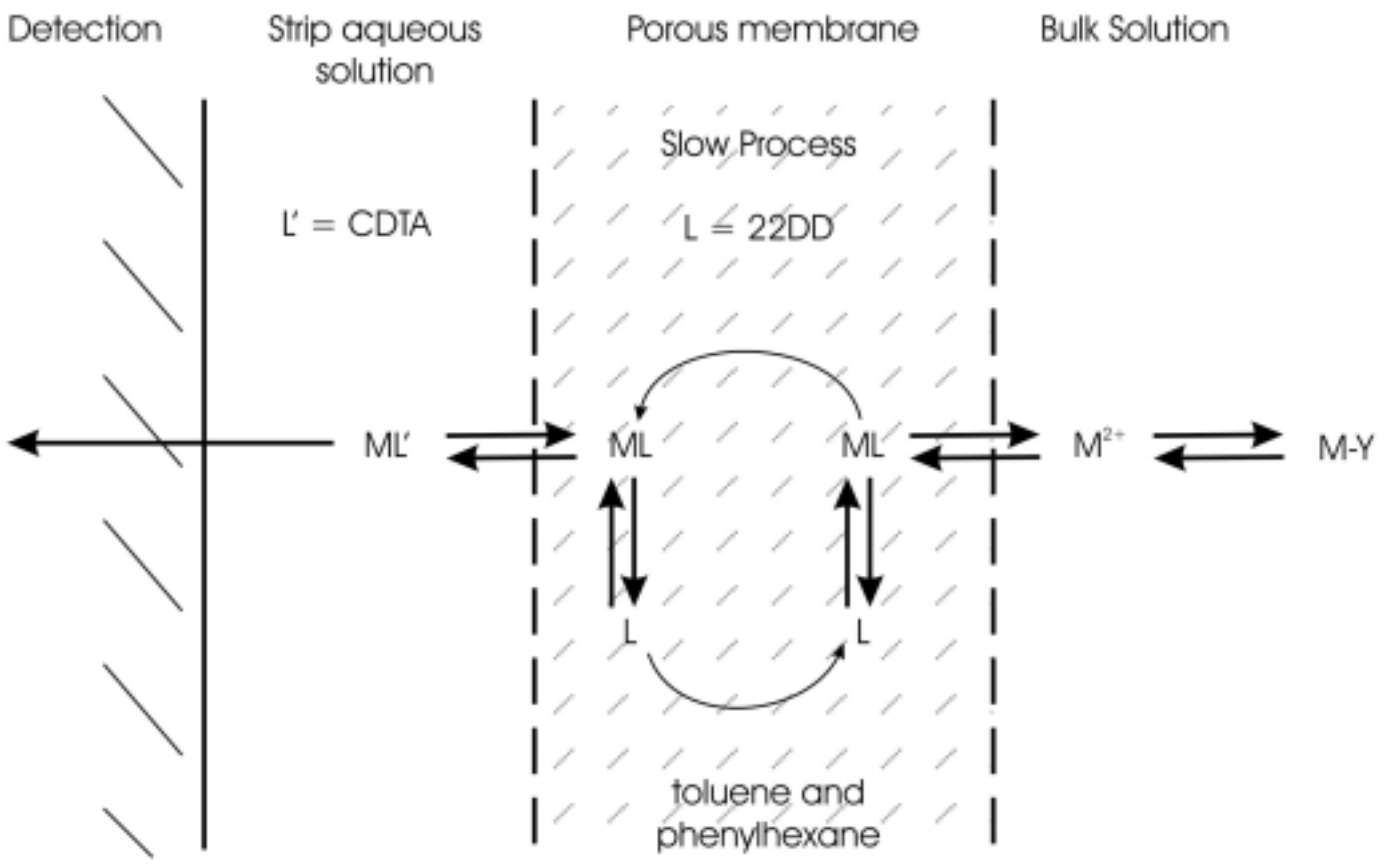
3.4.4 Other

The speciation techniques described above represent the most promising techniques at the current time for low-level determinations of free-metal concentrations in natural waters. Another technique involves the use of Ion-Specific Electrode (ISE). ISE techniques, which have been developed for Cu and Cd, are currently limited in their application due to poor detection limits. Additional techniques being utilized include batch treatment of water samples with ion exchange resins (Muller and Kester, 1991), liquid chromatography-inductively coupled plasma mass spectrometry (Vela and Caruso, 1993), the Wageningen Donnan Membrane technique (Temminghoff *et al.,* 2000) and electrospray ionization mass spectrometry (Schramel *et al.,* 1998; Ross *et al.,* 2000). These other approaches do not have the broad applicability of the above-mentioned techniques, however, and are thus not discussed in detail.

3.5 Summary

The relative proportions of the various metal species in natural waters depend on the concentrations of the metals and the concentrations and strengths of the various ligands present. A variety of analytical techniques has been used to quantify metal speciation in natural waters. Virtually all of the techniques suffer inadequacies that limit their general use. For example, most existing techniques must be carried out in a laboratory rather than *in situ*. Furthermore, most require expensive instrumentation that must be used by a highly trained operator. The DGT method, however, can be carried out *in situ* at modest cost by relatively untrained personnel. Thus, the DGT approach has tremendous potential for use as a routine water-quality monitoring tool.

Figure 3-2 **Schematic Representation of the Supported Liquid Membrane Preconcentration Technique** (from Buffle *et al.*, 1997)



4 DGT

4. DGT

4.1 Introduction

The collective understanding of trace metal cycling in the environment has been advanced significantly since the early 1980's with the advent of "ultra-clean" trace metal protocols for sample collection, handling and analysis (Bruland, 1979; Nriagu *et al*., 1993). Prior to this time, attempts to study the behaviour of trace metals in aquatic systems were hindered by problems of contamination, and as result, the data generated were often not representative. Despite improved sampling techniques and insight into trace metal behaviour and toxicity, the regulatory framework for the environmental assessment of water quality (*i.e.,* the reliance on total metal concentrations) has remained virtually unchanged. For example, it is acknowledged that total metal concentrations are a poor proxy for metal bioavailability, particularly where high suspended sediment loads exist.

Although it is recognized that measurements of total metals yield limited site-specific information with respect to bioavailability and toxicity, there is currently a dearth of economic and user-friendly tools for routine determinations of metal speciation. Efforts to ascertain metal toxicity typically utilize standard bioassay protocols. The use of polarographic techniques to assess metal speciation, effluent toxicity and complexation capacity have been used less frequently. The primary limitation with these latter approaches, however, relates to the potential for alterations to metal speciation between sample collection in the field and analysis in the laboratory. Sample turn-around, for example, is often on the order of days for both bioassays and speciation analyses. *In situ* bioassays and/or autonomous *in situ* samplers have been used on occasion, but these approaches are often prohibitively expensive, difficult to use routinely, and usually require operation by highly-trained personnel.

DGT (diffusive gradients in thin-film) represents a relatively new approach to water sampling which essentially obviates many of the limitations outlined above (Zhang and Davison, 1995). In general, DGT affords *in situ* determinations of metal speciation in aquatic systems by selectively accumulating labile metals. Accordingly, the technique allows inferences to bioavailability and toxicity. Furthermore, since DGT passively accumulates labile metals from solution while deployed *in situ*, contamination problems associated with water collection and filtration are eliminated. Given the speciation capabilities of DGT, in conjunction with its affordability and ease of use, DGT has considerable potential as a routine monitoring tool in mining-related environmental studies (McNee and Robertson, 1998).

The following Chapter 5 details the theory and application of DGT technology. Subsequent to a discussion of the theoretical considerations (Section 4.2), applications of the approach to assessments of speciation, toxicity, complexation kinetics and complexation capacity are discussed (Section 4.3). The section concludes with an overall assessment of the considerations for DGT application and associated technique limitations (Section 4.4), as well as identifying data gaps and areas for future research (Section 4.5).

4.2 DGT Theory and Design

The following section presents the theory and design of DGT. Following a detailed discussion of DGT theory, the calculations used to generated DGT values are described. The section concludes with a description of the DGT sampler with respect to configuration, deployment and analysis.

4.2.1 DGT Theory

The theory behind DGT is based on the diffusional characteristics of metals in a hydrogel and on the sorption properties of a metal-binding resin (Zhang and Davison, 1995**;** Davison *et al*., 2000). Specifically, the technique utilizes a hydrogel layer to control the diffusive transport of metals in solution to a cation-exchange resin. The laws of diffusion are well known, while the characteristics of the diffusive path afforded by the DGT sampler are reasonably well defined. In addition, since the cation exchange resin in DGT (Chelex) is selective for free and/or weakly complexed species, it affords a proxy for the labile fraction of metals in solution which is believed to approximate the bioavailable fraction. . Other methodologies have implicitly acknowledged the value of using resins such as Chelex (Paulson, 1986; Pai *et al.,* 1990; Chakrabarti, *et al*. 1994) or 8-hyroxyquinoline (Beauchemin *et al.,* 1987; Sturgeon *et al.,* 1982) to extract metals *in situ*, but most approaches typically involve the use of pumps or autonomous samplers. DGT differs from other resin techniques in that it uses the laws of diffusion to control the flux of metals to the resin layer. In this manner, the concentration of labile metal species in solution can be reasonably quantified (Zhang and Davison, 1995; Denny *et al*., 1999; Davison *et al*., 2000).

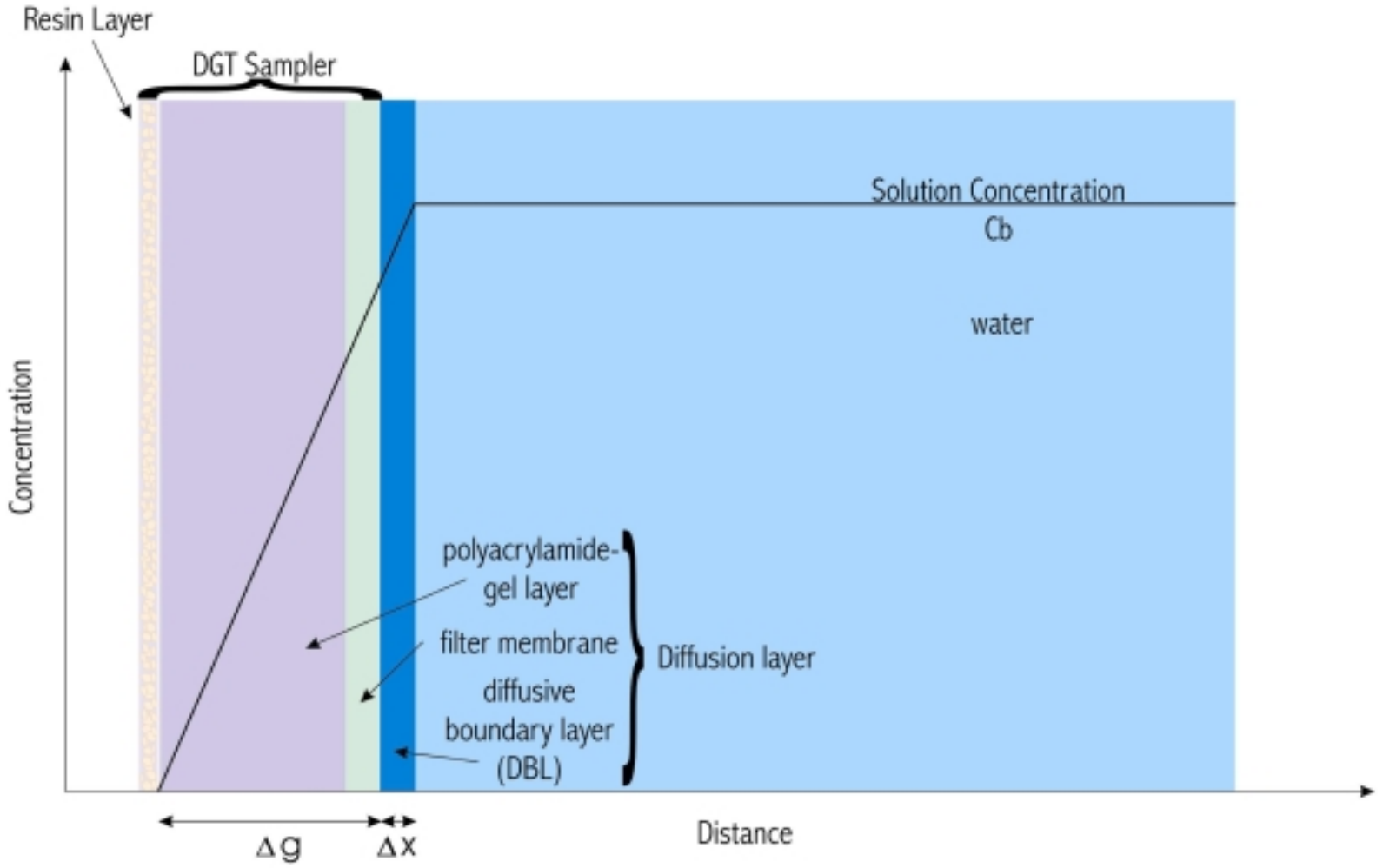
DGT utilizes a three layer system: 1) a resin-impregnated hydrogel layer; 2) a hydrogel diffusion-layer; and 3) and a filter membrane (Figure 4-1). The innermost two gel layers are fabricated from a polyacrylamide hydrogel. Polyacrylamide is a hydrous polymer consisting of acrylamide-polymer chains, typically linked with bis or agarose cross-linkers (Chramback, 1985). Chelex resin is a strong metal complexing agent and rapidly binds free and/or kinetically-labile metal species (Paulson, 1986; Pai *et al*., 1990).

Therefore, the resin layer serves as a sink for labile metal species which diffuse through the polyacrylamide diffusion-layer (Figure 4-1). The filter membrane (pore size = 0.45 μm) protects the fragile polyacrylamide surface and isolates it from particles.

During deployment of the DGT sampler, dissolved metal species in solution freely diffuse through the diffusive boundary layer (DBL), the filter membrane and the polyacrylamide gel-layer (Figure 4-1). The DBL is the layer of water adjacent to all solid surfaces where flow velocity is zero; its thickness varies inversely with flow but typically ranges from a single molecule to millimetre-order distances (Santschi *et al.,* 1983). Thus, there is a continuum of flow velocities adjacent to the solid surface ranging from a zero velocity very near the surface to a maximum velocity at some finite distance. Within the DBL zone adjacent to the DGT surface, mass transport occurs by molecular diffusion. Similarly, since approximately 90% of the polyacrylamide gel is water, and because the nature of the polymer matrix is open to movement at the molecular scale, transport of metals through the gel also occurs by molecular diffusion (Davison and Zhang, 1994; Krom *et al.,* 1994; Zhang and Davison, 1999). Most hydrated cations fall within the range of 0.2 to 0.3 nm diameter and thus diffuse through the DGT hydrogel, which hosts a functional pore size greater than 2 to 5 nm.

Although diffusion through the hydrogels can be relatively unconstrained, the gels used in DGT consist of ~10 % cross-linked polymer. This constrains the permeability of the gel and requires that neutral and charged diffusing ions follow a tortuous path, which lowers their effective diffusion coefficients. Such effects become more pronounced as the gel becomes tighter. Furthermore, ionic strength, pH, and solution composition can influence the rate of diffusion by affecting the behaviour of functional groups on the polyacrylamide, which may become ionized and interact specifically with diffusing charged species. Collectively, these features should reduce slightly the diffusion coefficients of ions relative to those in water. Waters with very low cation concentrations ($<2 \times 10^{-4}$ M) also pose a challenge. In such settings, the sodium form of polyacrylamide used in the gel samplers can establish steep negative concentration gradients of Na^+ from the gel to the sample water (Alfaro-De la Torre et al., 2000). To preserve electroneutrality, these are compensated by steepened concentration gradients of cations in the opposite direction. These enhance the flux of cations to the gel, causing their "labile" concentrations to be overestimated. In most mining-impacted regions, this is not of importance because surface waters typically have higher ionic strengths. But in pristine natural waters on the Canadian Shield, for example, this co-diffusion effect may be important. Such constraints need to be kept in mind in DGT field applications.

Figure 4-1 **Representation of the Concentration of Free Metal Species in a DGT Device and Adjacent Water During Deployment**



Within the Chelex gel-layer, labile metal species are rapidly removed from solution to metal-binding sites on the resin. Metal consumption within the resin layer results in an effective "zero" concentration of labile metal species adjacent to the resin surface (Figure 4-1). Due to the presence of this "metal sink", concentration gradients are established within the DGT unit, and extend into solution through the DBL (Figure 4-1). If differences between the diffusion coefficients in the DBL, the protective filter membrane, and the hydrogel are present, contrasting concentration gradients will be established, as shown in Fig. 4-1. The different gradients reflect the fact that at steady-state, the flux through each layer must be identical.

Metal ions accumulate in the Chelex layer by diffusing along the concentration gradients from solution to resin in accordance with Fick's laws of diffusion and the well-defined configuration of the DGT sampler (Figure 4-1). Diffusion of most ionic and molecular species over these distances (*i.e.,* < 1 mm) occurs on the order of seconds to minutes (Li and Gregory, 1974; Manheim, 1970). Thus, both steady-state and stable concentration gradients are established rapidly (*i.e.,* < 5 minutes) within the DGT sampler upon deployment in the sample medium. Therefore, while the DGT unit is deployed, labile metal species accumulate at a constant rate in a predictable and reproducible fashion. The concentration of labile metal species can then be calculated knowing the mass of metal accumulated on the resin, the diffusion coefficient for the metal, and the deployment time. A limit on the accuracy of the assayed concentration is imposed by uncertainty in the diffusion coefficient used in the calculation. For example, Alfaro-De la Torre et al. (2000) determined that the tracer diffusion coefficient of Cd in gel (D_{Cd}) was 0.9 that of water, while Zhang (cited in Alfaro-De la Torre et al (2000)) more recently derived a D_{Cd} of 0.8. The difference was ascribed to batch-to-batch variations in the agarose-derivative cross-linker. Thus, although estimated gel diffusion-coefficients are available for most environmentally-significant trace metals (Zhang and Davison, 1995), they are likely to be adjusted as research on the DGT technique continues.

The influence of the DBL is related to the flow conditions adjacent to the sampler surface. Specifically, the impact of the DBL is most pronounced in stagnant fluids because the thickness of the DBL is greatest when the interfacial velocity (flow) at the gel surface is lowest (Gundersen and Jorgensen, 1990). In low-current fluids, such as the deep waters of a lake or ocean, the DBL is often in the range of 0.5 to 1 mm thick (Santschi *et al.,* 1983). This thickness is comparable to that of the polyacrylamide layer within the DGT unit (typically 0.4 to 0.8 mm); in such cases, the DBL exerts a significant influence on the flux of metal to the resin layer. By contrast, in high-velocity fluids such as flowing streams, the DBL thickness can be negligible relative to the gel sampler thickness. Under such conditions (*i.e.,* cm/s order flow rates), the diffusive path length from solution to resin layer is negligibly influenced.

Direct measurements of the thickness of the DBL are difficult and impractical. Given that the thickness of the DBL varies inversely with flow, and since flow is variable in time and space, a single direct measurement of DBL thickness is not representative. Rather, where DBL thickness is deemed to be an issue (*i.e.,* in quiescent waters), it is more effective to measure it indirectly by using multiple DGT units with varying diffusive-gel thicknesses. Since the DBL thickness and the free metal concentration in solution are identical for DGTs with varying gel thicknesses, the DBL thickness can be determined mathematically. The calculation of the DBL correction factor is outlined in detail in Section 4.2.2. This calculation assumes that the diffusion coefficients adopted for the ions of interest are the same for all samplers irrespective of gel thickness. This assumption has not yet been rigourously tested. Further, changing the thickness of the gel layer will change the size of the pool of labile metal that can dissociate within the sampler and react with the Chelex; this could have an impact on the calculated labile metal concentration (Zhang and Davison, 1995), although in most cases, this should be very minor.

The manner in which DGT sequesters metals from solution serves to pre-concentrate metals. Specifically, the DGT sampler continually accumulates metals during the deployment period, such that the final mass of metal on the resin may be equivalent to that present in many millilitres or litres of water. Upon processing of the DGT for analysis, the total mass of metal on the resin is extracted into ~1 mL of acid. This pre-concentration process has two principal advantages. First, the method affords very low detection limits, with the effective sensitivity increasing with deployment time. Second, because DGT metals are extracted into a medium (usually nitric acid) which is desirable for direct injection into most analytical instruments (*e.g.*, ICP-MS and GFAAS), problems associated with matrix effects are obviated. Conventional analyses of high ionic-strength solutions, for example, generally involve labour-intensive extraction procedures which increase the chances for contamination. In addition, since Chelex does not preferentially adsorb the anions common to salt waters (*e.g.*, Cl^-, SO_4^{2-}, *etc.*), such constituents are effectively eliminated from the sample matrix.

4.2.2 Calculation of Concentration

The use of DGT for trace metal determinations is in many respects simpler than conventional water sampling methods. However, the calculation of actual DGT-metal concentrations is more involved. The concentration of labile metals in solution is calculated using the measured metal ion inventory on the resin, the sampler exposure time, and the temperature-corrected molecular diffusion coefficient for the metal of interest. This section explains how the “free” or “bioavailable” metal concentrations are determined using DGT.

Calculation of the free metal ion concentration is based on measurement of a metal flux (*e.g.*, g cm^{-2} s^{-1}) through the gel and involves the following steps. The flux of trace metals may be quantified by applying Fick's first law of diffusion as expressed by the following equation:

$$F = \frac{DC_b}{\Delta g} \quad (1)$$

where F is the flux of trace metal ions diffusing from the bulk solution to the resin layer, C_b is the concentration of the labile metal in bulk solution, Δg is the thickness of the diffusion layer (*i.e.*, gel + filter) and D is the temperature-corrected diffusion coefficient for the free hydrated metal ion in the gel (Zhang and Davison, 1995; Krom *et al.*, 1994). The equations below effectively assume a single average D for all three layers (the DBL, the filter, and the gel) and thus represent the simplest approach to the determination of C_b. Where D in the gel is close to that of water, this approach will introduce little inaccuracy. As D_{gel} diverges from that in water, however, some inaccuracy will be incorporated, particularly where the DBL is thick, as in stagnant waters; this can be minimized by adopting the more rigourous approach of Alfaro-De la Torre et al. (2000), in which different diffusion coefficients are assigned to the gel layer and the combined filter/DBL layer pair. Because the difference between the two approaches will be very small in terms of the C_b that each determines, we describe only the simpler calculation here.

Since flux is equal to mass (M) per unit area (A) per unit time (t), equation (1) can be rewritten as:

$$M = \frac{DC_b tA}{\Delta g} \quad (2)$$

Thus, the concentration of free metal ion in the solution (C_b) to be expressed as:

$$C_b = \frac{M\Delta g}{DtA} \quad (3)$$

where M is the mass of metal accumulated on the resin, t is the deployment time, and A is the surface area of the gel layer. The value of M is determined analytically, typically by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The thickness of the gel layer (Δg) is formed to precise specifications during the manufacturing process, but for accurate assays it should be measured prior to deployment because there can be slight batch-to-batch differences in thickness. The diffusion coefficients of metals in polyacrylamide gel have been measured (Davison and Zhang, 1995) and are typically less than those observed in water, reported by Li and Gregory (1974). As noted above, there can also be differences in diffusion coefficients from one batch of gel to the next. This imposes a constraint on accuracy that can probably be on the order of ±10 %. Because diffusion coefficients are temperature dependent, it is essential to measure the

temperature of the water in which the gel sampler is deployed. The only remaining variable is deployment time, which must be accurately recorded for a reliable measurement. Thus, based on the amount of metal measured on the resin and some simple calculations, the concentrations of labile metal in solution can be estimated.

In cases where the DBL thickness (x) is consequential, equation (3) becomes:

$$C_b = \frac{M(\Delta g + x)}{DtA} \quad (4)$$

Equation (4) has two unknowns (C_b and x); thus, in order to solve for both variables, a second equation is developed through deployment of an additional DGT unit having a different value for Δg (Figure 4-2). For example, if two samplers are deployed having gel layer thicknesses (Δg) of 0.4 and 0.8 mm, respectively, then:

$$C_b = \frac{M(\Delta g + x)}{DtA} = \frac{(M_{0.4})(0.4 + x)}{DtA} = \frac{(M_{0.8})(0.8 + x)}{DtA} \quad (5)$$

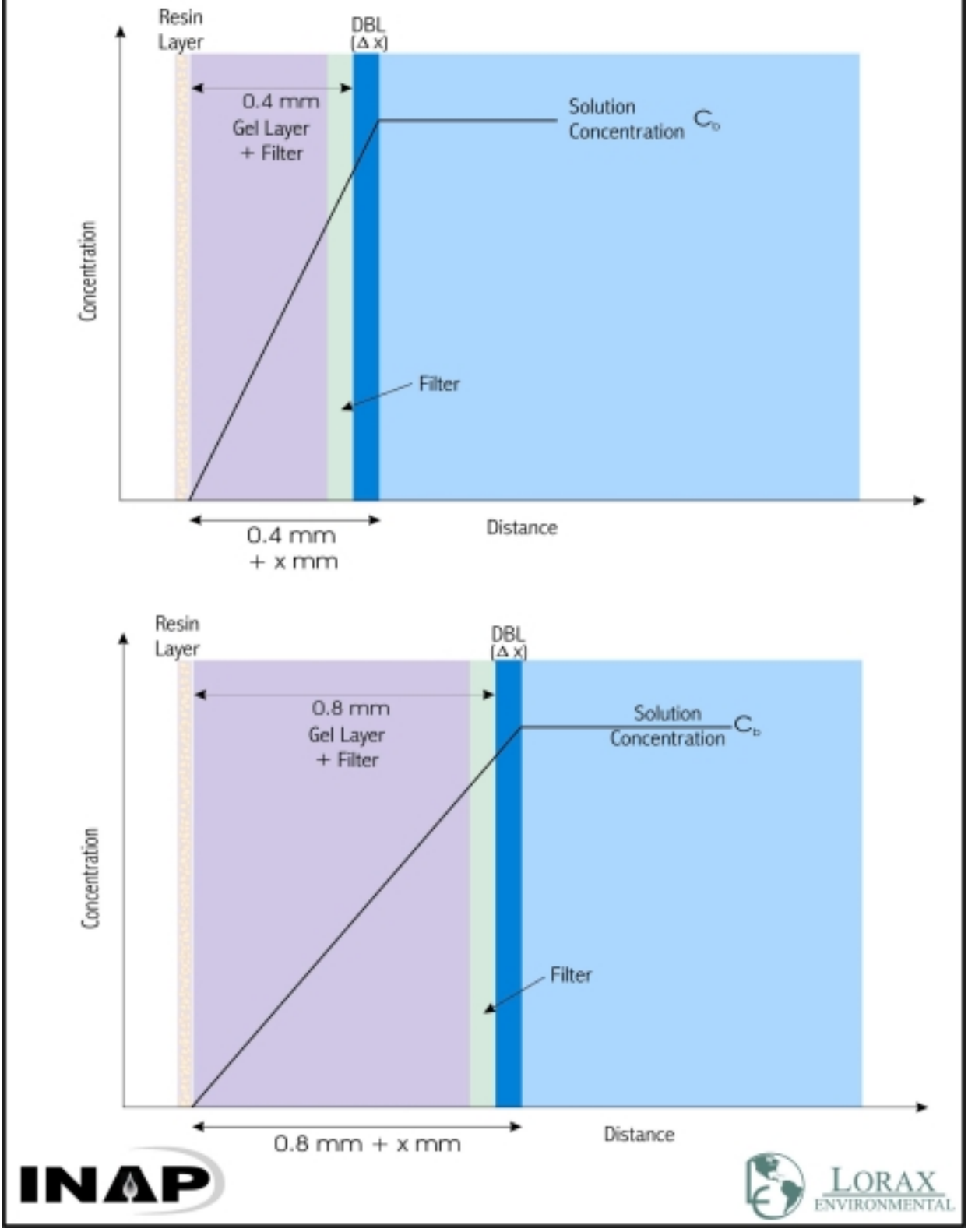
C_b, D, t and A are constants and $M_{0.4}$ and $M_{0.8}$ are measured, simplifying the equation to:

$$x = \frac{(M_{0.4})(0.4) - (M_{0.8})(0.8)}{(M_{0.8} - M_{0.4})} \quad (6)$$

The DBL thickness (x) can now be solved as can the labile metal concentration in solution (Figure 4-2). Note that this calculation ignores the possible effect introduced by increasing the size of the labile metal pool in the thicker gel layer. If significant concentrations of metal complexes in that pool dissociate within the gel and add to the Chelex-bound metal concentration, then C_b can be overestimated. Under these circumstances, Equation 5 would be difficult to solve accurately. Further work is required to determine the importance of this effect in C_b estimates. It should be of most significance where dissolved metal concentrations are low and thus should not be of concern in impacted mine-site regions.

In summary, determining free metal-ion concentrations using DGT requires measurements of: 1) the mass of metal accumulated on the resin; 2) the water temperature; and 3) the deployment time, followed by a series of flux calculations. In stagnant or slow-flowing waters it is necessary to deploy gel samplers of two different thicknesses to reduce uncertainties caused by the diffusive boundary layer. In some cases however, especially where ambient dissolved metals concentrations are low, other uncertainties will be introduced by the dual-thicknesses approach.

Figure 4-2 **Schematic Representation of Multiple Gel Thickness to Correct for Uncertain DBL**



4.2.3 The DGT Sampler

As outlined above, the DGT sampler utilizes a three-layer system, consisting of a resin-impregnated gel layer (metal-binding layer), an outer gel layer (diffusion layer) and a filter membrane. Each layer is cut to specifications and placed in sequence on a plastic base (Figure 4-3). A plastic outer-sleeve is placed over the base in order to secure the layers, to maintain an even surface, and to inhibit water ingress into the resin-gel. Hydrated DGT samplers are stored refrigerated in sealed plastic bags until immediately before deployment. Deployment typically involves suspension of the unit *in situ* or in a stirred solution in the laboratory (or in the field) for a fixed period of time. During the deployment interval, time and temperature are recorded. Upon retrieval, the DGT units are rinsed with distilled deionized water and stored refrigerated in sealed plastic bags to avoid dehydration and potential contamination.

Processing of DGT samplers is performed under class 100 clean-room conditions. The samplers are disassembled and the resin layer analyzed for metal content. Analysis can occur via: 1) acid-extraction of the resin layer followed by metal analysis of the extract (*e.g.*, via ICP-MS); or 2) through direct analysis of the resin beads themselves by techniques such as proton induced x-ray emission (PIXE; Davison *et al.,* 1997a) or laser ablation (Davison *et al.,* 2000). Metal determinations involving direct analysis of the Chelex beads by PIXE or laser ablation are comparatively non-destructive and afford the opportunity to observe spatial distributions of metals. This ability does not offer advantages for water column applications, but does provide the opportunity to study ultra-fine scale structure in certain environments such as microbial mats (Davison *et al.,* 1997a) or sediment pore waters (Harper *et al.,* 1999a) (see Chapter 5).

4.3 DGT Applications

DGT techniques afford wide-ranging applications in various fields of environmental chemistry which utilize measurements of free or labile metal species. The following sections describe the use of DGT as it applies to measurements of speciation (Section 2.3.1), toxicity (Section 4.3.2), complexation kinetics (Section 4.3.3) and complexation capacity (4.3.4). Examples of field and laboratory data are also presented.

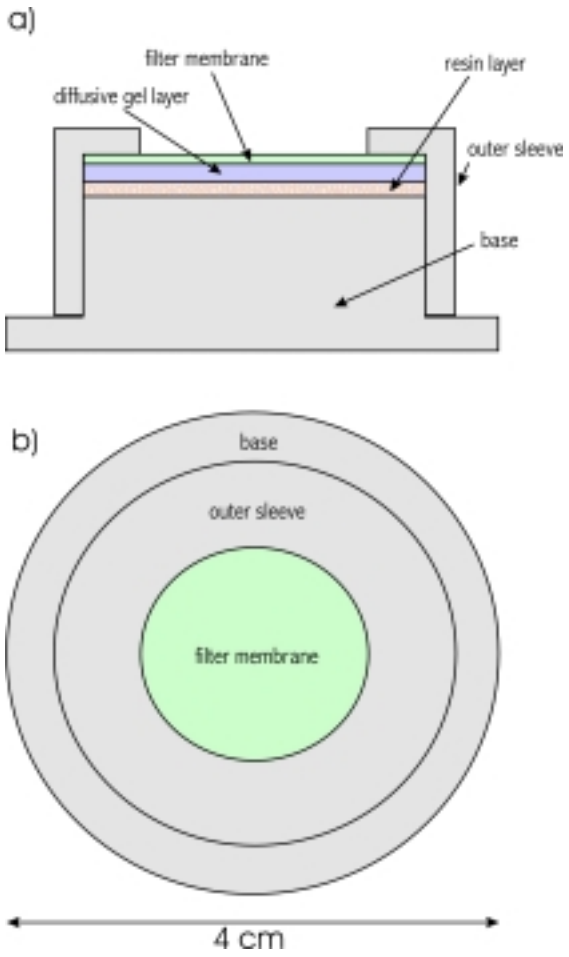


Figure 4-3: Schematic Cross-Section (a) and Plan View (b) of DGT Sampler

4.3.1 The Measurement of Speciation as Determined by DGT

As described above, the DGT sampler detects labile or free metals in solution. In reality, this includes metal ions which are free or weakly complexed and have relatively rapid dissociation kinetics, as well as to some degree, those complexed by ligands of intermediate strength.

Metal complexation in aquatic systems can be represented generically by equation (7), a simple equilibrium relationship,

$$M + L \leftrightarrow ML \tag{7}$$

where M, L and ML are the free metal, total ligand and metal-ligand complex, respectively. A stability constant, k, for the reaction can be defined by equation (8) as,

$$k = \frac{(ML)}{(M)(L)} \tag{8}$$

Metal-ligand interactions associated with DGT samplers can be broadly classified as falling into one of three classes: a) no ligand and/or weak ligands (small K), b) intermediate-strength ligands, and c) strong ligands (large K). The respective concentration distributions through a DGT sampler for the latter three scenarios are illustrated in Figure 4-4.

In the first case, where a metal in solution is either 100% free or only weakly complexed, the metal is free to interact via rapid dissociation with the strongly-sorbing Chelex resin. The Chelex acts as a ligand and quantitatively binds all the free metal, out competing any weak ligands for metal ions. The resulting concentration distribution is illustrated in Figure 4-4, distribution (a).

The metal-resin interaction is defined by equation (9):

$$M + R \leftrightarrow MR \tag{9}$$

where M, R and MR are free metal, resin site and metal-resin complexes, respectively. In the case described above, the equilibrium is strongly biased to the right side of the equation and effectively all of the free metal is bound to the Chelex resin. Where weak metal complexes are involved, the resin effectively out-competes the ligand for the metal. The dissociation kinetics of the ML complex are sufficiently rapid (i.e. the complex is labile) to allow binding by the resin (equation 10):

$$ML + R \leftrightarrow MR + L \tag{10}$$

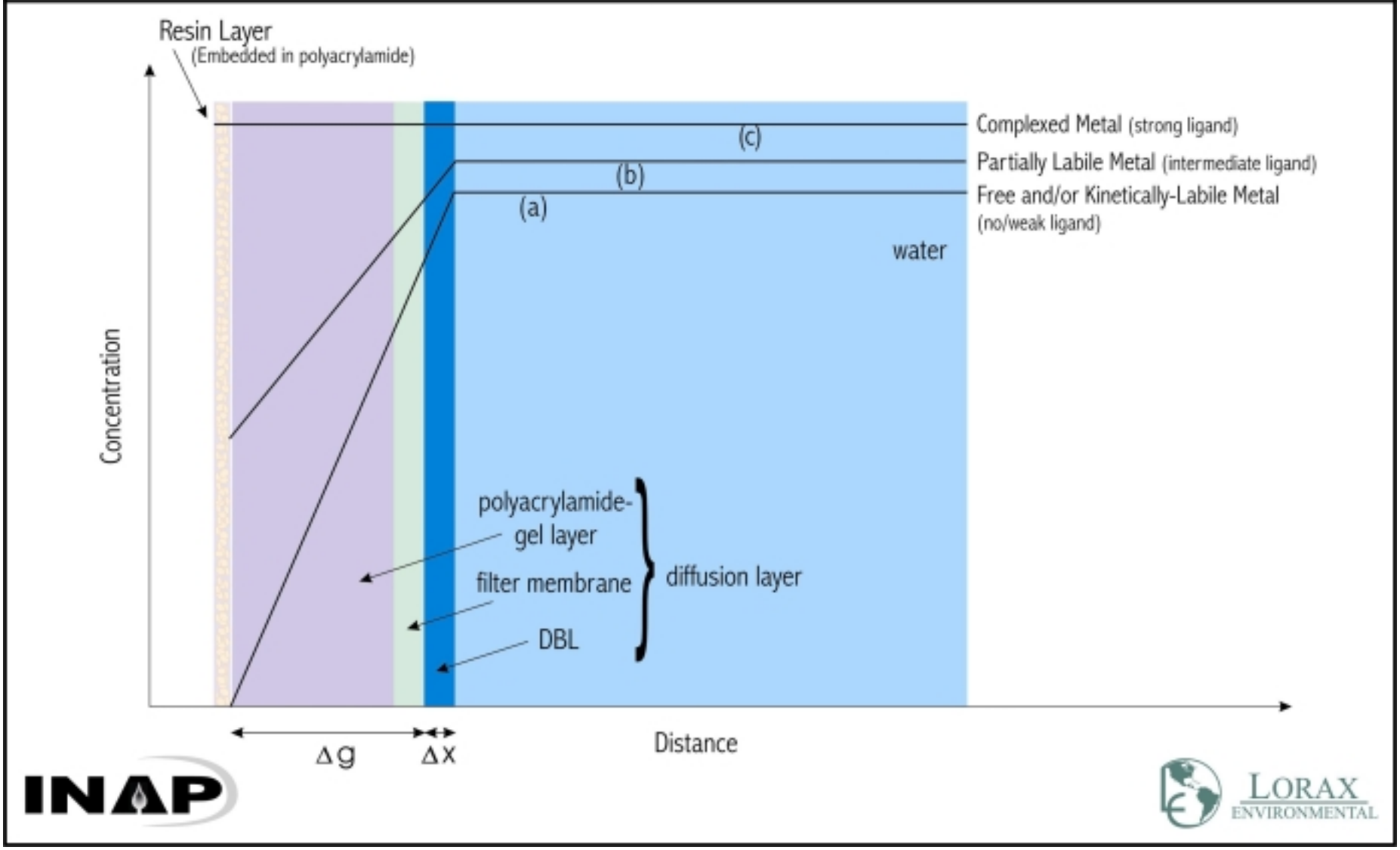
In the second case (example (b) in Figure 4-4), a ligand of intermediate strength is capable of binding the metal in solution more strongly. Since, the resin is capable of binding only free metals in solution, the quantity of metal sequestered by the DGT sampler is a function of the kinetics of ligand-metal exchange, and indirectly on gel thickness. With sufficiently rapid dissociation kinetics for ML, the concentration of the complexes will be lower in the resin layer. This will support diffusion of molecular species through the gel as a function of the square of the path length Δg, viz :

$$t = \frac{\Delta g^2}{2D} \tag{11}$$

where D is the diffusion coefficient and t is the characteristic reaction time. For a diffusive path length of 0.5 mm and a diffusion coefficient of 7 x 10^{-6} cm^2 s^{-1}, the diffusion time (t) of an ion is ~3 minutes, whereas a path length of 1 mm translates to a diffusion time of ~12 minutes. Dissociation kinetics of intermediate strength ligands must be on this order or faster to be detected by the DGT sampler (Zhang and Davison, 1995). Depending on the kinetics of dissociation of the metal ligand complex, the hypothetical concentration distribution of a metal ion in the DGT unit will approximate profile (b) (Figure 4-4).

Figure 4-4

Representation of the Concentration of Free, Partially-Labile and Strongly Complexed Metal Species in a DGT Device and Adjacent Water During Deployment



The final scenario represents the case in which the metal-ligand complex is so strong that there is effectively no uptake of metal in the resin layer, and thus no concentration gradient is established through the gel-layer (distribution (c) in Figure 4-4). In this case, there is no free metal in solution, and thus no uptake by the DGT sampler. Functionally, these three cases of ligand strength are illustrated by the data presented in Figure 4-5 (McNee and Martin, 1999).

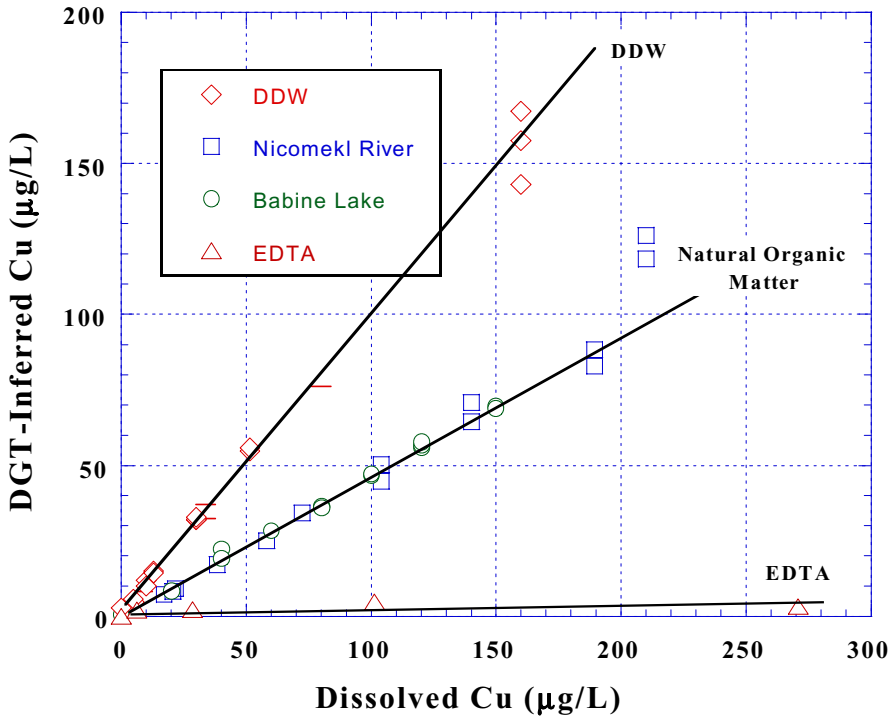


Figure 4-5: DGT-Cu vs. dissolved-Cu addition for various solutions: 1) distilled deionized water (DDW); 2) DOC-rich Nicomekl River water; 3) DOC-Rich Babine Lake water; and 4) DDW with excess EDTA (from McNee and Martin, 1999). The experiments with Babine Lake and Nikomekl River waters were carried out in each case using a mixture of 10% natural water and 90% DDW. pH in both experiments was ~6.5.

DGT data are presented for a range of Cu additions to solutions containing no ligands (DDW), intermediate-strength ligands (natural organic matter) and strong ligands (EDTA). In each case, the data are characterized by linear distributions of DGT-inferred Cu concentration *vs*. Cu addition. The slope of the regression is indicative of the strength of the ligand-metal complex. This notion is described in greater detail below within the context of metal complexing capacity (Section 4.3.4).

Although the DGT approach does not permit rigorous quantification of multiple metal species, the technique does permit a qualitative assessment of speciation beyond that of "free vs. complexed". For example, changing parameters such as type of resin or binding agent, the thickness of the diffusion layer and the effective pore-size of the diffusion layer can influence which species are detected or assimilated by the sampler. While such variations are somewhat academic and outside the scope of most mining applications, they are described below for the sake of completeness.

The Nature of the Polyacrylamide

Two aspects of the polyacrylamide diffusion-layer influence diffusion within the DGT sampler: 1) the thickness of the gel-layer; and 2) the degree of cross-linking within the polymer (*i.e.*, pore size). By varying these characteristics, it is possible to differentiate and quantify labile-inorganic and organically-bound metals (Zhang and Davison, 2000).

The rate of diffusion of ions through the gel, as well as the maximum size of metal complexes allowed to pass, is dependent on the effective pore size of the polymer and the size of the complex. The pore size of the gel can be varied by altering the polyacrylamide recipe. The "standard" composition of gel (Zhang and Davison, 1995) permits the diffusion of free ions and complexed species of up to several hundred thousand atomic mass units (Dunn, 1993). By increasing the content of cross-linker, the effective pore size can be reduced and tortuosity increased such that the diffusive transport of metals complexed by organic matter is impeded more than the diffusion of simple ions.

In order to measure both inorganic and organic metal species in an aquatic system, it is necessary to deploy multiple DGT units that have markedly different pore sizes. Specifically, one DGT unit would present an open matrix that allows the free diffusion of all species, while a second sampler would utilize a more restrictive pore size that retards the diffusion of most organic complexes (Davison and Zhang, 2000). When the two samplers are deployed under identical conditions, the mass of metal on each device (M_{DGT}) can be defined as the sum of the inorganic and organic species:

$$M_{DGT} = M_{inorg} + M_{org} \tag{12}$$

where M_{inorg} and M_{org} represent the masses of inorganic and organic metals sequestered by the sampler, respectively. In accordance with equation (2),

$$M_{inorg} = \frac{D_{inorg} C_{inorg} At}{\Delta g} \tag{13}$$

$$M_{org} = \frac{D_{org} C_{org} At}{\Delta g} \tag{14}$$

where C_{inorg} and C_{org} are the respective concentrations of labile inorganic and organic metal complexes in solution. Equation (12) becomes:

$$M_{DGT} = \frac{(D_{inorg}C_{inorg} + D_{org}C_{org})At}{\Delta g} \quad (15)$$

Rearranging, equation (15) becomes:

$$\frac{(M_{DGT})\Delta g}{At} = C_{org}\left(\frac{D_{org}}{D_{inorg}}\right) + C_{inorg} \quad (16)$$

Thus, plotting $\frac{(M_{DGT})\Delta g}{At}$ *vs.* $\left(\frac{D_{org}}{D_{inorg}}\right)$

should produce a linear relationship in which the slope yields the concentration of organic species (C_{org}), while the y-intercept represents the concentration of inorganic species (C_{inorg}) (Zhang and Davison, 1999; 2000).

Thus, if the diffusion coefficient of the organic species is known, the total amount of metal sequestered by the gel can be separated into a contribution from inorganic (rapidly-diffusing) and organic (slowly-diffusing) fractions. This approach has resulted in determinations of labile Cd concentrations that agree extremely well with independent determinations by anodic stripping voltammetry (Zhang and Davison, 2000).

An alternative parameter that may be altered in the DGT sampler is the thickness of the gel-layer. Since diffusive transport through the gel limits metal sequestration by the resin to those complexes that can dissociate on the timescale of diffusion, metal-ligand complexes with slow dissociation kinetics are largely undetected by the DGT approach. It is therefore possible to alter the types of species which are assimilated by the sampler by varying the thickness of this layer; a thinner gel-layer biases the sampler towards the exclusion of progressively less labile metal species (Davison and Zhang, 2000).

The Nature of the Resin

While most applications of DGT have involved the use of Chelex 100, and hence have focused on trace metals, it is possible in principle to use any one of a number of binding agents in order to measure a variety of solutes. For example, certain exchange resins have been used for ^{137}Cs and Sr (Davison *et al.,* 2000 Chang *et al.,* 1998). Cs, for example, has been detected using ammonium molybdophosphate (AMP) as the binding agent (Chang *et al.*, 1998) and ferrihydrite has been used to fix phosphate (Zhang *et al.,* 1998a; 1998b). Yet another application involves the use of silver iodide for the detection of free sulphide (Teasdale *et al.,* 1999; Teasdale, in press). Regardless of the specific binding

agent utilized, all approaches rely on molecular diffusion of labile species through the gel layer to quantify solute uptake.

4.3.2 Toxicity

Several investigations have demonstrated that DGT provides a quantitative measure of labile (free or kinetically-labile) metal species in aquatic systems (*e.g.*, McNee and Martin, 1999). Given that the toxicity of many metals (*e.g.*, Cu, Ni, Cd, Pb) has been shown to be a function of the free-ion activity (see Chapter 2), it is possible that that DGT provides a more representative indicator of bioavailability, and hence, toxicity than assays of total dissolved metal concentration. However, few data currently exist which directly compare measurements of DGT and toxicity to aquatic biota. In the following section, the results from a series of experiments conducted by Lorax are presented which relate metal toxicity to the metal fraction as defined by DGT. Specifically, experiments were conducted in which various test organisms (*Daphnia magna* and rainbow trout) were exposed to varying concentrations of dissolved copper, both in the presence and absence of copper complexing agents (ligands). Both synthetic and natural ligands were used. In each experiment, both DGT-Cu and dissolved Cu were determined. In this manner, toxicity measured via standard LC50 approaches could be compared with both dissolved and DGT-inferred metal concentrations.

Experiments using the synthetic ligand EDTA (ethylenediaminetetraacetic acid) were conducted with both *D. magna* and rainbow trout (*Salmo gairdneri*). In experiments with *D. magna*, forty-eight-hour bioassays were carried out with dissolved Cu concentrations ranging from 0 to ~500 μg/L, both with and without a 7-fold molar excess of EDTA (Figure 4-6). The respective concentrations were chosen to bracket the range of dissolved Cu levels encountered in contaminated waters, and to illustrate clearly the applicability of DGT in estimations of bioavailability in the presence of significant concentrations of complexing ligands. In the absence of EDTA, dissolved and DGT-inferred Cu concentrations generally agreed within 10%, indicating that in the absence of metal-complexing ligands, the DGT technique is capable of reasonably precise quantification of the dissolved Cu concentration (Figure 4-6a). With no EDTA added, daphnid survival decreased with increasing Cu concentrations; the LC_{50} was calculated to be ~12 μg/L (Figure 4-6b). Conversely, in the presence of excess EDTA, DGT-inferred concentrations were significantly reduced in comparison to the total dissolved fraction (Figure 4-6a). Such observations reflect the strength of Cu-EDTA complexes and lack of sorption of such species by the DGT device. In the presence of EDTA, *D. magna* exhibited 100% survival at all dissolved Cu concentrations, indicating that Cu-EDTA complexes were also unavailable to the test species (Figure 4-6b).

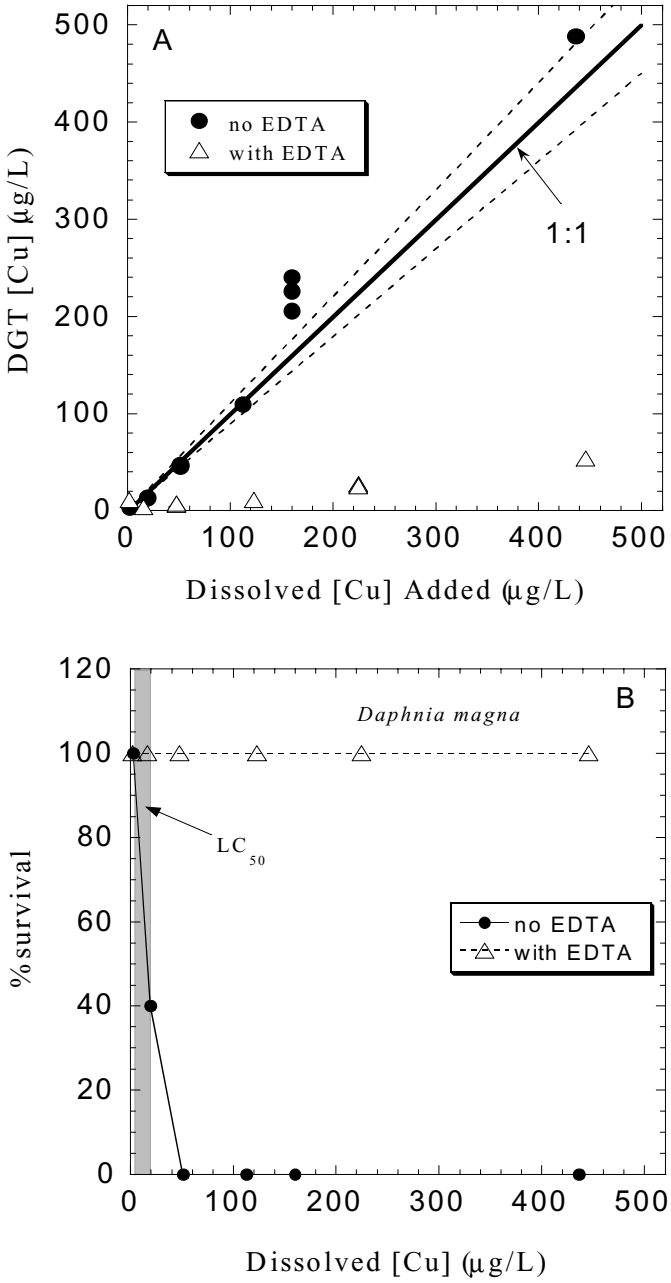


Figure 4-6: ***Daphnia magna*** **48 h bioassay: a) Copper concentrations determined by DGT vs. dissolved concentrations, for solutions with no EDTA and with a seven-fold molar excess of EDTA. The dashed lines represent 10% error boundary in 1:1 slope; b) percent survival in test cultures of** ***Daphnia magna*** **vs. dissolved Cu concentration for solutions without added EDTA and with a seven-fold molar excess of EDTA. Estimate of LC_{50} is represented by the shaded area (Lorax, unpublished data). For both a and b, test solutions (pH = 7.5) consisted of moderately hard (100 mg/L as $CaCO_3$), Chelex-cleaned reconstituted fresh water and was prepared by adding 96 mg/L $NaHCO_3$, 60 mg/L $CaSO_4 \cdot 2H_2O$, 60 mg/L $MgSO_4$, and 4.0 mg/L KCl .**

Similar to the daphnid experiment, bioassays using rainbow trout were carried out with dissolved Cu concentrations ranging from 0 to ~500 µg/L, both with and without a 7-fold molar excess of EDTA (Figure 4-7). As for the daphnid bioassay, dissolved and DGT-inferred Cu concentrations exhibited a near 1:1 relationship in the absence of EDTA (Figure 4-7a). With no EDTA, trout mortality increased progressively with the amount of Cu added; the LC_{50} was 53 µg/L (Figure 4-7b). In the presence of excess EDTA, DGT-inferred Cu concentrations were significantly reduced (Figure 4-7a); trout exhibited 100% survival throughout the entire concentration range (Figure 4-7b). The results are consistent with the daphnid experiment, and indicate that Cu-EDTA complexes are largely unavailable to both DGT and aquatic biota. In other words, the concentration of "available" metal recorded using the DGT approach is consistent with the "available-metal" moiety assimilated by the aquatic organisms used in the Lorax experiments.

Experiments using natural ligands were conducted with waters collected from the Nicomekl River (Figure 4-8; Lorax, unpublished data), a meandering watercourse that drains low-relief marshland regions of the Fraser River delta, near Vancouver, B.C. The waters are heavily stained, and contain abundant DOC(~8 mg/L). Forty-eight-hour bioassays with *D. magna* were conducted using Nicomekl River water to which was added dissolved Cu concentrations ranging from 0 to 220 µg/L. As a control, identical bioassays were conducted in Nicomekl-free growth medium (Chelex-cleaned, reconstituted moderately hard freshwater as described in Figure 4-6) with Cu concentrations ranging from 0 to ~50 µg/L. In the absence of Nicomekl water (*i.e.*, growth medium only), dissolved and DGT-inferred Cu concentrations agreed to within ~10%, demonstrating that all of the Cu added was in a labile form (Figure 4-8a). In this ligand-free scenario, the LC_{50} was determined to occur at a dissolved Cu concentration of 10.8 µg/L (Figure 4-8b). In the presence of DOC-rich Nicomekl River water, DGT-inferred Cu concentrations were significantly reduced (Figure 4-8a). The reduction of DGT-inferred Cu by roughly a factor of two reflects the formation of Cu-organic complexes which are not sequestered as readily by the DGT device in comparison to the free metal ion (Figure 4-8a). Cu bound to organic matter will also diffuse more slowly than inorganic complexes of Cu or free ion, further reducing the sequestration of the metal by the gel-resin. Toxicity towards daphnids in Nicomekl River waters was reduced considerably in comparison to the growth medium. *D. magna* exhibited 100% survival at dissolved Cu concentrations up to ~100 µg/L, decreasing to 0% survival at a concentration of 210 µg/L (Figure 4-8b). The corresponding LC_{50} was calculated to occur at a dissolved Cu concentration of 170±20 µg/L. Such enhanced survival is consistent with the reduction in the concentration of labile-Cu in the river medium as determined by DGT.

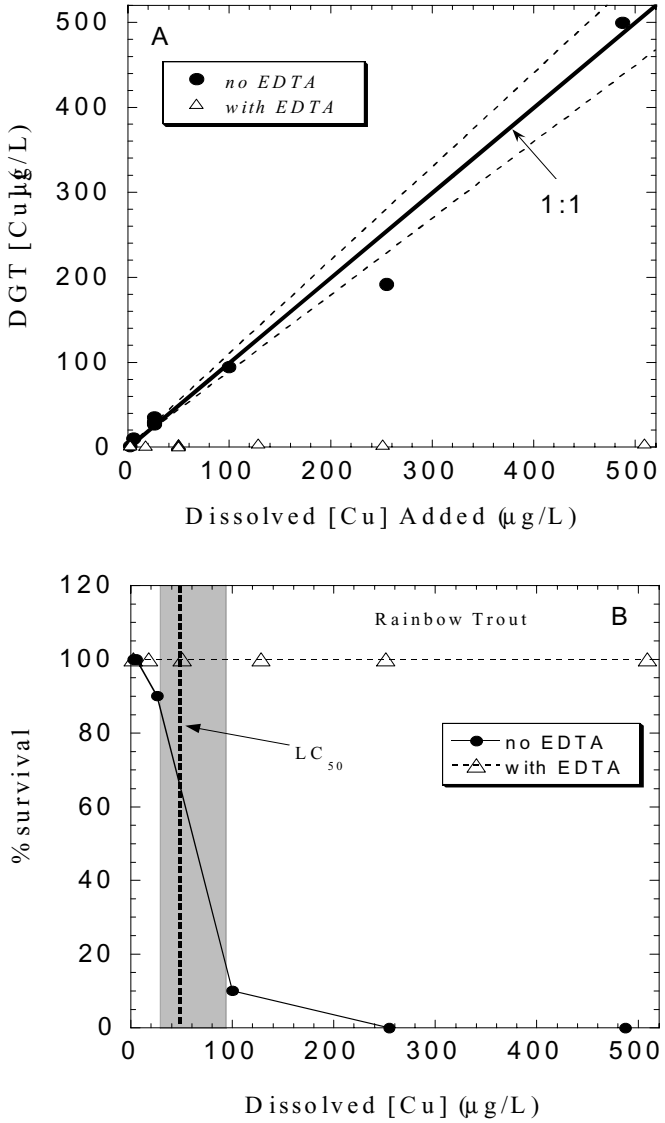


Figure 4-7: Trout 96 h Bioassay, run at pH = 7.5: a) copper concentrations determined by DGT vs. dissolved concentrations, for solutions with no EDTA and with a seven-fold molar excess of EDTA. The dashed lines represent 10% error boundary in 1:1 slope; b) percent survival in test cultures of Rainbow Trout vs. dissolved Cu concentration for solutions without added EDTA and with a seven-fold molar excess of EDTA. The LC_{50} is denoted by the dashed vertical line, while the uncertainty in this value (90% confidence interval) is represented by the shaded area (Lorax, unpublished data). The composition of the solutions was the same as that described in the caption to Figure 4-6.

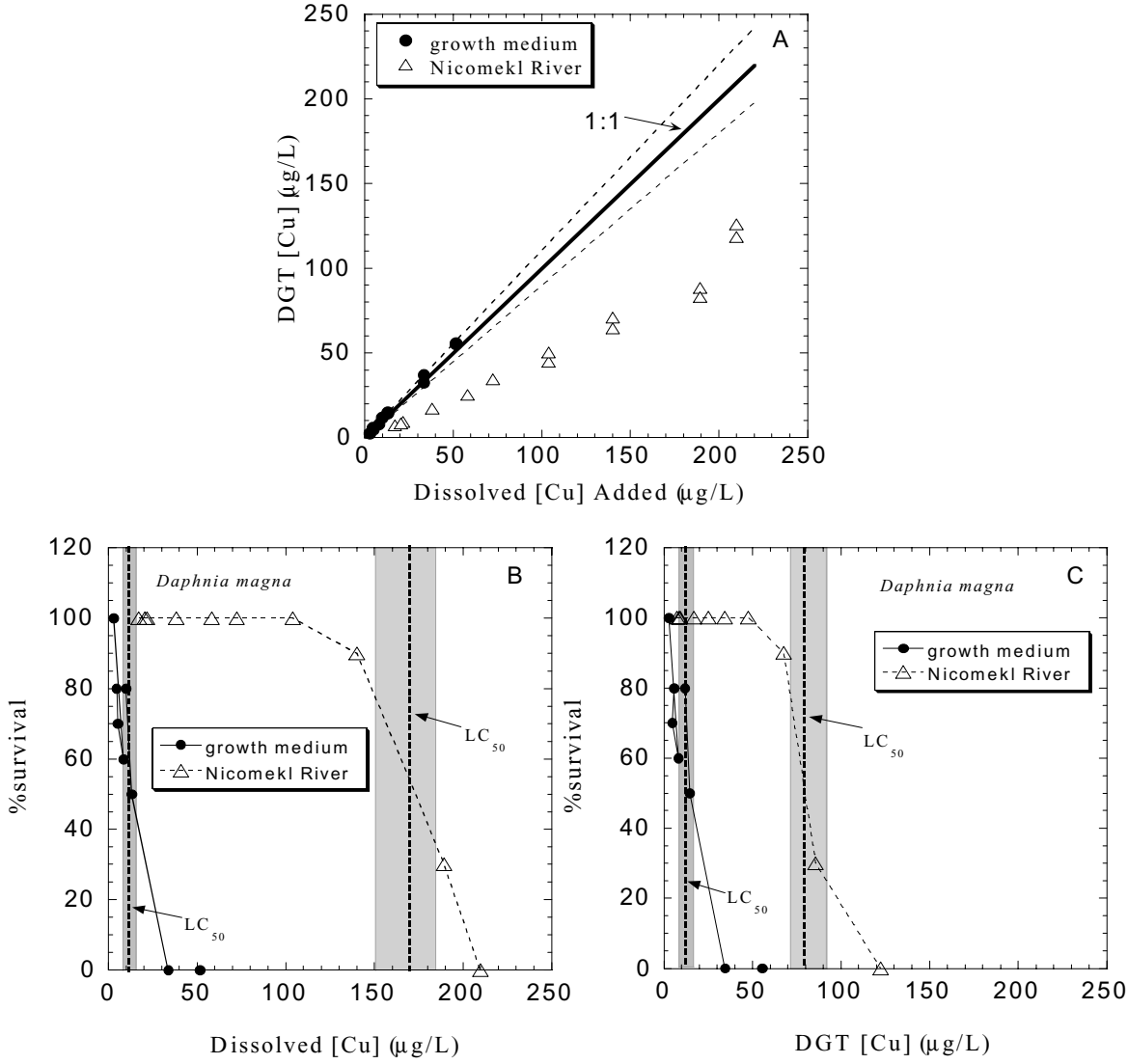


Figure 4-8: Nicomekl River *Daphnia magna* Bioassay: a) copper concentrations determined via DGT vs. dissolved concentrations for growth medium and DOC-rich Nicomekl water (the dashed lines represent 10% error boundary in 1:1 slope); b) percent survival in test cultures of *D. magna* vs. dissolved Cu concentration for growth medium and in Nicomekl River water; c) percent survival in a *D. magna* test culture vs. DGT-inferred Cu concentrations, in growth medium and in Nicomekl River water. LC_{50} values are denoted by the dashed vertical lines, while the uncertainty in these values (90% confidence interval) is represented by the shaded areas (Lorax, unpublished data). Growth medium consisted of Chelex-cleaned moderately-hard reconstituted freshwater as described in Figure 4-6.

The DGT-inferred concentrations at which mortality occurred in the river waters were significantly higher than the levels at which mortality occurred in the absence of added ligands (Figure 4-8c); the LC_{50} in the river medium (DGT-Cu = 80 μg/L) was approximately 7 times greater than that calculated for the growth medium (DGT-Cu = 11 μg/L). Such observations imply that DGT sequesters some metal species that are unavailable to aquatic biota, i.e. labile species, not just free ion. In this example, therefore, DGT provides a conservative estimate of toxicity.

In summary, the DGT/bioassay experiments suggest that DGT provides a more representative measure of metal bioavailability, and hence toxicity, than conventional measurements of dissolved species. Specifically, the results indicate that metal uptake by DGT and aquatic biota is reduced in the presence of metal-complexing ligands. The results also highlight the limited utility of total and total dissolved Cu concentrations as a proxy for the bioavailable fraction in waters that contain significant concentrations of Cu-complexing ligands.

4.3.3 Kinetics of Complexation

The principal application of DGT in environmental studies has been its use as an *in situ* tool for assessing metal speciation in aquatic systems. As outlined above, DGT provides a general measure of the biologically available, or "labile" concentration of metals in solution. In addition to speciation measurements, DGT may also be used to examine the kinetics (*i.e.*, rate) of metal-ligand complexation. When a metal is added to a solution containing metal-complexing ligands, the concentration of free-metal ion will progressively decrease until an equilibrium is established between ion and ligand. Since DGT provides a measure of the labile-metal concentration, the rate at which this fraction decreases can be used to quantify the rate of the association reaction. Kinetic considerations have considerable relevance to predictions of metal toxicity in receiving waters into which mining-related effluents are discharged. The following section discusses the applicability of DGT to studies of metal-ligand complexation kinetics, with emphasis on the application to the mining industry. The discussion is supported by experimental data acquired by Lorax.

Metal speciation in natural waters varies markedly, and is affected by several variables including the types and concentrations of metals and ligands present, salinity, pH, pE, biological processes, particle concentration, *etc*. Accordingly, the speciation of metals originating from mining-related sources will respond to the specific physical and chemical conditions of the receiving waters. The “free ion” concentration (and hence, toxicity, according to the Free Ion Activity Model (Campbell, 1995) can be significantly reduced, for example, when ligands (*e.g.*, dissolved organics) are present in excess of the metal concentrations. Given the importance of metal speciation in determining toxicity,

an understanding of the former is fundamental to establishing more accurate estimates of assimilative capacity, which can be used to develop site-specific discharge criteria. In this respect, an important consideration is the rate at which uncomplexed (and potentially toxic) metals in effluent are transformed into complexed (less toxic) metal by the ligands in the receiving waters. While thermodynamic data provide information with respect to tendency of a reaction to occur, kinetics dictate the rate of metal-ligand reactions. Kinetic considerations have particular relevance to the discharge of mining-related effluents, the toxicity of which may vary over time and space (*e.g.*, downstream) in the receiving environment. This is particularly important for metals such as Ni, which are characterized by slow complexation kinetics (Bedsworth and Sedlak, 1999).

In order to demonstrate the ability of DGT to assess the kinetics of metal complexation, two experiments were conducted with mine effluent (Lorax, unpublished data). In the first experiment, a synthetic ligand (EDTA) was added in varying concentrations to the mine effluent and the DGT-Ni fraction measured after 24 h and 14 days. The EDTA was added such that a "free metal " gradient was established, with progressively less free metal available with increasing EDTA concentration. Measured DGT-Ni concentrations were compared to those predicted assuming instantaneous equilibrium between EDTA and the metals present. Inspection of the results indicates that one day after EDTA addition, DGT-inferred Ni concentrations were significantly higher than the predicted equilibrium values (Figure 4-9). Although there is little difference between predicted and observed (by DGT) values at [EDTA] < 1 mg/L, the DGT-Ni value of ~36 µg/L observed at an EDTA concentration of 3 mg/L is over two orders of magnitude greater than the predicted value. Similarly, at [EDTA] = 10 mg/L, the predicted $[Ni]_{free}$ is far lower than that assayed by DGT. In contrast, the DGT-inferred Ni concentrations after 14 days were much closer to the levels “predicted” (Figure 4-9). The DGT results demonstrate the apparent importance of Ni-ligand exchange kinetics with EDTA.

In the second experiment, a range of EDTA concentrations was added to mine effluent and the DGT-inferred concentrations determined in all solutions after a 24-hour incubation period. In this manner, the time-dependence of DGT-inferred Ni concentration could be modelled as a function of the EDTA concentration, assuming first-order reaction kinetics. Such modelling provides evidence of slow reaction kinetics for the formation of Ni-EDTA complexes (Figure 4-10). With increasing concentrations of EDTA, DGT-inferred Ni concentrations decrease gradually and non-linearly from a value equal to the dissolved concentration (~65 mg/L) at no EDTA to a minimum of ~3 µg/L in the presence of 18 mg/L EDTA (Figure 4-10). The non-linearity of the response curve suggests that the rate of Ni-EDTA complex formation is kinetically controlled, and follows first-order kinetics . Specifically, Ni-EDTA complex formation is faster at higher concentrations of EDTA, with the concentration of uncomplexed Ni (*i.e.*, DGT-inferred)

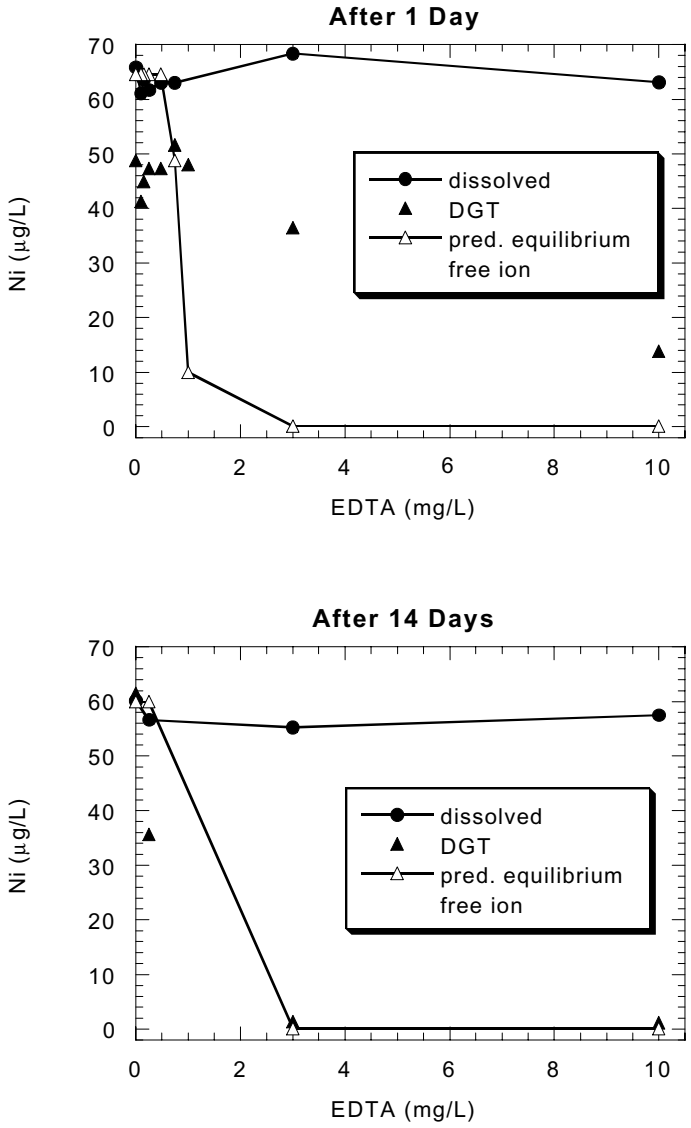


Figure 4-9: Dissolved, DGT-inferred and predicted (at equilibrium) Ni concentrations in mine effluent, 1 and 14 d after addition of EDTA. The mine effluent consisted of circumneutral pH tailings pond overflow. Predicted Ni concentrations represent those based on thermodynamic equilibria.

increasing with decreasing EDTA concentration (Figure 4-10). If kinetics were not important (*i.e.*, assuming an instantaneous reaction), the concentration of DGT-inferred Ni would decrease linearly with increasing EDTA concentration to values <0.1 µg/L. Thus, although Ni-EDTA complexes are very strong, the kinetics of formation appear to be slow, with complete equilibration requiring days to weeks. Such observations have been reported elsewhere (Bedsworth and Sedlak, 1999; Hudson, 1998).

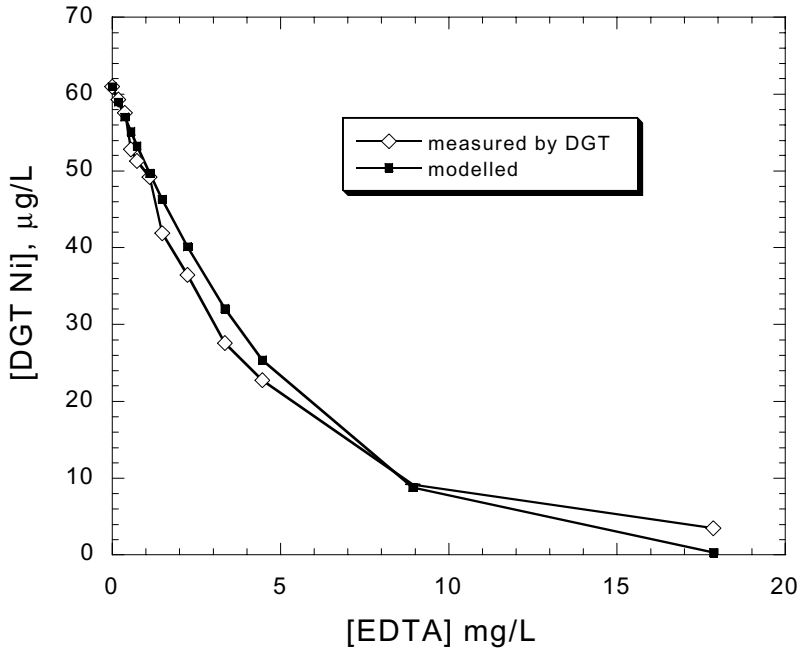


Figure 4-10: Measured "DGT"-Ni and modelled "free"-Ni as a function of EDTA concentration. Measured values represent the DGT fraction after a 24 h incubation period with EDTA. The model assumes complexation occurring for 24 hours according to first-order kinetics with a Ni complexation rate constant that is directly proportional to the EDTA concentration. Note that the precise rate law for Ni-EDTA complex formation is not known, but first-order reaction kinetics are likely.

In summary, the results suggest that DGT offers potential to assess the kinetics of formation of metal-ligand complexes. The experimental results also indicate that speciation (and hence toxicity) cannot be predicted from equilibrium considerations alone. This DGT application has several potential applications in the mining sector. For example, at sites which discharge effluent to receiving waters, an understanding of how rapidly introduced metals interact with natural ligands could aid in:

1. determination of effluent toxicity over time;
2. assessment of effluent toxicity with distance downstream of the discharge point;
3. the development of site specific discharge limits;
4. determining the time interval required for effluent treatment processing before discharge to the environment. EDTA, for example, may be added to effluent process streams to reduce the bioavailability (*i.e.*, toxicity) of the final discharge. Given the slow reaction kinetics of some metals with EDTA (*e.g.*, Ni), a sufficient time period is necessary to ensure equilibration; and

5. validating toxicity tests outlined by regulatory bodies (*e.g.*, MISA). For example, systems which exhibit slow reaction kinetics between metals and ligands in receiving waters may yield differing LC50 values depending on the time period between sample collection and toxicity tests. The kinetics of metal complexation will dictate the rate at which toxicity is ameliorated, and thus, will aid in determining most appropriate time interval in which to conduct toxicity tests.

4.3.4 Complexation Capacity

The ability of an aquatic system to complex metals in solution (*i.e.*, complexation capacity) if often used in environmental impact studies to assess the assimilative capacity of receiving waters (Carter *et al*., 1992; Pardo *et al.*, 1994; Yang *et al.*, 1999). It is commonly accepted that in the presence of complexing agents (most commonly dissolved organic matter), metal toxicity is ameliorated, and thus the assimilative capacity of the receiving environment is enhanced. Furthermore, it is also understood that the degree to which toxicity abatement takes place depends largely on the quantity and type of organic matter in receiving waters. Accordingly, estimates of assimilative capacity are sometimes made in which a metal-specific complexing capacity is assigned to a receiving water body; that value is then applied to adjust receiving-water criteria. Unfortunately, the notion of complexing capacity is often misunderstood to the degree that a measured value is broadly used to adjust receiving-water criteria without regard to considerations of both ligand concentration and ligand affinity.

There are two salient considerations in regards to the degree of metal complexation: 1) the concentration of the ligands present; and 2) the ligand binding strength for the metal of interest. For example, a large concentration of a weak or intermediate-strength ligand (*i.e.,* one that allows a significant fraction of free metal in solution) is often less effective at ameliorating toxicity than a low concentration of a strong ligand. Figure 4-11 illustrates this notion with three hypothetical cases: no ligand/weak ligand, a ligand of intermediate strength, and a strong ligand. The plot is a representation of "free" metal concentration as a function of incremental metal additions to the ligand-containing solution. In the case of no ligand or a weak ligand, all of the metal added to solution is accounted for in the "free" metal inventory. "Free" metal is used here to connote organically-uncomplexed ions, but may include hydroxo-complexes. Given this caveat, there is no effective complexing capacity and the slope of the data regression is unity (*i.e.*, 1:1). In the case of an intermediate ligand, only a fraction of the added metal can be allocated to the free inventory. Once all ligand sites are saturated (the break in the slope; Figure 4-11), the adsorption capacity is exceeded and any additional metal is quantitatively added to the free inventory (*i.e.,* the slope of the line adopts a 1:1 slope at dissolved M^{2+} values >~180 μg/L). In the strong ligand case, very little of the added metal occurs in the free pool until all sites are saturated, at which point any additional metal is quantitatively added to the free inventory.

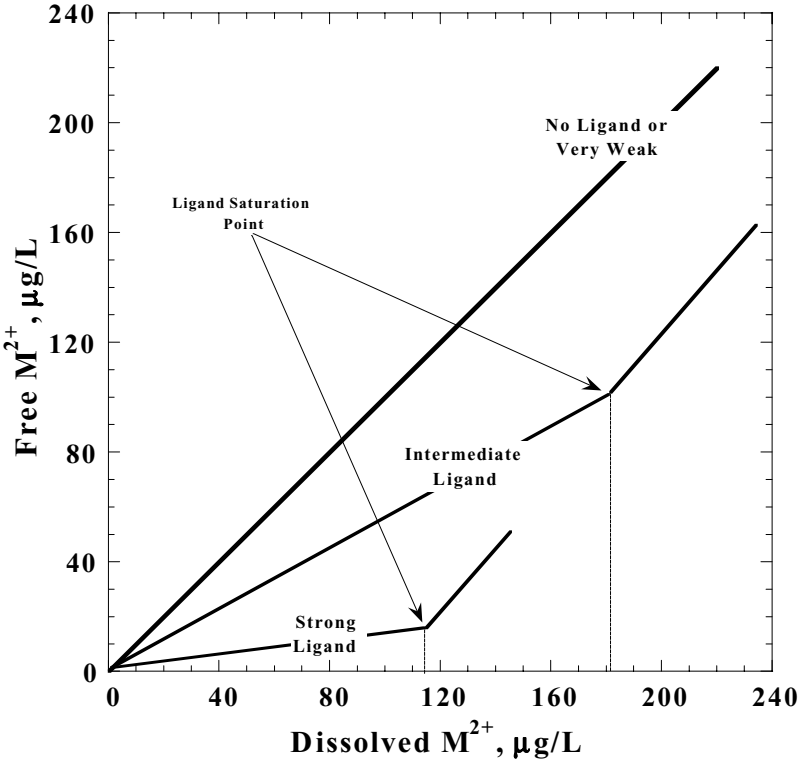


Figure 4-11: Hypothetical representations of metal complexing capacity for ligands of varying strengths and concentrations

Complexation-capacity data were calculated for Nicomekl River water (Lorax, unpublished data), which contains dissolved organic concentrations of ~8 mg/L. Varying amounts of dissolved Cu were added to the river water, with DGT samplers deployed in each solution (Figure 4-12). In all solutions, the DGT-inferred Cu concentration was 50% of the dissolved concentration, suggesting that: 1) 50% of the Cu was present as free ions; or 2) all of the Cu was organically complexed and the diffusion coefficient of the Cu-organic complexes was 50% lower than that of free Cu. In either case, ligand sites appear to have been saturated at a dissolved Cu concentration of ~200 µg/L, although this supposition is based on a single replicated sample. In this case, the Cu complexing capacity could be described as having a complexation "strength" of 50% to a maximum dissolved Cu concentration of 200 µg/L.

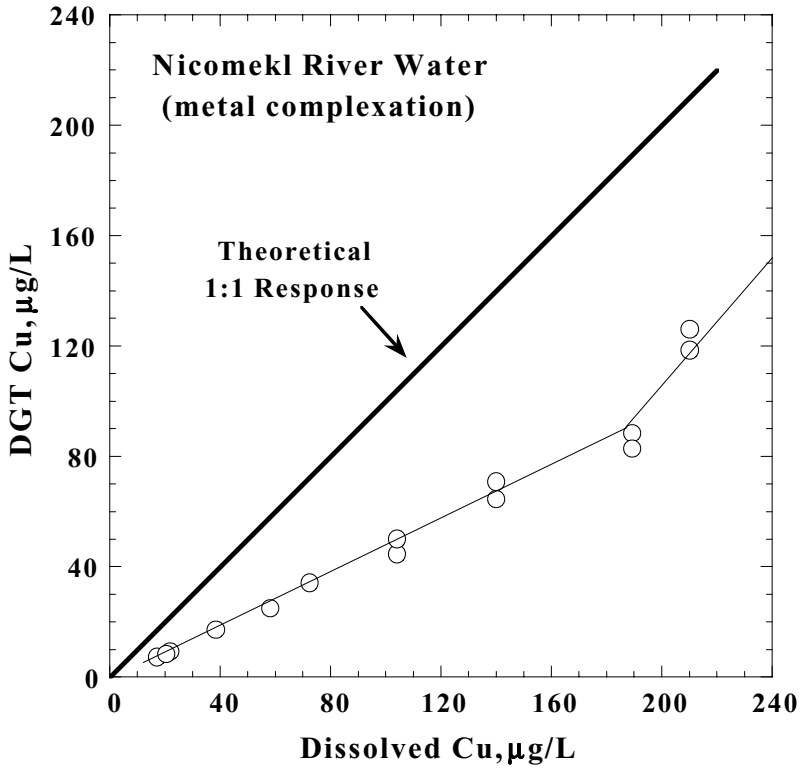


Figure 4-12: Copper complexing capacity data for the Nicomekl River, B.C. Natural DOC from this system can be represented as a ligand of intermediate strength. Nicomekl River waters are characterized by circumneutral pH.

4.4 Considerations and Limitations

DGT is a promising tool for various applications in aquatic settings where quantification of the free-ion, or more accurately, labile-metal, activity is required. It offers the advantages of relative ease of use, reliability in a spectrum of environments (*e.g.*, varying salinity), low risk of contamination, and high sensitivity (*i.e.*, potential for low detection limits). However, in order to acquire meaningful data, certain considerations and limitations must be acknowledged, most of which can be easily negotiated if the site-specific characteristics of the sampled environment are considered, as discussed in the following section.

Diffusive Boundary Layer

As discussed in Section 4.2.2, the presence of the diffusive boundary layer serves to increase the diffusive path-length over which ions must travel in order to be fixed by the DGT sampler. The thickness of the DBL under conditions of flow (*i.e.*, order cm/sec) is sufficiently small such that impacts on metal uptake are negligible. However, as flow diminishes, the DBL thickens and multiple samplers having differing gel-layer thicknesses must be deployed in order to calculate the DBL correction factor (see Section

4.2.2 for DBL correction). Note that dissociation kinetics for certain metal complexes will affect the mass of metal sequestered by the resin in a DGT sampler. Thicker gel-layers will permit more extensive dissociation of metal and its uptake by the Chelex. This poses a slight complication, as discussed earlier (Section 4.3.1).

Biofouling

A related consideration for long-duration deployments of DGT samplers in natural waters is that of biofouling, or the progressive accumulation of algal growth on the active sampler surface. The primary impact of such growth arises from its influence on the DBL; algal films typically limit metal transport to molecular diffusion. If long-term deployments in productive waters are anticipated , it is prudent to deploy multiple configurations of DGT samplers in order to correct for any deviation of DBL thickness. In addition, biofilms can serve as sorption sites for dissolved metals, and as a result, can lead to underestimates in the free-metal ion concentration measured by DGT. In general, DGT deployment times should be minimized by taking into account the nature of the environment sampled and the sensitivity of the analytical methods employed.

Temporal Integration of Data

Implicit within the DGT approach is the notion of time integration. DGT integrates the concentration of labile metals in the test solution throughout the deployment period. Under quiescent conditions, where variability in water quality is low, the data afforded by DGT are comparable to more conventional sampling approaches, which are more instantaneous in nature. In other words, an instantaneous grab sample is representative of a time-integrated sample only when the variability in water quality is low on the time-scales of DGT deployment. If, however, an aquatic system hosts some degree of variability (such as is often the case where effluents are discharged into a natural water course), then grab samples are not comparable to the time-integrated assay yielded by DGT.

These notions are illustrated in Figure 4-13, which presents variation in a hypothetical water quality parameter with time. The shaded regions represent intervals of deployment for a DGT sampler, while the open circles represent water quality as determined by a grab sample collected at the onset of sampler deployment.

The two deployments illustrated in Figure 4-13 show how, in a watercourse that has significant variability, a comparison between an instantaneous grab sample and a DGT sampler can lead to erroneous interpretations that have no relation to bioavailability. In the first deployment, the gel sampler concentration (dashed line) is greater than that of the water quality parameter. In the second deployment, the reverse is true. In both cases, the concentrations of the water quality parameter, as determined by one grab sample, do not

reflect the conditions "seen" by the DGT sampler. The significance of this effect depends on the magnitude and frequency of the variability and is minimized in "chemically-quiescent" systems such as larger rivers and lakes, or where external forcings on water quality are minimal. Nonetheless, it is important to note the distinction between the two types of sampling approaches.

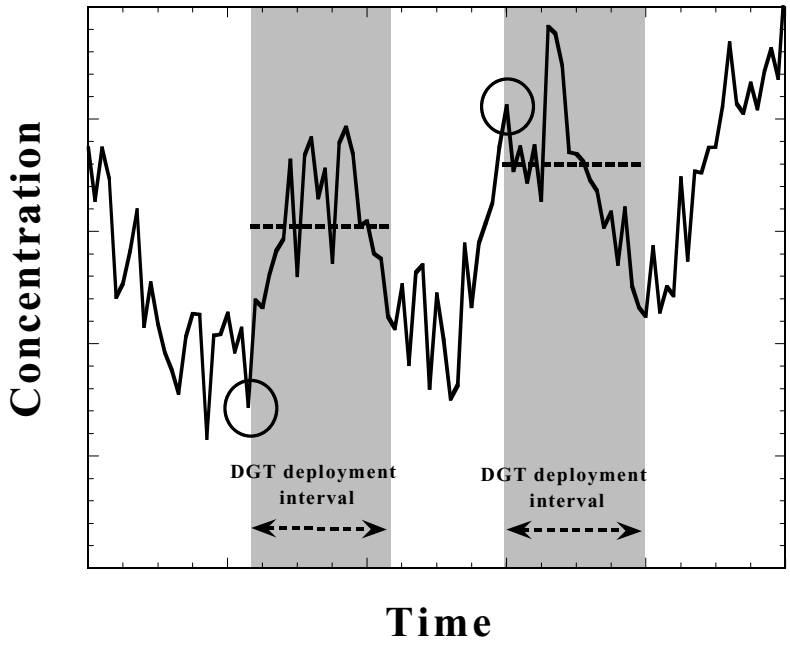


Figure 4-13: Concentration of a hypothetical water quality parameter with time. The shaded regions represent the deployment interval of a DGT sampler while the circles reflect water quality as determined by grab samples collected at the commencement of deployment. The horizontal dashed lines through the shaded zones represent the integrated DGT concentration.

In order to compare grab samples with *in situ* DGT values directly, it is necessary to understand the nature of variability in a system; this can be accomplished only through high-frequency sampling during DGT deployment. Direct comparisons of DGT and grab-sample values can be achieved by deploying DGT samplers directly in an aliquot of the grab sample. Such non *in situ* uses of DGT can provide useful information in situations where an *in situ* time-integrated signal is not desired.

pH

A limitation to the DGT sampler is the reduced performance of Chelex-resin at both high and low pH. While Chelex is a robust and tolerant resin, it functions best in the pH range of 5 to 9. Below pH 5, the adsorptive capacity of the resin diminishes, and at pH values greater than 9 the resin is prone to swelling, thus impacting the physical state of the resin layer (Paulson, 1986; Pai *et al*., 1990).

Saturation of Complexing Capacity of Resin

The mass of Chelex resin contained within the metal-binding gel layer affords considerable complexation capacity for sorbed metals. However, the complexation capacity of the resin layer is finite. Thus, given a large enough inventory of metals in solution and sufficient deployment period, saturation of the resin will occur. The time to reach saturation will vary greatly, depending on the metals present in solution, metal concentration, temperature and thickness of the diffusion layer. Limitations of resin saturation can be easily overcome as long as site-specific factors are considered when determining appropriate deployment periods.

Low Ionic Strength Solutions

Recent research suggests that DGT may overestimate the labile metal fraction in low ionic-strength solutions (Alfaro-De la Torre *et al.*, 2000). Specifically, in solutions that contain major ion concentrations $< 2 \times 10^{-4}$ M, concentrations of Cd and Ni measured by DGT were consistently higher than comparable total dissolved measurements. Such observations were attributed to an increase in metal diffusion coefficients associated with electrical effects induced by charge imbalances across the diffusive layer. The results have implications with respect to the use of DGT in low ionic strength media (*e.g.*, pristine, soft-water lakes). However, additional research is required to quantify more precisely the effects of such processes.

Ineffectiveness of DGT for Some Metals/Metalloids

DGT in its present form (*i.e.*, Chelex resin) is capable of measuring the labile concentration of a wide spectrum of trace metals in aquatic systems. Many metal cations (*e.g.*, Cu, Ni, Zn, Fe, Pb) exhibit a strong affinity for Chelex exchange sites. Chelex is limited, however, with respect to metals which commonly exist as oxyanions in solution (*e.g.*, As, Mo, Se, Sb) and which may not be preferentially adsorbed by the resin (Pai *et al.*, 1990). Such constraints are at worst short-term limitations, however, and will be rectified with the incorporation of alternative resins (*e.g.*, anionic exchange resins). Indeed, species-specific DGT applications have already been developed for Cs, Sr, H_2S and PO_4^{3-}.

Limitations of Free-Ion Activity Model

The parallels between metal uptake by DGT and aquatic biota relate to the Free-Ion Activity Model (FIAM) (see Chapter 2); uptake is related to the concentration of free and kinetically-labile metals in solution. The general similarity in uptake mechanisms explains why DGT appears to provide a better proxy of bioavailability than other traditional measurements of dissolved or total metals. However, there are exceptions to the FIAM which apply to DGT. Some non-polar neutrally-charged species (*e.g.*, AgCl,

$HgCl_2$), for example, are lipid-soluble, and as a result, are able to diffuse freely across cellular membranes. In this manner, metal uptake occurs in the absence of metal binding to transmembrane transporters (Gutknecht, 1981; Phinney and Bruland, 1994). The presence of synthetic organic ligands (*e.g.*, dithiocarbamate, 8-hydroxyquinoline) and low molecular-weight ligands (*e.g.*, citrate) has also been shown to increase the cellular uptake of several trace metals (see Chapter 2). The uptake of these aforementioned species by DGT may also be constrained, although DGT will measure neutral complexes if they are labile.

Another factor worthy of consideration is water hardness. The toxicity of metals to aquatic organisms, for example, generally decreases with increasing water hardness (*i.e.*, increasing concentrations of Ca and Mg) (see Chapter 2). Accordingly, in solutions of varying water hardness, the activity of free metal ions may remain constant while the toxicity towards aquatic biota may vary. This limitation of the FIAM also has implications with respect to the use of DGT.

4.5 Data Gaps and Future Research

The application of DGT to studies of metal speciation in aquatic systems has advanced considerably since its relatively-recent implementation. DGT techniques can be applied to explore various aspects of environmental chemistry in various test media (*e.g.*, water, sediments and soils). However, further research is necessary to rigourously defend interpretations inferred from DGT measurements in varying environments. In particular, the application of this technique to mining-impacted systems requires further examination. A summary of data gaps and future research is briefly highlighted below.

- More work is required to assess the precise nature of species measured by DGT in various aquatic environments. In particular, attention should be given to the effects of ligand competition with Chelex, size exclusion (*i.e.*, polyacrylamide pore-size), and differential diffusion of inorganic *vs.* organic species. Assessments of the metal species measured by DGT, therefore, require rigorous comparison with other speciation techniques (*e.g.*, ASV, AdCSV, SLM; see Chapter 3).

- The relationship between DGT values and toxicity to aquatic biota requires further study. The link between DGT and toxicity has been briefly examined with respect to copper via comparisons of DGT-Cu concentrations and LC_{50} values as determined by bioassay methods (see Section 4.3.2). However, this topic requires additional study, and should be expanded to include other metals of environmental concern (*e.g.*, Zn, Cd, Ni, Pb, *etc.*).

- Mining-related effluents present complex solutions hosting a spectrum of organic and inorganic agents which have the potential to complex trace metals, thereby influencing metal bioavailability and toxicity. Considerable work, therefore, is needed to assess the utility of DGT in systems influenced by mining-related discharges, and to determine how DGT values vary in response to the presence of different ligands. In particular, cyanide compounds represent ubiquitous components of most gold-mine waste streams and form strong complexes with many metals. The interactions between metal-CN complexes and Chelex are poorly constrained, and thus require examination. Mine-site discharges are also host to a variety of organic reagents (*e.g.*, pine oil, cresol, alcohols, xanthates, *etc*.) which undoubtedly influence metal uptake by DGT.

5 DGT Sediment Probe

INAP
International Network for Acid Preventi

5. DGT Sediment Probe

5.1 Introduction

In this section, the theory and design of the DGT sediment probe are described and its applicability to mining-related environmental studies is discussed. Although the scope of the INAP project is focused on the DGT water sampler, a literature review was also conducted on the DGT sediment probe and is included for completeness. The DGT sediment probe may be utilized in sediment toxicity and sediment/tailings reactivity assessments.

The principles of metal speciation and its affect on bioavailability and toxicity have been described in Chapters 2 and 3. These concepts also apply to pore waters, where the biological response (bioavailability) is suggested to be proportional to the free metal ion activity in pore solution. Bioavailability of metals in pore waters can therefore be decreased by complexation of metals by soluble inorganic (*e.g.*, F^-, Cl^-, HCO_3^-, SO_4^{2-}, HPO_4^{2-}, HS^-, polysulphides *etc*.) and organic (*e.g.*, humic substances) ligands. In addition, metal-binding phases in sediments (*e.g.*, sulphides, particulate organic matter and iron and manganese oxyhydroxides), may release "exchangeable" metals to pore waters. Where steady-state conditions may exist, chemical equilibrium is established between the solid phase and adjacent pore waters, with the concentration and speciation of metals in pore waters being controlled by pore water chemistry (*i.e.*, redox state, pH, ligands, *etc*.). Accordingly, the DGT sediment probe may be used to assess labile metal concentrations in pore waters or to determine the flux of exchangeable metals remobilized from the solid phase. A complication in the application of the sediment probe is that organisms may not "see" the same pore water as the probe. This is because many benthic organisms create their own microenvironments (see, for example , Warren et al., 1998, and Hare et al., 2001), often by irrigating their burrow with oxic water drawn from above. Thus, caution must be used in assessments of pore-water toxicity as they relate to specific groups of benthic organisms.

Following this brief introduction, existing techniques for conducting sediment toxicity and sediment reactivity assessments are reviewed (Section 5.2). The theory and application of the DGT sediment probe (Section 5.3) and a brief summary (Section 5.4) are then presented.

5.2 Existing Techniques for Assessing Sediment Geochemistry and Toxicity

Currently, there are several established techniques available for assessing the subaqueous chemical reactivity of sediment/tailings and sediment toxicity. Descriptions of such methods are outlined in the following sections.

5.2.1 Sediment/Tailings Reactivity Assessment

Previous assessments of the post-depositional cycling of metals in contaminated sediments and submerged tailings and their exchange with the overlying water column have principally relied on two pore water sampling techniques: 1) extraction of pore waters from sectioned sediment cores by centrifugation or squeezing; and 2) dialysis arrays. Coring/extraction techniques are subject to artifacts associated with sample handling and processing (*e.g.*, preserving speciation). A more recent and improved method is the use of dialysis arrays (peepers), which sample pore waters passively by allowing equilibration of water-filled peeper cells with adjacent pore waters. The peeper design affords sub-centimetre-scale resolution of dissolved constituents across the sediment-water interface. Peepers have been used to assess the subaqueous chemical reactivity of sediment/tailings in the field (*e.g.*, Tessier *et al.*, 1989; MEND, 1995; Pedersen *et al*. 1997; Vigneault et al., 2001; Martin *et al*., 2001) and in the laboratory (*e.g*., Sahami and Riehm, 1998; Lorax, 1999).

5.2.2 Toxicological Assessment

The three main sources of metals for benthic organisms (infauna and epifauna) are considered to be pore waters, overlying water and food. Dissolved metal concentrations in pore waters are considered to be the best proxy for toxicity toward a variety of benthic invertebrates (Ankley *et al*., 1994), although they may not apply well in the case of organisms that irrigate their burrows (e.g. Hare et al., 2001). Existing techniques for assessing sediment toxicity have been designed to assess metal concentration and speciation in the three potential metal sources, or more commonly, to determine whole-sediment toxicity by field or laboratory studies. A brief summary of techniques and test methods utilized in toxicological and environmental assessments is provided in the following paragraphs.

Techniques such as anodic stripping voltammetry (ASV) (see Chapter 3) have been used to determine labile metal species in soil and sediment pore waters (*e.g.,* Sauvé *et. al*., 1997). These studies have attempted to assess the bioavailability of specific metals by determining the concentration of free ionic species. Voltammetric techniques are

typically conducted in the laboratory subsequent to pore water extraction, and are therefore prone to potential artifacts associated with the preservation of speciation.

A new *in situ* voltammetric stripping technique has been developed for natural waters and pore waters, and incorporates measures to eliminate problems of electrode fouling by natural organic matter and/or inorganic colloids (Tercier and Buffle, 1998a; Tercier-Waeber *et al.,* 1999). Since this technique provides *in situ* determinations of metal speciation, it obviates problems associated with alterations to metal speciation during sampling, transport and processing of pore waters. However, there is a lack of data at present to permit full validation of this method.

Other techniques used to determine speciation in soil and sediment pore waters include competitive chelation (*e.g.*, Xue and Sigg, 1994), ion exchange (*e.g.,* Sunda, 1984) and cathode stripping voltammetry (*e.g.,* van der Berg, 1998). Alternatively, microelectrodes that determine pH, O_2, H_2S and CO_2 (Revsbech and Jørgensen, 1986) have been utilized to constrain water chemistry (*i.e.,* redox and pH conditions), which together with well known thermodynamic principles, may be used to predict the speciation of metals in water and pore water samples.

The laboratory and *in situ* speciation techniques for pore waters and overlying waters described above are labour-intensive, non-routine and do not consider particulate-bound metals which are ingested by organisms. Therefore, such methods do not provide reliable indicators of whole-sediment toxicity.

Sequential extraction procedures have been developed to determine the phase-specificity and potential availability of metals associated with soils and sediments (*e.g*., Tessier *et al*., 1979; Savvides *et al.* 1995; Tessier *et al*., 1996). For example, Savvides *et al.* (1995) studied the partitioning of metals (Cu, Cr, Ni, Pb, Zn and Fe) in contaminated marine sediments to determine the fraction of sediment-bound metals that are potentially available to biota. Limitations of such extraction procedures have been described by Nirel and Morel (1990). The main disadvantage of this approach is the operationally defined and non-specific nature of the extractants used, which makes direct extrapolations to bioavailability tenuous.

Since the bioavailability of sedimentary contaminants ingested by deposit-feeders will depend on their solubilization under digestive biochemical conditions, "biomimetic techniques" aimed at mimicking biological uptake, have been conducted. For example, Mayer *et al*. (1996) studied Cu and Pb solubility by digestive fluids extracted from marine invertebrates to determine metal bioavailability. Laboratory radiotracer studies aimed at elucidating metal uptake mechanisms by biota have also been conducted.

Gagnon and Fisher (1997), for example, determined the relative uptake of particulate-bound and dissolved mercury by a marine bivalve (*Mytilus edulis)*. The main limitations of such biomimetic and radiotracer studies are the laborious and non-routine nature of these techniques.

Geochemical modelling has been used to predict metal partitioning and speciation, which in turn can be used to predict bioavailability. The simplest approach adopted has been to normalize sediment metal contents to variables such as acid volatile sulphide, particulate organic carbon, and iron and manganese oxyhydroxides (*i.e.,* metal-binding phases). For example, a method for assessing the bioavailability of five metals (Cd, Cu, Pb, Ni and Zn) in anoxic sediments used by the USEPA involves determining the SEM (simultaneously extracted metals) and AVS (acid volatile sulphide) ratio derived from a cold-acid extraction of sediment samples. In cases where the SEM:ASV molar ratio is greater than one, the sediments are considered potentially toxic, whereas if the ratio is less than one, the sediments are considered non-toxic. The limitations of the sedimentary phase-ratio approach are outlined in O'Day *et al*. (2000). The principal objections to the USEPA approach are the non-phase specific, operationally-defined extraction and the assumption that all metals will precipitate as sulphides (in fact, metal removal in pore waters can also occur via sorption and precipitation processes associated with clays, carbonates, phosphates and/or oxyhydroxides minerals).

Geochemical speciation models have been used to calculate partitioning of metals between sediment and pore water (*e.g.*, PHREEQE, Parkhurst, 1980; FITEQL, Herbelin and Westall, 1996; MIMEQL^{+}, Schecher and M^{c}Avoy, 1994; MINTEQA2, Allison *et al*., 1991; WHAM, Tipping, 1995). As summarized by Koretsky (2000), surface complexation models (*e.g.,* constant capacitance model, the diffuse layer model, the triple layer model) have also been used in predicting speciation and solid-liquid partitioning of metals and provide significant advantages over empirical models (*e.g.,* the Langmuir and Freundlich isotherm). Although the modelling approach is useful in understanding the mechanism of metal cycling between pore waters and the solid phase, its main weakness is the limited thermodynamic database on which the models are based.

Whole-sediment techniques have been used to provide a holistic measure of sediment toxicity to selected organisms. Laboratory toxicity testing with whole-sediment exposures (*e.g.,* amphipods) and water column species in extracted pore waters and overlying water samples are routinely used to assess sediment toxicity to test organisms (*e.g.*, Chapman *et al.,* 1998). The main problem associated with these tests is the preservation of speciation due to changes in water chemistry (*i.e.,* redox and pH changes) during sample handling, transport and processing (Burton, 1992). *In situ* toxicity tests involving enclosures, artificial streams and ponds, and caging experiments with various

species are less common as they are logistically difficult, costly and non-routine. Biomarkers (*e.g.,* Pedersen *et al*., 1997) and bioaccumulation measurements (*e.g.,* Ankley, 1994) have also been used to assess exposure of organisms to contaminated sediment. These latter two approaches are relatively new and require further research and validation work.

Holistic studies of sediment toxicity have also been conducted by characterizing the existing biological community in contaminated sediments (*e.g*., Ellis and MacDonald, 1998) and comparing it to the benthic community at an unimpacted site. These studies provide a holistic assessment of the effect of contamination on benthic communities but do not elucidate cause and effect relationships. Simplistic approaches such as determination of metal concentrations in sediment and tissue of aquatic organisms have also been utilized to assess the toxicity of contaminated sediments in aquatic systems (*e.g.,* Luoma *et al.,* 1990). The weakness of the latter approach is that no information regarding the mechanism of metal uptake or site-specific controls on metal bioavailability (and toxicity) can be gleaned from the data.

Presently, it is considered that the best approach for assessing toxicological impacts of contaminated sediments is the integration of the various methods via a "weight of evidence" approach (Ingersoll *et al*., 1995). This would involve conducting several of the studies described above and using all the results to determine the actual or potential toxicity of sediments to aquatic organisms.

5.3 Theory and Application of the DGT Sediment Probe

The theory and application of the DGT sediment probe has been documented in approximately a dozen scientific publications over the past five years. The design and theory of the DGT sediment probe are outlined in Section 5.3.1. A brief summary of the application of the DGT probe to sediment and soil pore water studies is presented in Section 5.3.2.

5.3.1 DGT Sediment Probe Theory and Design

The technique of diffusive equilibrium in a thin-film (DET), which was the precursor to the DGT technique, was developed approximately ten years ago (Davison *et al*., 1991). This method is based on establishing diffusive equilibrium between a well-characterized polyacrylamide gel layer and the adjacent pore waters. DET is analogous to dialysis arrays, but provides better spatial resolution for major ions (*i.e.,* sub-millimetre scale for DET versus sub-centimetre scale for dialysis arrays). Field and laboratory pore water studies have been conducted which have yielded sub-millimetre resolution profiles of

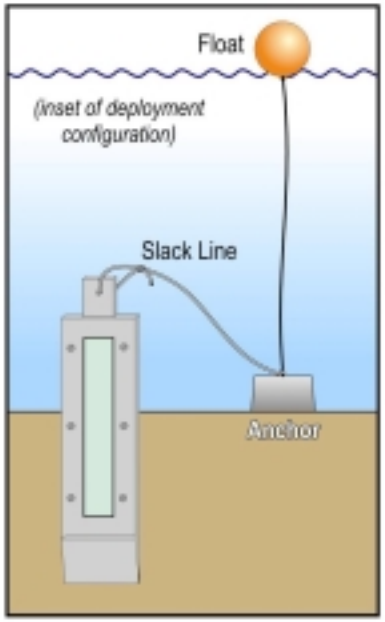
iron, manganese, nitrate, sulphate, chloride and ammonia-N (Davison *et al*., 1991; Davison *et al*., 1994; Krom *et al*., 1994; Shuttleworth *et al*. 1999). Previous DET studies have shown good recoveries and good agreement with conventional pore water techniques. Due to analytical limitations, however, DET does not permit the assessment of many trace elements which exist at low concentrations in pore waters.

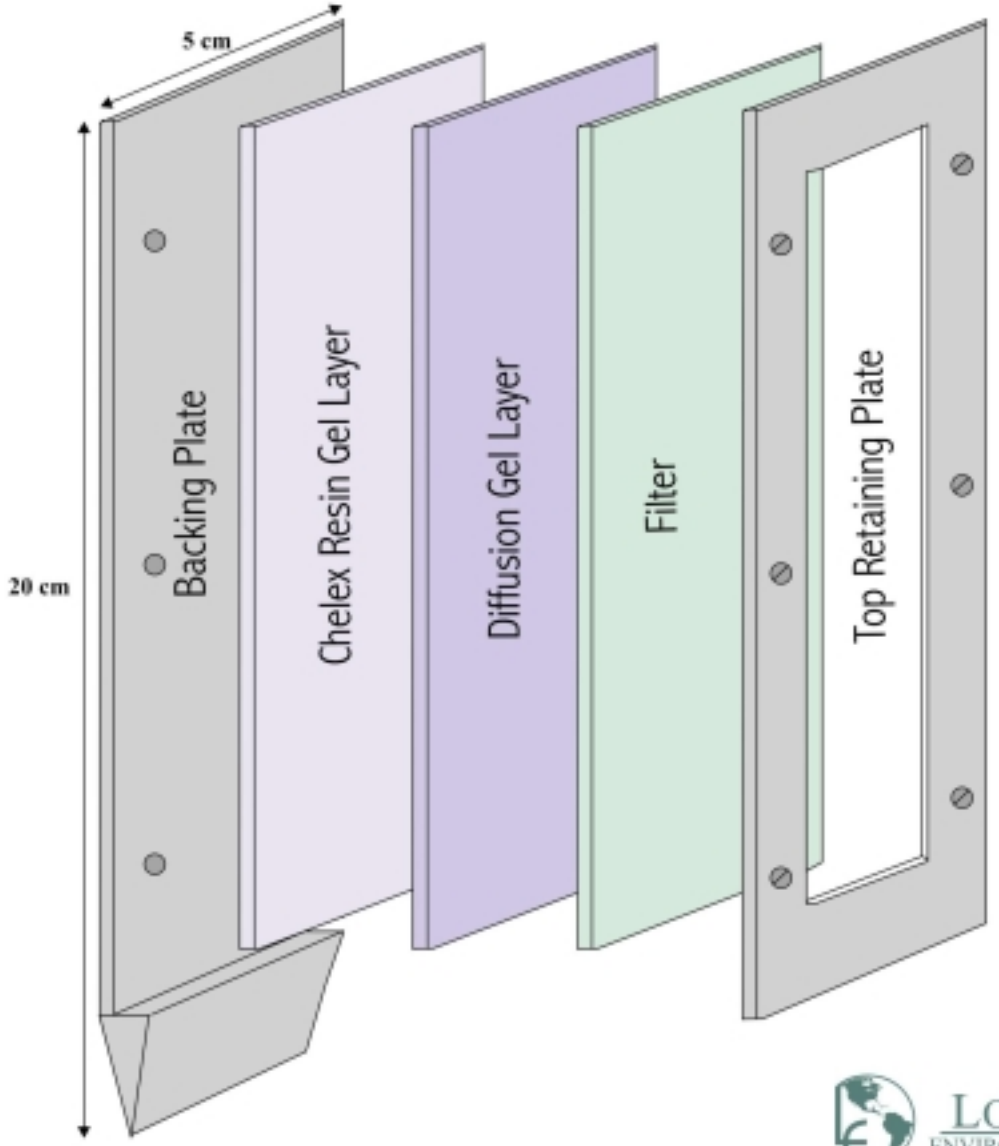
The DGT sediment technique was developed in order to determine trace metal concentrations in pore waters at sub-millimetre spatial resolution. A schematic of the DGT sediment probe and common deployment configuration is presented in Figure 5-1. The DGT sediment probe consists of three layers sandwiched between Perspex back and front plates and held together by plastic clips. An ion-exchange resin layer (Chelex-100) is separated from the pore waters by an ion-permeable polyacrylamide (>95% water content) layer and a cellulose filter (typically, with a collective thickness of 0.8 mm). The gel layer and filter exclude large organic-metal complexes and colloids (>50 nm) from diffusing to the resin.

The mode of DGT function is identical to that described for the water sampler described in Chapter 4. During deployment, dissolved metals in pore waters diffuse through the filter and gel layers. On contacting the resin layer, the metals are removed from solution by binding to the resin. The consumption of metals at the resin surface sets up a concentration gradient in the diffusion gel layer, the magnitude of which determines the accumulation rate of metals on the resin. After deployment (typically 24 hours), the filter and gel diffusion-layer are discarded and the mass of the metals on the resin is determined. The resin gel layer may be sliced, eluted with acid and the metal content determined by atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS). For higher spatial resolution, the resin gel is dried onto a cellulose-nitrate filter and the mass of metal per unit area of the filter is determined using proton induced X-ray emissions (PIXE). Subsequent to determining the mass of metals accumulated per unit area, the quantity is normalized to the deployment time to give a time-averaged flux of metals from pore waters to the resin.

The DGT technique provides higher spatial resolution than other pore water assessment techniques (*i.e.*, dialysis arrays and pore water extraction from sediment cores). For example, Davison *et al*. (1997a) conducted *in situ* measurement of Zn, Mn, Fe and As (III) by DGT in stream sediments and microbial mat pore waters at 100-micron vertical intervals. In contrast to other *in situ* pore water methods, DGT provides a measure of labile metal species.

Figure 5-1 **Schematic of Gel Sediment Probe and Deployment Configuration**





Provided that porewater metal concentrations at the porewater-diffusion-layer interface remain the same during DGT deployment and knowing the diffusion characteristics of the gel layer (*i.e.*, thickness of diffusive layer and published molecular diffusion coefficients in water), time of deployment and mass of metal on the resin, the concentration of metal in the pore water may be calculated using Fick's first law of diffusion (analogous to the DGT water sampler described in Chapter 4). However, there are two complications in this approach that are unique to the sediment probe, these are: 1) perturbation of pore water concentrations; 2) lack of precise estimates of the diffusion coefficients for species within the gel itself; and 3) vertical diffusion of metals within the diffusive (gel) layer. These complicating factors are discussed below.

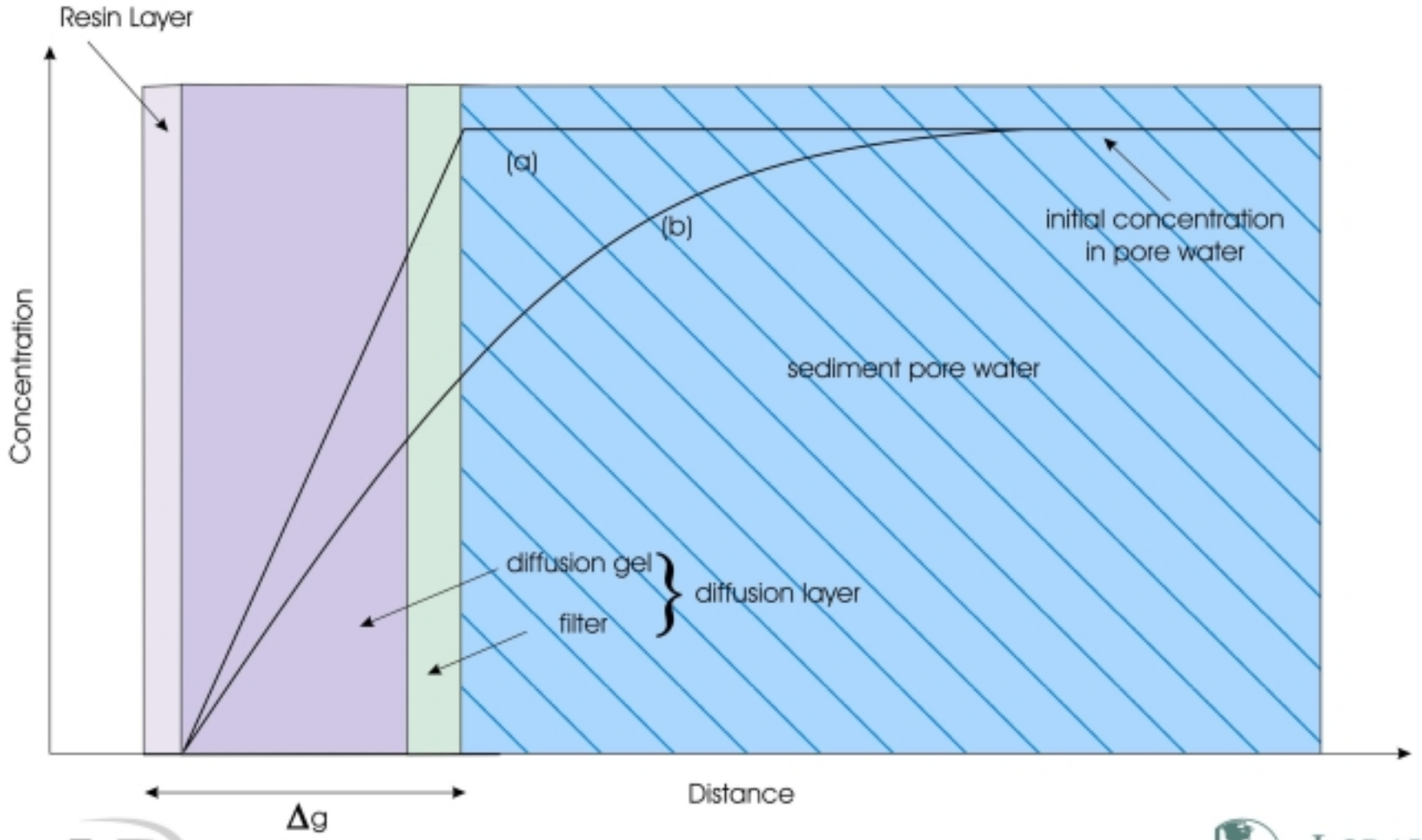
The introduction of the DGT probe perturbs the pore water system in a well-characterized fashion (by introducing a local sink) while simultaneously recording a response. Metals diffuse from pore waters to the DGT device where they are bound to the resin. The DGT device therefore induces a flux from pore water, which may deplete the local pore water concentration. The magnitude of this local depletion in pore water concentrations depends on the re-supply rate of "exchangeable" metals to the pore water from the solid phase (due to changes in equilibrium between pore water and solid phase). The processes which remove exchangeable metals to the solid phase include adsorption, absorption and surface precipitation, collectively referred to as "sorption"; metals are remobilized by desorption, the reverse process. Based on the observed kinetics of metal remobilization in DGT studies, re-supply of metals to pore waters is controlled by these sorption/desorption processes (Harper *et al.*, 1998).

Based on the relative flux of a given metal from pore waters to the DGT and the rate of re-supply to pore waters from the solid phase, local pore water concentrations may be affected in three possible ways (Zhang *et al.*, 1998c). The cases where pore waters are well buffered and the re-supply from the solid phase maintains constant pore water concentrations, will be referred to in the subsequent discussion as the "sustained case". The "partially sustained case" refers to the situation where there is some re-supply, but is insufficient to prevent some decrease in local pore water concentrations. The "unsustained case" refers to the situation where there is no significant re-supply from the solid phase and that local pore water concentrations decrease with time during DGT deployment.

The sustained case occurs when there is both a fast rate of, and high capacity for, re-supply to pore waters from the solid phase. Pore water concentrations are therefore not depleted during DGT deployment. This is shown schematically in Figure 5-2 (a), where pore water metal concentrations remain constant during DGT deployment.

Figure 5-2

Representation of the Concentration of an Ionic Species in a DGT Device and Adjacent Porewater During Deployment, for (a) sustained case and (b) partially-sustained case (from Harper *et al.*, 1999a)



Determination of pore water metal concentrations using DGT is based on Fick's first law of diffusion. Pore water concentrations (C) may be calculated, according to:

$$C = M\Delta g/(DtA)$$

Where, M is the mass of the metal accumulated in the resin layer, Δg is the thickness of the diffusive layer (Figure 5-2), D is the diffusion coefficient of a given metal ion and A is the area of the gel membrane exposed to pore waters. Note that D is typically assumed to be the same in the gel as it is in water. This is not necessarily true; D may well be less in the gel than in pure water. Ideally, D_{gel} should be determined experimentally for each species of interest.

The partially sustained case occurs when there is some re-supply from the solid phase, but is insufficient to prevent depletion of local pore water concentrations. This is shown schematically in Figure 5-2 (b), where pore water concentrations adjacent to the DGT device decrease during deployment, and thus the rate of metal binding by the resin decreases over time. In this case, the flux of metal from pore water to the DGT probe represents a remobilization flux from solid phase to solution.In the unsustained case, there is no re-supply of metal from the solid phase. This occurs because there is either no exchangeable metal bound to the solid phase or that the rate of desorption from the solid phase is slow, resulting in an insignificant re-supply rate to pore waters during the deployment period. In this case, DGT data represent the diffusive flux from adjacent pore waters to the DGT sediment probe.

Two different approaches have been adopted to differentiate between the three cases outlined above. Firstly, DGT-derived pore water concentrations may be compared to independent pore water determinations using conventional techniques (*e.g.,* Zhang *et al*., 1995a, b). The main disadvantage of this approach is the incompatible spatial resolution of DGT (sub-millimeter scale) and conventional techniques (sub-centimeter scale). Secondly, data from DGT devices of varying gel thickness have been used to distinguish between the three possible cases (*e.g.,* Hooda *et al*., 1999). By varying the thickness of the diffusive layer, the flux to the resin is effectively altered (*i.e.,* uptake by DGT decreases with increasing thickness of the gel layer). Hooda et al. (1999) claimed that in a graph of the measured mass of metal per unit area versus reciprocal of diffusive (gel) layer thickness (M vs. $1/\Delta g$), a linear plot represents the sustained case and a non-linear relationship indicates that re-supply to pore waters from the solid phase is less than DGT uptake. Of course, increasing the gel thickness will also increase the fraction of labile metal measured by DGT, as noted earlier in this report (Section 4.3.1), and this needs to be taken into account.

The second consideration unique to the DGT sediment probe is that vertical pore water concentration profiles may become relaxed, particularly where large chemical gradients foster vertical diffusion in the gel layer. The extent of this relaxation is dependent on gel layer thickness and the chemical gradient in pore waters (Harper *et al*., 1999a; 1999b). This artifact of the technique can be minimized by decreasing the gel thickness in high chemical gradients (Davison *et al*., 1994).

5.3.2 DGT Sediment Probe Applications

The DGT sediment probe has great potential as a tool to improve assessment techniques in two areas of interest to the mining industry: subaqueous sediment/tailings reactivity and sediment toxicity studies. The DGT sediment probe has undergone extensive testing and verification in order to minimize artifacts associated with use of the device. For example, oxygenation artifacts in anoxic pore waters (*e.g*., FeOOH formation in the gel due to the presence of molecular oxygen in the gel and in the lattice of the Perspex probe) have been overcome by deoxygenating the DGT sediment probe prior to deployment (Davison *et al*., 1994; Davison *et al.*, 1997b). DGT sediment probes have also been used in a number of studies to elucidate sediment and soil pore water chemistry and re-supply rates from the solid phase. A summary of the published scientific literature is presented in the following paragraphs.

Zhang *et al*. (1995a; 1995b) conducted *in situ* measurement of Fe, Mn, Cu, Ni, Cd and Zn at sub-millimetre resolution in lacustrine pore waters. Zn and Cd in pore waters were well buffered by rapid equilibria with the solid phase allowing measurement of their concentrations. Mn was not released to pore waters and therefore supply to DGT was solely by diffusion. Ni, Cu and Fe represented intermediate cases with a partial re-supply from the solid phase and provided a direct measure of the mean *in situ* flux from solid to solution phase for a 24 h deployment period.

Davison *et al*. (1997) made *in situ* measurements of Zn, Mn, Fe and As (III) by DGT in stream sediments and microbial mats at 100-micron resolution. This study showed the importance of the high spatial resolution achievable by DGT in determining fluxes of metals across the sediment-water boundary, as remobilization typically occurs in the top few millimeters of the sediments.

Harper *et al*. (1998a) used a two-dimensional numerical model (combining existing solid-solution interaction model and a simple diffusion model) and DGT data to study sorption rate constants. In cases where there is no rapid re-supply from the solid phase, DGT provides a remobilization flux from solid to solution (areal flux). The authors developed a method to express DGT data as a volumetric flux so that they can be related to the mass

of the solid phase. This enables the DGT flux to be expressed as a desorptive flux per unit volume of sediment (*i.e.*, mol/cm^3/s).

Zhang, *et al*. (1998c) and Hooda *et al*. (1999) conducted laboratory studies to determine labile metal concentrations in soil solutions and the bioavailability of metals (Cd, Co, Cu, Ni, Pb and Zn) bound to the solid phase using DGT, while Harper *et al*. (1999a) and Shuttleworth *et al*. (1999) studied the effects of horizontal heterogeneity and microniches on interpretation of pore water data and process rate (flux) determinations.

5.4 Summary

The DGT sediment probe is a potentially powerful tool for investigations of subaqueous sediment/tailings reactivity and sediment toxicity. The flux of metal from pore water to the DGT probe may be used either to calculate in some cases a concentration of the labile fraction in pore waters or to quantify a remobilization flux from solid to solution.

The main advantage of DGT over conventional pore water sampling techniques is the high spatial resolution (sub-millimetre resolution) and minimal sampling artifacts. In respect to toxicity assessments, DGT provides both *in situ* labile (bioavailable) metal concentrations and the remobilization flux from the solid phase. This information may be used to determine the bioavailable metal inventory both in pore waters and the solid phase. The main advantage of DGT over existing toxicity techniques (*e.g.*, pore water extractions, sequential extractions and solid phase chemistry) is that the metal inventory potentially available to benthic organisms may be estimated under realistic field conditions. In addition, DGT deployment procedures are relatively simple and inexpensive compared to other techniques.

Although extensive work has been conducted on the internal QA/QC for the DGT sediment probe, additional work is required prior to its routine application to environmental issues faced by the mining industry. The main areas in which further research are required are:

- speciation studies in pore waters to determine more precisely the nature of metal species measured by DGT; and
- correlation with conventional toxicity techniques to determine the utility of the DGT probe as an *in situ* toxicity indicator given a spectrum of different types and behaviours of benthic organisms.

Since comprehensive toxicological assessments are presently conducted by integration of several methods, the DGT sediment probe may become an important tool once additional speciation and toxicity work has been conducted. For example, integration of data from

DGT and other techniques (*e.g.*, bioassays, community structure assessments, *etc*.) may result in significant improvements in both toxicity assessments and in understanding the mechanisms of metal bioavailability.

References

References

Absil, M. C. P., J. J. Kroon, *et al.* (1994). "Availability of copper from phytoplankton and water for the bivalve Macoma balthica. II. Uptake and elimination from ^{64}Cu-labelled diatom and water." Mar. Biol. **118**: 129-135.

Achterberg, E. P. and C. Braungardt (1999). "Stripping voltammetry for the determination of trace metal speciation and *in situ* measurements of trace metal distributions in marine waters." Anal. Chim. Acta **400**: 381-397.

Achterberg, E. P. and C. M. G. Vandenberg (1994). "Automated Voltammetric System for Shipboard Determination of Metal Speciation in Sea-Water." Anal. Chim. Acta **284**: 463-471.

Ahner, B. A. and F. M. M. Morel (1995). "Phytochelatin production in marine algae. 2. Induction by various metals." Limnol. Oceanogr. **40**: 658-665.

Ahner, B. A., F. M. M. Morel, *et al.* (1997). "Trace metal control of phytochelatin production in coastal waters." Limnol. Oceanogr **42**(3): 601-608.

Alberic, P., E. Viollier, *et al.* (2000). "Interactions between trace elements and dissolved organic matter in the stagnant anoxic deep layer of a meromictic lake." Limnol. Oceanogr **45**(5): 1088-1096.

Alfaro-De la Torre, M. C., P. Y. Beaulieu, *et al.* (2000). "*In situ* measurement of trace metals in lakewater using the dialysis and DGT techniques." Anal. Chim. Acta **418**(1): 53-68.

Allison, J.D., Brown, D.S. and Novo-Gradac, K.J. (1991). "MINTEQA2/PRODEFA2, a geochemical assessment model for environmental systems: version 3.0 user's manual". U.S. Environmental Protection Agency, Athens, Ga.

Anderson, D. M. and F. M. M. Morel (1979). "Copper sensitivity of *Gonyaulax tamarensis*." Limnol . Oceanogr. **23**: 283-295.

Anderson, S. L., F. M. M. Morel, *et al.* (1978). "Growth limitation of a coastal diatom by low zinc ion activity." Nature **276**: 70-72.

Ankley, G.T., Thomas, N.A., Di Toro, D.M., Hansen, D.J., Mahony, J.D., Berry, W.J., Swartz, R.C., Hoke, R.A., Garrison, A.W., Allen, H.E., and Zarba, C.S. (1994). "Assessing potential bioavailability of metals in sediments: a proposed approach." Environ. Man.. **18:** 331-337.

Baillieul, M. and R. Blust (1999). "Analysis of the swimming velocity of cadmium-stressed Daphnia magna." Aquat. Toxicol. **44**(4): 245-254.

Barata, C., D. J. Baird, *et al.* (1998). "Influence of genetic and environmental factors on the tolerance of Daphnia magna Straus to essential and non-essential metals." Aquatic Toxicology **42**(2): 115-137.

Beauchemin, D., McLaren, J. W., Mykytiuk, A. P. and Berman, S. S. (1987). "Determination of trace metals in a river water reference material by inductively coupled plasma mass spectrometry." Anal. Chem. **59**: 778 - 783.

Bedsworth, W. W. and D. L. Sedlak (1999). "Sources and Environmental Fate of Strongly Complexed Nickel in Estuarine Waters: The Role of Ethylenediaminetetraacetate." Environ. Sci. Technol **33**: 926-931.

Bervoets, L. and R. Blust (1999). "Bioavailability of cadmium and zinc to midge larvae under natural and experimental conditions: Effects of some environmental factors." Belg. J. Zool. **129**(1): 269-284.

Bervoets, L. and R. Blust (2000). "Effects of pH on cadmium and zinc uptake by the midge larvae Chironomus riparius." Aquat. Toxicol. **49**(1-2): 145-157.

Boyle, E. A., F. Sclater, *et al.* (1976). "On the marine geochemistry of cadmium." Nature **263**(5572): 42-44.

Bruland K. W. (1992). Complexation of cadmium by natural organic ligands in the central North Pacific. Limnol. Oceanogr. **37**: 1008-1017.

Bruland K. W., Franks R. P., Knauer G. A., and Martin J. H. (1979). Sampling and analytical methods for the determination of copper, cadmium, zinc and nickel at the nanogram per liter level in sea water. Anal. Chim. Acta **105**: 233-245.

Bruland, K. W. (1989). "Complexation of zinc by natural organic ligands in the central North Pacific." Limnol. Oceanogr. **34**: 269-285.

Buffle, J., K. J. Wilkinson, *et al.* (1997). "*In situ* monitoring and speciation of trace metals in natural waters." Annali di Chimica **87**: 67-82.

Burton, G.A. (1992). "Sediment collection and processing: factors affecting realism". In Sediment Toxicity Assessment. Ed: G.A. Burton. Lewis Publishers, Chelsea, Mich. pp. 37-66.

Campbell P. G. C. (1995). "Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model." In Metal Speciation and Bioavailability in Aquatic Systems (ed. A. Tessier and D. R. Turner). John Wiley and Sons. pp. 45-102.

Carter, R. J., A. Hoxey, *et al.* (1992). "Complexation capacity of sediment humic acids as a function of extraction technique." Sci. Tot. Env. **125**: 25-31.

Carvalho, R. A., M. C. Benfield, *et al.* (1999). "Comparative bioaccumulation studies of colloidally-complexed and free-ionic heavy metals in juvenile brown shrimp *Penaeus aztecus* (Crustacea : Decapoda : Penaeidae)." Limnol. Oceanogr. **44**(2): 403-414.

Chakrabarti, C. L., Y. J. Lu, *et al.* (1994). "Kinetic-studies of metal speciation using Chelex cation-exchange resin - application to cadmium, copper, and lead speciation in river water and snow." Environ. Sci. Technol. **28**(11): 1957-1967.

Chang, J. C., P. B. Taylor, *et al.* (1981). "Use of the Microtox assay system for environmental samples." Bull. Environ. Contam. Toxicol. **26**(2): 150-156.

Chang, L., W. Davison, *et al.* (1998). "Performance characteristics for the measurement of Cs and Sr by diffusive gradients in thin films (DGT)." Anal. Chim. Acta. **368**(3): 243-253.

Chapman, P. M., F. Y. Wang, *et al.* (1998). "Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation." Can. J. Fish. Aquat. Sci. **55**(10): 2221-2243.

Cheng, J. G., C. L. Chakrabarti, *et al.* (1994). "Chemical speciation of Cu, Zn, Pb and Cd in rain water." Anal. Chim. Acta **288**(3): 141-156.

Chramback, A. (1985) "The Principles of Quantitative Gel Electrophoresis". Weinheim, Germany.

Coale, K. H. and K. W. Bruland (1988). "Copper complexation in the northeast Pacific." Limnol. Oceanogr. **33**: 1084-1101.

Codina, J. C., A. Perez-Garcia, *et al.* (1993). "A comparison of microbial bioassays for the detection of metal toxicity." Arch. Environ. Contam. Toxicol. **25**(2): 250-254.

Cotton, F. A. and G. Wilkinson (1980). Advanced Inorganic Chemistry, John Wiley & Sons.

Craig, A., L. Hare, *et al.* (1999). "Experimental evidence for cadmium uptake via calcium channels in the aquatic insect *Chironomus staegeri*." Aquat. Toxicol. **44**(4): 255-262.

Croot, P. L., J. W. Moffett, *et al.* (2000). "Production of extracellular Cu complexing ligands by eukaryotic phytoplankton in response to Cu stress." Limnol. Oceanogr. **45**(3): 619-627.

Davis, J. A. and J. O. Leckie (1978). "Effect of adsorbed complexing ligands on trace metal uptake by hydrous oxides." Environ. Sci. Technol. **12**(12): 1309-1315.

Davison, W., G. W. Grime, *et al.* (1991). "Distribution of dissolved iron in sediment pore waters at submillimetre resolution." Nature **352**: 323-324.

Davison, W. and H. Zhang (1994.). "*In situ* speciation measurements of trace components in natural waters using thin-film gels." Nature **367**: 546-548.

Davison, W. and H. Zhang (2000). "*In situ* measurements of labile species in waters and sediments using DGT". In Chemical Sensors in Oceanography. M. Varney (Ed.), Gordon and Breach.

Davison, W. Fones, G., Harper, M., Teasdale, P and Zhang, H. (2000) "Dialysis, DET and DGT: *in situ* diffusional techniques for studying water, sediments and soils". In: In situ Chemical Analyses in Aquatic Systems. Eds. J. Buffle and G. Horvai. Wiley.

Davison, W., G. R. Fones, *et al.* (1997a). "Dissolved metals in surface sediment and a microbial mat at 100 um resolution." Nature **387**: 885-888.

Davison, W., H. Zhang, *et al.* (1997b). "*In situ* procedures for measuring chemical speciation and availability of trace metals in soils and sediments". In: Contaminated Soils. R. Prost. Paris, INRA.

Davison, W., H. Zhang, *et al.* (1994). "Developing and applying new techniques for measuring steep chemical gradients of trace metals and inorganic ions at the sediment-water interface." Mineral. Mag. **58a**: 215-216.

Denney, S., J. Sherwood, *et al.* (1999). "In situ measurements of labile Cu, Cd, and Mn in river waters using DGT." Sci. Tot. Environ. **239**(1-3): 71-80.

Donat, J. R., K. A. Lao, *et al.* (1994). "Speciation of dissolved copper and nickel in South San-Francisco Bay - a multimethod approach." Anal. Chim. Acta **284**(3): 547-571.

dosSantos, M. M. C., M. L. S. Goncalves, *et al.* (1996). "Cadmium and lead complexation by anodic stripping voltammetry with a mercury microelectrode." Electroanalysis **8**(2): 178-182.

Dunn, M. J. (1993). Gel Electrophoresis: Proteins. Bios Scientific. Oxford, UK.

Ellis, D.V. and MacDonald, V.I. (1998). "Rapid preliminary assessment of seabed biodiversity for the marine and coastal mining industry". Mar. Geores. Geotech. **16:** 307-319.

Engel, D.W., W. Sunda and B.A. Fowler (1981). "Factors affecting trace metal uptake and toxicity to estuarine organisms. I. Environmental parameters. In: Biological Monitoring of Marine Pollutants, F. J. Vernberg et al. (Eds.). Academic Press, New York.

Errecalde, O. and P. G. C. Campbell (2000). "Cadmium and zinc bioavailability to *Selenastrum capricornutum* (Chlorophyceae): accidental metal uptake and toxicity in the presence of citrate." J. Phycol. **3**: 473-483.

Errecalde, O., M. Seidl, *et al.* (1998). "Influence of a low molecular weight metabolite (citrate) on the toxicity of cadmium and zinc to the unicellular green alga Selenastrum capricornutum: An exception to the free-ion model." Wat. Res. **32**(2): 419-429.

Fisher, N. S. (1986). "On the reactivity of metals for marine phytoplankton." Limnol. Oceanogr. **31**: 443-449.

Florence, T. M. (1982). "The speciation of trace elements in waters." Talanta **29**: 345-364.

Franklin, N. M., J. L. Stauber, *et al.* (2000). "pH-dependent toxicity of copper and uranium to a tropical freshwater alga (*Chlorella* sp.)." Aquat. Toxicol. **48**(2-3): 275-289.

Gagnon, C. and N. S. Fisher (1997). "Bioavailability of sediment-bound methyl and inorganic mercury to a marine bivalve." Environ. Sci. Tech. **31**(4): 993-998.

Gledhill, M., M. Nimmo, *et al.* (1999). "The release of copper-complexing ligands by the brown alga *Fucus vesiculosus* (Phaeophyceae) in response to increasing total copper levels." J. Phycol. **35**(3): 501-509.

Gundersen, J. K. and B. B. Jorgensen (1990). "Microstructure of diffusive boundary layers and the oxygen uptake of the sea floor." Nature **345**: 604-607.

Gutknecht, J. (1981). "Inorganic mercury (Hg^{2+}) transport through lipid bilayer membranes." J. Membrane Biol. **61**: 61-66.

Hao, O. J., C.-J. Shin, *et al.* (1996). "Use of Microtox test for screening industrial wastewater toxicity." Wat. Sci. Technol. **34**(10): 43-50.

Hare, L. and A. Tessier (1996). "Predicting animal cadmium concentrations in lakes." Nature **380**: 430-432.

Hare, L., A. Tessier and L. Warren (2001). "Cadmium accumulation by invertebrates living at the sediment-water interface". Environ. Toxicol. Chem. 20, 880-889.

Harper, M. P., W. Davison, *et al.* (1998). "Kinetics of metal exchange between solids and solutions in sediments and soils interpreted from DGT measured fluxes." Geochim. Cosmochim. Acta. **62**(16): 2757-2770.

Harper, M. P., W. Davison, *et al.* (1999a). "Estimation of pore water concentrations form DGT and DET techniques." *Anal. Chem. Acta.* **398.**: 329-340.

Harper, M., W. Davison, *et al.* (1999b). "Estimation of pore water concentrations from DGT profiles: a modelling approach." Aquat. Geochem. **5**: 337-355.

Herbelin, A.L. and Westall, J.C. (1996). "FITEQL – a computer program for determination of chemical equilibrium constants from experimental data". Rep. 96-01, Department of Chemistry, Oregon State University, Corvallis, Oreg.

Hill, S. J. (1997). "Speciation of trace metals in the environment." Chem. Soc. Rev. **26**(4): 291-298.

Hirose K. and Sigimura Y. (1985). "Role of metal-organic complexes in the marine environment - A comparison of the copper and ligand titration methods". Mar. Chem. **16**: 239-247.

Hodson, P. V., B. R. Blunt, *et al.* (1978). "pH-induced changes in blood lead of lead-exposed rainbow trout (*Salmo gairdneri*)." J. Fish. Res. Board Can. **35**: 437-445.

Hollis, L., K. Burnison, *et al.* (1996). "Does the age of metal-dissolved organic carbon complexes influence binding of metals to fish gills?." Aquat. Toxicol. **35**: 253-264.

Hooda, P. S., H. Zhang, *et al.* (1999). "Measuring bioavailable trace metals by diffusive gradients in thin films (DGT): soil moisture effects on its performance in soils." Euro. J. Soil Sc*i.* **50**: 1-10.

Hudson, R. J. M. (1998). "Which aqueous species control the rates of trace metal uptake by aquatic biota? Observations and predictions of non- equilibrium effects." Sci. Tot. Env. **219**(2-3): 95-115.

Ingersoll, C.G., Ankley, G.T., Benoit, D.A., Brunson, E.L., Burton, G.A., Dwyer, F.J., Hoke, R.A., Landrum, P.F., Norberg-King, T.J. and Winger, P.V. (1995). "Toxicity and bioaccumulation of sediment-associated contaminates using freshwater invertebrates: a review of methods and applications". Environ. Toxicol. Chem., **14:** 1885-1894.

Jackson, T. A. and T. Bistricki (1995). "Selective scavenging of Cu, Zn, Pb, and As by Fe and Mn oxyhydroxide coatings in lakes polluted with mine and smelter wastes: results of energy dispersive X-ray micro-analysis." J. Geochem. Explor. **52**: 97-125.

Jansen, R. A. G., H. P. Van Leeuwen, *et al.* (1998). "Speciation and lability of zinc(II) in river waters." Environ. Sci. Tech. **32**(24): 3882-3886.

Karbassi, A. R. and S. Nadjafpour (1996). "Flocculation of dissolved Pb, Cu, Zn and Mn during estuarine mixing of river water with the Caspian Sea." Env. Poll. **93**(3): 257-260.

Keller, O. C. and J. Buffle (2000). "Voltammetric and reference microelectrodes with integrated microchannels for flow through microvoltammetry. 2. Coupling the microcell to a supported liquid membrane preconcentration technique." Anal. Chem. **72**(5): 943-948.

Keller, O. C., S. Poitry, *et al.* (1994). "A hollow-fiber supported liquid membrane system for metal speciation and preconcentration - Calculation of the response-time." J. Electroanal. Chem. **378**(1-2): 165-175.

Koretsky, C. (2000). "The significance of surface complexation reactions in hydrologic systems: a geochemist's perspective." J. Hydrol. **230**(3-4): 127-171.

Kozelka, P. B. and K. W. Bruland (1998). "Chemical speciation of dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island." Mar. Chem. **60**: 267-282.

Krom M.D., Davison P., Zhang H., and Davison W. (1994) "High-resolution pore water sampling with a gel sampler". Limnol. Oceanogr. **39**(8), 1967-1972.

Krom, M.D., Davison, P., Zhang, H and Davison, W. (1995). "High resolution pore water sampling using a gel sampler: an innovative technique". Limnol. Oceanogr., **39:** 1967-1972.

Laporte, J. M., J. P. Truchot, *et al.* (1997). "Combined effects of water pH and salinity on the bioaccumulation of inorganic mercury and methylmercury in the shore grab *Carcinus maenas*." Mar. Poll. Bull. **34**(11): 880-893.

Laxen, D. P. H. (1985). "Trace metal adsorption / coprecipitation on hydrous ferric oxide under realistic conditions." Wat. Res. **19**(10): 1229-1236.

Leal, M. F. C., M. T. S. D. Vasconcelos, *et al.* (1999). "Copper-induced release of complexing ligands similar to thiols by *Emiliania huxleyi* in seawater cultures Limnol. Oceanogr **44**: 1750-1762.

Lee, J. G., B. Ahner, *et al.* (1996). "Export of cadmium and phytochelatin by the marine diatom *Thalassiosira weissfloglii*." Environ. Sci. Tech. **30**: 1814-1821.

Legras, S., C. Mouneyrac, *et al.* (2000). "Changes in metallothionein concentrations in response to variation in natural factors (salinity, sex, weight) and metal contamination in crabs from a metal-rich estuary." J. Exp. Mar. Biol. Ecol. **246**(2): 259-279.

Li, Y. H. and S. Gregory (1974). "Diffusion of ions in sea water and in deep-sea sediments." Geochmim. Cosmochim. Acta. **38**: 703-714.

Lorax, (1999). "Subaqueous Reactivity of Marcopper Tailings". Prepared for Placer Dome Asia Pacific. Prepared by Lorax Environmental Services Ltd.

Luoma, S. N., R. Dagovitz, *et al.* (1990). "Temporally intensive study of trace metals in sediments and bivalves from a large river-estuarine system: Suisun Bay/delta in San Francisco Bay." Sci. Tot. Env. **97/98**: 685-712.

Manheim, F. T. (1970). "The diffusion of ions in unconsolidated sediments." Earth Planet. Sci. Lett. **9**: 307-309.

Martin, A. J., J. J. McNee and Pedersen, T.F.. (2001). "The reactivity of sediments impacted by metal-mining in Lago Junin, Peru." J. Geochem. Explor.: 175-187

Mayer, L. M., Z. Chen, *et al.* (1996). "Bioavailability of sedimentary contaminants subject to deposit-feeder digestion." Environ.Sci. Technol. **30**: 2641-2645.

McGeer, J. C. and C. M. Wood (1998). "Protective effects of water Cl on physiological responses to waterborne silver in rainbow trout." Can. J. Fish. Aquat. Sci. **55**(11): 2447-2454.

McNee, J. J. and Martin, A. J (1999*).* "The measurement of biologically-available metals in aquatic systems and mine effluents – a new approach" *Presented at "Tailings and Mine Waste, 99", Fort Collins, CO.*

McNee, J. J. and Robertson, J. D (1998) "Development of a new sampling device to measure biologically-available metals in natural waters and mine effluents". CIM Journal: *CIM Paper No. 8135*.

MEND (1995). "GEOCHEMICAL ASSESSMENT OF SUBAQUEOUS TAILINGS DISPOSAL IN BUTTLE LAKE, B.C., 1993 STUDY PROGRAM", MEND REPORT 2.11.4A.

Meyer, J. S. (1999). "A mechanistic explanation for the In(LC50) vs. In(hardness) adjustment equation for metals." Env. Sci. Tech. **33**(6): 908-912.

Meyer, J. S., R. C. Santore, *et al.* (1999). "Binding of nickel and copper to fish bills predicts toxicity when water hardness varies, but free-ion activity does not." Env. Sci. Tech. **33**(6): 913-916.

Miller, W. E., S. A. Peterson, *et al.* (1985). "Comparative toxicology of laboratory organisms for assessing hazardous waste sites." J. Environ. Qual. **14**: 29-32.

Moffett, J. W. and L. E. Brand (1996). "The production of strong Cu chelators by marine cyanobacteria in response to Cu stress." Limnol Oceanogr. **41**: 388-395.

Muller, F. L. L. and D. R. Kester (1991). "Measurement of the different forms of zinc in Narragansett Bay water based on the rate of uptake by a chelating resin." Mar. Chem. **33**: 171-186.

Nirel, P.M.V. and Morel, F.M.M. (1990). "Pitfalls of sequential extractions". Wat. Res. **24:** 1055-1056.

Nordstrom, D. K. (1996). "Trace Metal Speciation in Natural Waters: Computational vs. Analytical." Wat. Air Soil Poll. **90**: 257-267.

Nriagu J. O., Lawson G., Wong H. K. T., and Azcue J. M. (1993). "A protocol for minimizing contamination in the analysis of trace metals in Great Lakes waters". J. Great Lakes Res. **19**(1), 175-182.

Nriagu, J. O., H. K. T. Wong, *et al.* (1981). "Particulate and dissolved trace metals in Lake Ontario." Wat. Res. **15**: 91-96.

Nriagu, J. O., H. K. T. Wong, *et al.* (1982). "Deposition and chemistry of pollutant metals in lakes around the smelters at Sudbury, Ontario." Environ. Sci. Technol. **16**: 551-560.

O'Day, P. A., S. A. Carroll, *et al.* (2000). "Metal speciation and bioavailability in contaminated estuary sediments, Alameda Naval Air Station, California." Environ. Sci. Tech **34**(17): 3665-3673.

Pai S. C., Fang T. H., Chen C. T. A., and Jeng K. L. (1990). "A low contamination Chelex-100 technique for shipboard pre-concentration of heavy metals in seawater". Mar. Chem. **29**: 295-306.

Pardo, R., E. Barrado, *et al.* (1994). "Voltammetric complexation capacity of waters of the Pisuerga River." Wat. Res. **28**: 2139-2146.

Parkhurst, D. L., D. C. Thorstenson, *et al.* (1980). "PHREEQE--A computer program for geochemical calculations": U.S. Geological Survey Water Resources Investigations Report.

Parthasarathy, N. and J. Buffle (1994). "Capabilities of Supported Liquid Membranes for Metal Speciation in Natural-Waters - Application to Copper Speciation." Anal. Chim. Acta **284**(3): 649-659.

Paulson A. J. (1986) "Effects of flow rate and pretreatment on the extraction of trace metals from estuarine and coastal seawater by Chelex-100." Anal. Chem. **58:** 183 - 187.

Pedersen, S.N., Lundebye, A.K. and Depledge, M.H. (1997). "Field application and metallothionein and stress protein biomarkers in the shore crab *(Carcinus maenas)* exposed to trace metals". Aquat. Toxicol. **37:** 183-200.

Pedersen, T.F., McNee, J.J., Flather, D.H., Mueller, B., Sahami, A.R., Pelletier, C.A. (1997). "Geochemistry of submerged tailings in Buttle Lake and the Equity Silver tailings pond, British Columbia, and Andersen Lake, Manitoba: what have we learned?" Proceedings, Fourth International Conference on Acid Rock Drainage. Vancouver, June 1997.

Petronio, B. M., B. Cosma, *et al.* (1993). "A Multimethod Approach to Study Humic Compounds and Metal Speciation in Marine Sediment Samples." International Journal of Environmental Analytical Chemistry **54**(1): 45-56.

Phinney, J. T. and K. W. Bruland (1997). "Effects of dithiocarbamate and 8-hydroxyquinoline additions on algal uptake of ambient copper and nickel in South San Francisco Bay water." Estuaries **20**(1): 66-76.

Playle, R. C. (1998). "Modelling metal interactions at fish gills." Sci. of the Tot. Env. **219**(2-3): 147-163.

Prygiel, J., A. Rosso-Darmet, *et al.* (1999). "Use of oligochaete communities for assessment of ecotoxicological risk in fine sediment of rivers and canals of the Artois-Picardie water basin (France)." Hydrobiologia **410**: 25-37.

Rainbow, P. S., C. Amiard-Triquet, *et al.* (2000). "Observations on the interaction of zinc and cadmium uptake rates in crustaceans (amphipods and crabs) from coastal sites in UK and France differentially enriched with trace metals." Aquat. Toxicol. **50**(3): 189-204.

Reinfelder, J. R., N. S. Fisher, *et al.* (1998). "Trace element trophic transfer in aquatic organisms: A critique of the kinetic model approach." Sci. Tot. Env. **219**(2-3): 117-135.

Reinfelder, J. R., R. E. Jablonka, *et al.* (2000). "Metabolic responses to subacute toxicity of trace metals in a marine microalga (*Thalassiosira weissflogii*) measured by calorespirometry." Environ. Toxicol. Chem. **19**(2): 448-453.

Revsbech N.P. and Jørgensen B.B. (1986) "Microelectrodes: their use in microbial ecology". In: Advances in Microbial Ecology (K.C. Marshall, *ed.*) pp. 293-352.

Roditi, H. A., N. S. Fisher, *et al.* (2000). "Uptake of dissolved organic carbon and trace elements by zebra mussels." Nature **407**(6800): 78-80.

Roesijadi, G. (1992). "Metallothioneins in metal regulation and toxicity in aquatic animals." Aquat. Toxicol. **22**: 81-114.

Roesijadi, G. and B. Fowler (1992). "Purification of invertebrate metallothioneins" In: Metallobiochemistry, Part B: Metallothionein and related molecules. J. F. Riordan and B. L. Vallee. San Diego, Academic Press.

Ross, A. R. S., M. G. Ikonomou, *et al.* (2000). "Characterization of dissolved tannins and their metal-ion complexes by electrospray ionization mass spectrometry." Anal. Chim. Acta **411**(1-2): 91-102.

Roy, R. and P. G. C. Campbell (1995). "Survival-Time Modeling of Exposure of juvenile Atlantic Salmon (Salmo-Salar) to Mixtures of Aluminum and Zinc in Soft-Water at Low Ph." Aquat. Toxicol. **33**(2): 155-176.

Sahami, A.R., and Riehm, D (1999). "An assessment of the subaqueous reactivity of sulphide-rich mine tailings from the Petaquilla Property, Panama". The Canadian Mining and Metallurgical (CIM) Bulletin. **92**(1035): 77-80.

Santschi, P. H., P. Bower, *et al.* (1983). " Estimates of the resistance to chemical transport posed by the deep-sea boundary layer." Limnol. Oceanogr. **28**: 899-912.

Sauve, S., M. B. McBride, *et al.* (1997). "Speciation of lead in contaminated soils." Environ. Poll. **98**(2): 149-155.

Savvides, C., A. Papadopoulos, *et al.* (1995). "Sea sediments contaminated with heavy metals: Metal speciation and removal." Water Science and Technology **32**(9-10): 65-73.

Sawamoto, H. (1999). "Adsorptive stripping voltammetry of metal ions (Review)." Bunseki Kagaku **48**(2): 137-150.

Schecher, W.D. and McAvoy, W. (1994). "MINEQL+, user's manual". Environmental Research Software, Hallowell, Me.

Schramel, O., B. Michalke, *et al.* (1998). "Analysis of metal species by using electrospray ionization mass spectrometry and capillary electrophoresis electrospray ionization mass spectrometry." J. Chromatography **819**(1-2): 231-242.

Sedlak, D. L., J. T. Phinney, *et al.* (1998). "Strongly complexed Cu and Ni in wastewater effluents and surface runoff." Environ. Sci.Technol. **31**(10): 3010-3016.

Sholkovitz, E. R. (1976). "Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater." Geochim. Cosmochim. Acta **40**: 831-845.

Shuttleworth, S. M., W. Davison, *et al.* (1999). "Two-dimensional and fine structure in the concentrations of iron and manganese in sediment pore-waters." Environ. Sci. Technol. **33**: 4169-4175.

Sigg, L. (1985). "Metal transfer mechanisms in lakes; the role of settling particles". In: Chemical Processes in Lakes. W. Stumm, Wiley-Interscience.**:** 283-310.

Snell, T. W. and G. Persoone (1989). "Acute toxicity bioassays using rotifers: II. A freshwater test with *Branchionus rubens.*" Aqat. Toxicol. **14**: 81-92.

Soegianto, A., M. Charmantier-Daures, *et al.* (1999). "Impact of cadmium on the structure of gills and epipodites of the shrimp *Penaeus japonicus* (Crustacea : Decapoda)." Aquatic Living Resources **12**(1): 57-70.

Spry, D. J. and J. G. Weiner (1991). "Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review." Env. Pollution **71**: 243-304.

Stumm, W. and J. J. Morgan (1981). Aquatic Chemistry. New York, Wiley-Interscience.

Sturgeon, R. E., Berman, S. S., and Willie, S. N. (1982). "Concentration of trace metals from sea water by complexation with 8-hydroxyquinoline and adsorption on C_{18}-bonded silica gel." Talanta **29**: 167-171.

Sunda, W. G. (1991). "Trace metal interactions with marine phytoplankton." Biol. Oceanogr. **6**: 411-417.

Sunda, W. G. and P. A. Gillespie (1979). "The response of a marine bacterium to cupric ion and its use to estimate cupric ion activity in seawater." J. Mar. Res. **37**: 761-772.

Sunda, W. G. and R. R. L. Guillard (1976). "The relationship between cupric ion activity and the toxicity of copper to phytoplankton." J. Mar. Res. **34**: 511-529.

Sunda, W. G. and S. A. Huntsman (1992). "Feedback interactions between zinc and phytoplankton in seawater." Limnol. Oceanogr. **37**: 25-40.

Sunda, W. G. and S. A. Huntsman (1998). "Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems." Sci. Tot. Env. **219**: 183-208.

Sunda, W. G., P. A. Tesier, *et al.* (1987). "Effects of cupric and zinc ion activities on the survival and reproduction of marine copepods." Mar. Biol. **94**: 203-210.

Sunda, W.G. (1984). "Measurement of manganese, zinc and cadmium complexation in seawater using chelex ion exchange equilibria". Mar. Chem., **14:** 365-378.

Taillefert, M., C.-P. Lienemann, *et al.* (2000). "Speciation, reactivity and cycling of Fe and Pb in a meromictic lake." Geochim. Cosmochim. Acta **64**: 169-183.

Teasdale, P. R., S. Hayward, *et al.* (1999.). "In situ, high-resolution measurement of dissolved sulfide using diffusive gradients in thin films with computer-imaging densitometry." Analytical Chemistry. **71**(11): 2186-2191.

Temminghoff, E. J. M., A. C. C. Plette, *et al.* (2000). "Determination of the chemical speciation of trace metals in aqueous systems by the Wageningen Donnan Membrane Technique." Anal. Chim. Acta. **417**(2): 149-157.

Tercier, M. L., J. Buffle, *et al.* (1998a). "Novel voltammetric in-situ profiling system for continuous real-time monitoring of trace elements in natural waters." Electroanalysis **10**(6): 355-363.

Tercier-Waeber, M. L., C. Belmont-Hebert, *et al.* (1998b). "Real-time continuous Mn(II) monitoring in lakes using a novel voltammetric in situ profiling system." Environ. Sci. Technol. **32**(10): 1515-1521.

Tercier-Waeber, M. L., J. Buffle, *et al.* (1999). "Submersible voltammetric probes for in situ real-time trace element measurements in surface water, groundwater and sediment-water interface." Measurement Science & Technology **10**(12): 1202-1213.

Tercier-Waeber, M. L., J. Buffle, *et al.* (2000). "Carrier-mediated transport of toxic elements through liquid membranes - Transport of Cd(II) from high salinity chloride medium through supported liquid membrane containing TBP/cyclohexane." J. Membrane Sci. **177**(1-2): 41-47.

Tessier, A., D. Fortin, *et al.* (1996). "Metal sorption to diagenetic iron and manganese oxyhydroxides and associated organic matter: narrowing the gap between field and laboratory measurements." Geochem. Cosmochim. Acta **60**(3): 387-404.

Tessier, A., F. Rapin, *et al.* (1985). "Trace metals in oxic lake sediments: possible adsorption onto iron oxyhydroxides." Geochem. Cosmochim. Acta **49**: 183-194.

Tessier, A., P. G. C. Campbell, *et al.* (1979). "Sequential Extraction procedure for the speciation of particulate trace metals." Anal. Chem. **51**(7): 844-851.

Tessier, A., R. Carignan, *et al.* (1989). "Partitioning of Zn between the water column and the oxic sediments in lakes." Geochim. Cosmochim. Acta. **53**: 1511-1522.

Tessier, A., Y. Couillard, *et al.* (1993). "Modelling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis.*" Limnol. Oceanogr. **38**: 1-17.

Tipping, E. (1995). "WHAM – a chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances". Comp. Geosci., **20:** 973-1023.

Tipping, E., S. Lofts, *et al.* (1998). "Modelling the chemical speciation of trace metals in the surface waters of the Humber system." Sci. Tot. Environ. **210**(1-6): 63-77.

Toussaint, M. W., T. R. Shedd, *et al.* (1995.). "A comparison of standard acute toxicity tests with rapid-screening toxicity tests." Environ. Toxicol. Chem. **14**(5): 907-915.

Van der Berg, G.A. (1998). "Determination of metal speciation in seawater using cathodic stripping voltammetry". In Metals in Surface Waters. Eds: H.E. Allen, W. Garrison, and G.W. Luther. Ann Arbor Press, Chelsea, Mich. Pp. 133-153.

Vega, M., R. Pardo, *et al.* (1995). "Pseudopolarographic Determination of Stability-Constants of Labile Zinc-Complexes in Fresh-Water." Anal. Chim. Acta **310**(1): 131-138.

Vela, N. P. and J. A. Caruso (1993). "Potential of Liquid-Chromatography - Inductively-Coupled Plasma-Mass Spectrometry for Trace-Metal Speciation." J. Anal. Atomic Spectrometry **8**(6): 787-794.

Vigneault, B. P.G.C. Campbell, A. Tessier, and R de Vitre (2001). "Geochemical changes in sulfidic mine tailings stored under a shallow water cover. Wat. Res. 35: 1066-1076.

Vigneault, B., A. Percot, *et al.* (in press). "Permeability changes in model and phytoplankton membranes in the presence of aquatic humic substances." Environ. Sci. Technol.

Wang, W. X. and N. S. Fisher (1996). "Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: Effects of food composition." Limnology and Oceanography **41**(2): 197-207.

Wang, W. X. and N. S. Fisher (1999). "Delineating metal accumulation pathways for marine invertebrates." Sci. Tot. Environ. **238**: 459-472.

Wang, W. X. and N. S. Fisher (1999a). "Assimilation efficiencies of chemical contaminants in aquatic invertebrates: A synthesis." Environ. Toxicol. Chem. **18**(9): 2034-2045.

Wang, W. X. and R. C. H. Dei (1999b). "Factors affecting trace element uptake in the black mussel *Septifer virgatus*." Mar. Ecol. Prog. Ser. **186**: 161-172.

Wells, M. L., P. B. Kozelka, *et al.* (1998). "The complexation of 'dissolved' Cu, Zn, Cd and Pb by soluble and colloidal organic matter in Narragansett Bay, RI." Mar. Chem. **62**: 203-217.

Wilhelm, S. W. and C. G. Trick (1994). "Iron-limited growth of cyanobacteria: multiple siderophore production is a common response." Limnol. Oceanogr. **39**: 1979-1984.

Wilkinson, K. J., P. M. Bertsch, *et al.* (1993). "Surface complexation of aluminum on isolated fish gill cells." Environ. Sci. Technol **27**: 1132-1138.

Xue, H. and Sigg, L. (1994). "Zinc speciation in lakewaters and its determination by ligand-exchange with EDTA and differential pulse anodic stripping voltammetry". Anal. Chim. Acta, **284:** 505-515.

Xue, H. and W. G. Sunda (1997). "Comparison of [Cu2+] Measurements in Lake Water Determined by Ligand Exchange and Cathodic Stripping Voltammetry and by Ion-Selective Electrode." Environ. Sci. Technol. **31**: 1902-1909.

Yang, Y. Y., D. Wu, *et al.* (1999). "Determination of complexation capacity of trace metal-organic in natural water." J. Env. Sci. **11**: 124-128.

Zamuda, C. D. and W. G. Sunda (1982). "Bioavailability of dissolved Cu to the American oyster *Crassostrea virginica*. I. Importance of chemical speciation." Mar. Biol. **66**: 77-85.

Zamuda, C. D., D. A. Wright, *et al.* (1985). "The importance of dissolved organic compounds in the accumulation of copper by the American Oyster, *Crassostrea virginica.*" Mar. Env. Res. **16**: 1-12.

Zhang, H. and W. Davison (1995). "Performance characteristics of the technique of diffusion gradients in thin-films (DGT) for the measurements of trace metals in aqueous solution." Anal. Chem. **67**: 3391-3400.

Zhang, H. and W. Davison (1999). "Diffusional characteristics of hydrogels used in DGT and DET techniques." Anal. Chem. Acta. **398**: 329-340.

Zhang, H. and W. Davison (2000). "Direct in situ measurements of labile inorganic and organically bound metal species in synthetic solutions and natural waters using DGT." Anal. Chim. Acta. **72**: 4447-4457.

Zhang, H., W. Davison, *et al.* (1995a). "*In situ* high resolution measurements of fluxes of Ni, Cu, Fe and Mn and concentrations of Zn and Cd in porewaters by DGT." Geochim. Cosmochim. Acta. **59**(20): 4181-4192.

Zhang, H., W. Davison, *et al.* (1995b). "New *in situ* procedures for measuring trace metals in pore waters. Remediation, and Containment of Contaminated Sediments". In: Dredging, Remediation, and Containment of Contaminated Sediments. K. R. Demars, G. N. Richardson, R. N. Yong and R. C. Chaney. Philadelphia. 1995, American Society, ASTM STP 1293: 170-181.

Zhang, H., Davison, W., Gadi, R. and Kobayashi, T. (1998a). "*In situ* measurement of dissolved phosphorus in natural waters using DGT", Anal Chim. Acta., **370**: 29-35.

Zhang, H., W. Davison, *et al.* (1998b). "In situ measurements of dissolved phosphate in natural waters using DGT." Anal. Chim. Acta. **370**(1): 29-38.

Zhang, H., W. Davison, *et al.* (1998c). "In situ measurement of solution concentrations and fluxes of trace metals in soils using DGT." Env. Sci. Technol. **32**(5): 704-710.

Zhou, X. and P. J. Wangersky (1989). "Production of Copper-Complexing Organic Ligands by the Marine Diatom *Phaeodactylum tricornutum* in a Cage Culture Turbidostat." Marine Chemistry **26**: 239-259.

Part II

DGT Application

INAP
International Network for Acid Prevent

Executive Summary

Executive Summary

In natural waters, trace metals exist in a variety of organic and inorganic forms, spanning the spectrum of large organic complexes to simple hydrated molecules. The bioavailability, and hence toxicity, of metals in aquatic systems is strongly dependent on the nature of the metal species present. The widely accepted Free-Ion Activity Model (FIAM) posits that metal bioavailability in aqueous systems is controlled by the free ionic activity of metals rather than by the total or dissolved concentration. This is due to the fact that free metal ions (*e.g.,* Cu^{2+}) are readily taken up by aquatic organisms, whereas particulate and strongly complexed metals are not. In most cases, complexation with organic ligands reduces metal bioavailability as most organic-metal complexes are not readily transported across cell membranes. Accordingly, techniques that attempt to quantify the bioavailable or labile fraction of metals in aqueous systems are potentially more valuable to the prediction of impacts to aquatic biota.

The technique of diffusion gradient in thin-films (DGT) has been developed over the past ten years by academic researchers in order to provide a method for *in situ* determinations of "labile" metal species in natural waters. Since it is the labile fraction of the total metal inventory that is considered most bioavailable to aquatic organisms, DGT has the potential to become an important tool in toxicological and environmental assessments; however, additional validation/verification work is required to advance the DGT sampler as a reliable *in situ* toxicity indicator for routine use by industry. This Part II presents the results of a suite of validation and intercomparison experiments.

In an attempt to define better metal species measured by existing techniques, laboratory experiments were conducted to compare metal speciation data as determined by DGT, ASV, and to a lesser extent ion-selective electrode (ISE) methods. In a second stage, the speciation results were compared to a biological response using cultures of *Daphnia magna* in defined ligand-bearing solutions. The latter component was conducted to evaluate the various techniques as indicators of metal toxicity performed using DGT.

Copper (Cu), cadmium (Cd) and zinc (Zn) were the metals chosen for evaluation in this study. Two well-characterized ligands, EDTA (Ethylenediaminetetraacetic acid) and NTA (nitrilotriacetic acid), were used in the experiments. EDTA is widely used as a metal chelator in industrial processes and has a strong affinity for a wide variety of metals, while NTA is a well characterized moderately strong (weaker than EDTA) ligand. In addition to the experiments with synthetic solutions, the speciation of Cu and Cd was assessed in natural waters rich in dissolved organic carbon (DOC). This water was collected from the Nicomekl River, a meandering stream that receives runoff from peat

bogs and agricultural lands in southern Greater Vancouver. Bioassays using the copepod *Daphnia magna* were conducted on the experimental solutions in which the dissolved, DGT-detectable and ASV-detectable concentrations had been determined.

In the presence of an excess of the strong ligand EDTA, labile Cu, Cd and Zn concentrations, as measured by both DGT and ASV approaches, are substantially lower than total dissolved (0.45 μm filtered) metal concentrations. However, DGT-labile concentrations are consistently higher than those assayed using ASV. Chemical equilibrium modeling (using MINEQL+) of Cu- and Zn-containing solutions suggests that the DGT approach overestimates the labile concentration while the ASV technique provides a more reasonable estimate. When total dissolved metal concentrations exceed the complexing capacity of the EDTA in solution, both methods provide accurate determinations of the labile concentration, suggesting that both approaches can quantify metal complexing capacity in the presence of strong ligands.

In the presence of an excess of the moderately strong ligand NTA, labile Cu and Cd concentrations as determined by ASV are substantially lower than the dissolved metal concentrations. DGT, by contrast, measures concentrations ~25% lower than total dissolved levels. The different results returned by each technique are attributed to two factors: (a) the Chelex resin used in the DGT samplers is a strong complexing agent, and readily adsorbs free metal ions at the resin-solution interface, thus supporting ongoing diffusion from the bulk solution through the gel; (b) ASV samples the solution on a timescale of a fraction of a second, whereas DGT samples over ten or more minutes, allowing kinetic adjustments that are not witnessed by ASV. In other words, the much greater time required for the metal to diffuse from solution to the detection surface in the DGT approach permits dissociation of more slowly dissociating complexes and thus leads to detection of a larger "labile" concentration by the DGT technique. However, when the metal concentrations exceed the complexing capacity of the added weak ligand NTA, both ASV and DGT provide accurate determinations of the labile metal concentration.

Data obtained from experiments using DOC-rich natural waters suggest the presence of both strong and weak ligands. Under such conditions DGT and ASV delivered similar results. In a dilute solution of DOC-rich Nicomekl River water (90% "moderately hard water" and 10% river water), ASV-labile Cu concentrations were undetectable (<0.05 μg/L) under the conditions of the experiment (Appendix A) when the dissolved Cu concentration was below ~50 μg/L (0.8 μM), indicating the presence of a ligand with a high affinity for Cu. At higher dissolved concentrations the ASV-labile concentration increased, but not as quickly as the dissolved concentration, suggesting the presence of a weaker ligand at a concentration above 0.8 μM. The DGT-labile concentrations were

consistently ~30% of the dissolved concentrations, considerably higher than the ASV-labile values, probably reflecting the two factors summarized above.

Bioassays for Cu and Zn using *Daphnia magna* offer additional insight as to which method provides a better measure of metal availability to biota. In the Cu-amended mixture of "moderately hard water" and Nicomekl River water, ASV severely underestimated availability to *D. magna* while DGT predicted within 30% the LC_{50} concentration of copper. In Zn-amended EDTA-containing solution, both the ASV and DGT approaches yielded reasonable estimates of metal availability. These data are consistent with the suggestion that the ASV approach provides a measure of “free” metal ion, but fails to detect an additional fraction of “labile” metal that is available to biota. The DGT approach detects this additional “labile” fraction, although it appears to slightly overestimate the concentration.

Table of Contents

Table of Contents

LIST OF TABLES

LIST OF FIGURES

1 Introduction

1. Introduction

Trace metals exist in a variety of inorganic and organic forms in aquatic systems, ranging from simple hydrated molecules to large organic complexes. The biological availability (*i.e.*, toxicity) of metals in aquatic systems is strongly dependent on the aqueous species present (Part I, Chapter 2). Determination of the chemical form (speciation) of metals in the environment is therefore a critical component to ultimately predicting impacts to aquatic organisms.

A variety of techniques exist to quantify metal speciation; however, each of these isolates an operationally-defined fraction of the total metal concentration and there is presently a dearth of intercomparison data. The purpose of the work described in this report is to intercompare the technique of Diffusive Gradients in Thin-film (DGT) with other techniques for assessing metal speciation, and to determine which technique provides a better indication of metal toxicity. This introduction presents brief discussions of: 1) the influence of metal speciation on bioavailability; 2) controls on "labile" metal concentrations; 3) metal speciation techniques utilized, and 4) experimental design for this study.

Throughout this part of the report, references are made to unpublished data collected by Lorax between 1997 and 1999. These data were collected under a two-year research and development program jointly funded by the Science Council of B.C., Lorax Environmental and Placer Dome Inc. Extensive quality assurance/quality control data for copper concentrations, as determined by DGT, in both the absence and presence of ligands have been accumulated and are referenced in this report.

1.1 Metal Bioavailability Controlled by Labile Species

It has been demonstrated that the bioavailability (and hence toxicity) of many metals in aqueous systems (*e.g.*, Cu, Zn, Fe, Mn, Cd) is reduced when metal-complexing ligands are present. The "Free Ion Activity Model" (FIAM) suggests that only free metal ions [M^{n+}] are transported across biological membranes (Anderson *et al.*, 1978; Campbell, 1995). Species that fit this label include not only free metal ions, but also hydrated aquo complexes and kinetically-labile inorganic complexes that dissociate rapidly to free metal ions. In the following discussions, the free metal ion concentration plus the concentration of the weakly complexed species in solution, M′ (Cu′, Cd′, Zn′, *etc.*), will be referred to as "labile" metal species.

1.2 Controls on Labile Metal Concentration

The labile metal concentration in solutions containing complexing ligands depends on the concentrations of both metal and ligand, as well as on the strength of the stability constant (K) of the metal-ligand complex. The ensuing discussion of metal-ligand complexation theory will aid in the understanding of the experimental data and technique comparisons presented in subsequent chapters.

Considering a simple solution with only one metal and one ligand present, the reaction between labile metal species (M′) and a ligand (L) may be expressed as:

$$M' + L \leftrightarrow ML, \qquad \text{(Eq. 1-1)}$$

The stability constant that describes the strength of complexation can be described as:

$$K_{L/M'}^{Cond} = \frac{[ML]}{[M'][L]}, \text{ where} \qquad \text{(Eq. 1-2)}$$

$K_{L/M'}^{Cond}$ = conditional stability constant between the metal and ligand

[ML] = activity of complexed metal;

M′ = sum of the free metal ion activity and those of the weakly-complexed inorganic (labile) species; and

[L] = activity of free ligand

To a first approximation, when the ligand concentration greatly exceeds the metal concentration, a plot of the labile metal concentration versus the total dissolved metal concentration yields a line with a slope:

$$\frac{1}{K[L]+1} \qquad \text{(Eq. 1-3)}$$

Thus, if the concentration of ligand is known, the strength of the metal-ligand complex (as defined by K) can be determined from the slope. For example, when a strong ligand (large K) is present, the plot of labile metal concentration versus dissolved metal concentration yields a low sloping line that remains close to the x-axis (Figure 1-1). When a weak ligand (small K) is present, that same line slopes more steeply (Figure 1-1).

This simplified example overlooks some processes that complicate the interpretation of metal speciation data, including the effects of: 1) ions of multiple elements on the complexing ability of the ligand; 2) formation of multiple complexes with the ligand; 3) multiple metals; and 4) multiple ligands. Furthermore, the linear relationship breaks

down when the metal concentration approaches the ligand concentration, because the concentration of free ligand [L] ceases to be approximated by the total ligand concentration and thus changes with increasing dissolved metal concentration. Due to these complications in speciation calculations, computer programs are typically used for such computations.

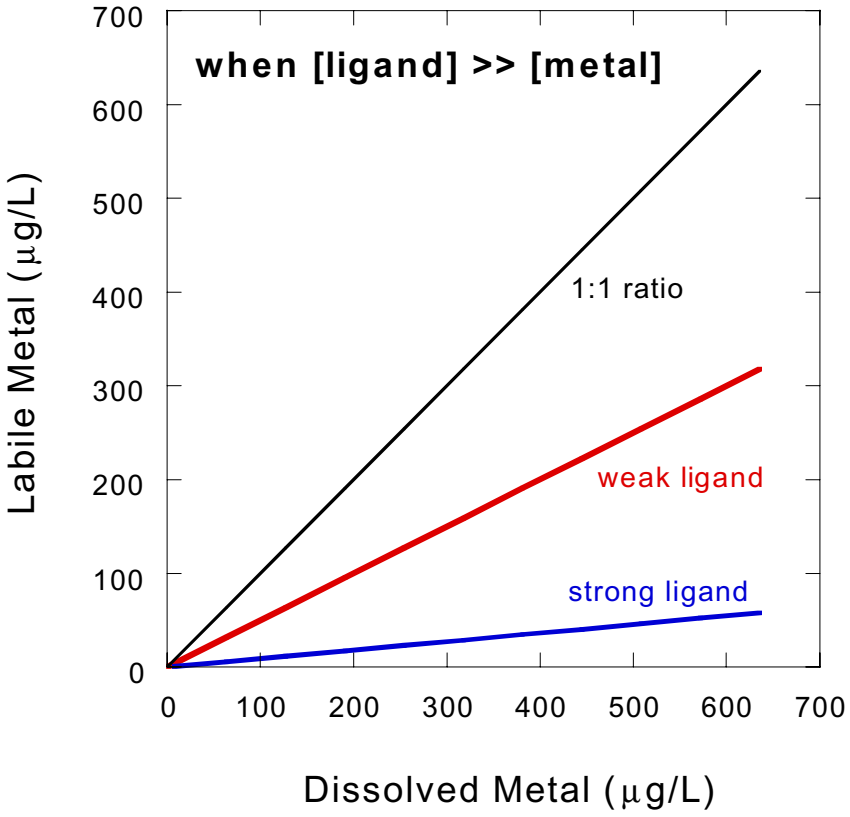


Figure 1-1: A simplified simulation showing how the labile metal ion concentration compares to the dissolved metal concentration in the presence of both "weak" and "strong" ligands. In this example it is assumed that the concentration of the ligand greatly exceeds the metal concentration.

The deviation from linearity as the metal concentration approaches the ligand concentration is well illustrated using MINEQL+, a well-known chemical speciation program (Schechler and McAvoy, 2001). Using copper as a generic example, speciation is simulated for a fixed concentration (7.88 µM) of either a weak or strong ligand (Figure 1-2). In this example, the Cu concentration varied from 0 to 1300 µg/L (0 to 20 µM); at low Cu concentrations each of the simulated datasets is reasonably well described as a straight line (as in the simplified example in Figure 1-1). The slope increases with decreasing ligand strength, and as the dissolved metal concentrations approach the ligand concentration the trends deviate from linearity. This is because the free ligand

concentration decreases as the total metal concentration approaches the total ligand concentration (Equation 1-3). Once the ligand concentration is exceeded (to the right of the dashed line in Figure 1-2), the trends begin to approximate another straight line parallel to the 1:1 line, because all of the additional metal exists as labile species. These profiles are merely theoretical, but their characteristics will serve as useful models against which to compare the actual speciation data presented in the following chapters.

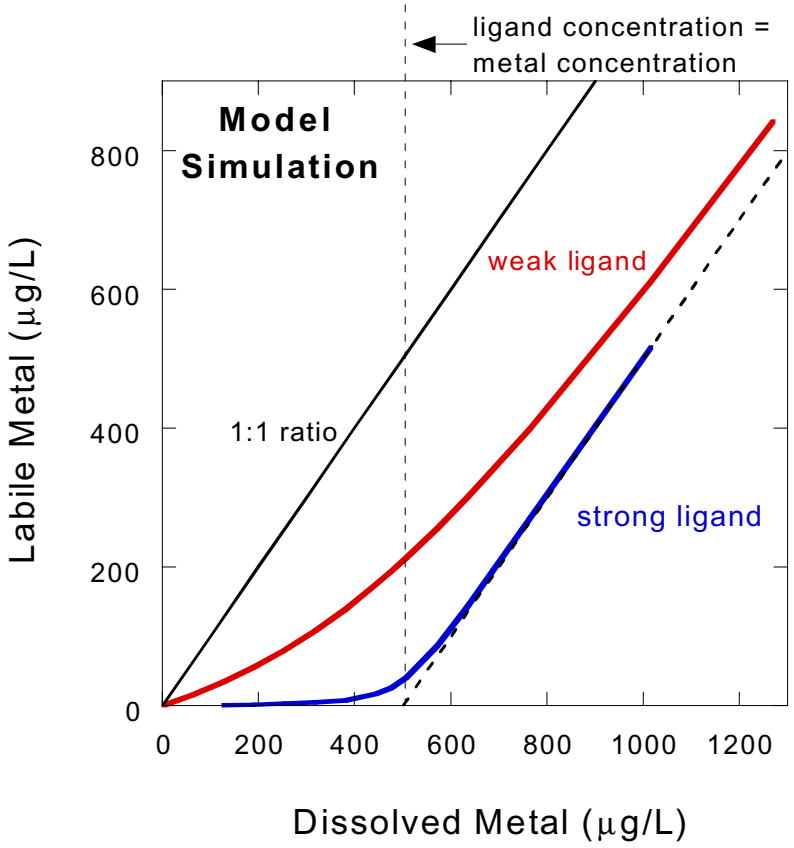


Figure 1-2: Labile metal ion concentration versus dissolved metal ion concentration, as simulated using the modelling program MINEQL+. Labile ion concentration is calculated in this example as the sum of inorganic species. For both the strong and weak ligand cases, the ligand concentration was fixed at 7.88 µM. Thus the ligand concentration and metal concentration (in this case Cu) are equal when the metal concentration was equal to 500 µg/L (dashed vertical line).

The "complexing capacity" can be defined as the total concentration of complexing sites on the ligands that complex the metal of interest in a given solution. It is important to note that labile metal is present even when the dissolved metal concentration is less than the "complexing capacity" (Figure 1-2). This is true for both the strong and weak ligand cases. The complexing capacity can be determined by extending a line through the linear

portion of the data, and is equal to the concentration at which this line intercepts the x-axis (Figure 1-2). In subsequent chapters the term complexing capacity is used to refer to the dissolved concentrations at which the ligand concentration is equal to the metal concentrations (dashed vertical line in Figure 2-1).

Another approach, termed "Langmuir linearization", is particularly useful for determining complexing capacity (ligand concentration), when a single weak ligand is present. This approach utilizes the fact that a plot of the ratio of labile to bound metal versus labile metal yields a straight line, with a slope equal to $1/L_T$ and an intercept of $\frac{1}{K_{L/Cu}^{Cond} \cdot L_T}$. Thus, from such a plot, the ligand concentration and strength can be determined. Because the focus of this report is on intercomparisons of speciation techniques and not on determinations of complexation capacity or ligand strength, Langmuir linearization is not discussed in further detail.

1.3 Speciation Techniques

Speciation determinations were made in this study using two different techniques: 1) DGT; and, 2) Anodic Stripping Voltammetry (ASV). These methods are discussed in detail in Part I, Chapter 3. As a consequence, only a very brief summary of each technique will be provided in this section.

In the DGT approach, metals are quantified using a ~4 cm-diameter disk-shaped device that contains a filter membrane underlain by a polyacrylamide gel layer, consisting of 98% water, which is in turn underlain by a trace-metal-adsorbing gel-embedded resin (Chelex). Metals diffuse from solution across the filter and gel layers to the underlying resin, where metal sorption takes place. The resin only adsorbs free and kinetically-labile metal ions. In ASV, the metal ion of interest is deposited on a mercury electrode via the reduction of the metal to a metallic state and subsequent amalgamation with the mercury. This is followed by a voltammetric scan, during which the mercury-bound metal is oxidized and the current produced is determined. The identity of the metal is dictated by the potential (voltage) of the peak, while the concentration is proportional to the current.

Each of these methods quantifies a fraction of the total metal concentration that includes the free metal ion as well as those complexed species that dissociate to free metal ion at a fast rate relative to the timescale of the measurement. These techniques can therefore yield different results, in part due to differences in the timescales of the measurements. For example, the timescale of the ASV method is on the order of ~0.1 seconds (Zhang and Davison, 2000), while the timescale of the DGT method is on the order of ~10 minutes (the time required for diffusion of the metal species across the 0.8 mm-thick gel layer; Zhang and Davison, 2000). Based on these considerations alone, the DGT method

would be expected to quantify slowly-dissociating species better than the ASV method, and thus the DGT approach would detect a larger fraction of the dissolved concentration than would the ASV technique.

1.4 Experimental Design

In an attempt to better define metal species measured by existing techniques, laboratory experiments were conducted to compare metal speciation data as determined by DGT, ASV, and to a lesser extent ion-selective electrode (ISE) methods. In a second stage, the speciation results were compared to a biological response using cultures of *Daphnia magna* in defined ligand-bearing solutions. The latter component was conducted to evaluate the various techniques as indicators of metal toxicity. Detailed experimental and analytical procedures are described in Appendix A.

Copper (Cu), cadmium (Cd) and zinc (Zn) were the metals chosen for evaluation in this study. Two well-characterized ligands, EDTA (Ethylenediaminetetraacetic acid) and NTA (nitrilotriacetic acid), were used in the experiments. EDTA is widely used as a metal chelator in industrial processes and has a strong affinity for a wide variety of metals, while NTA is a well characterized moderately strong (weaker than EDTA) ligand. Stability constants for EDTA and NTA complexes of Cu, Cd and Zn are provided in Table 1-1.

Table 1-1:
Stability Constants for Formations of Complexes from Metals and Ligands (from Morel and Hering, 1993)[1]

Metal	log K- EDTA	log K- NTA
Cu	20.5	14.2
Cd	18.2	11.1
Zn	18.3	12.0

[1]Values given for zero ionic strength at 25ºC.

In addition to the experiments with synthetic solutions, the speciation of Cu and Cd was carried out using natural waters rich in dissolved organic carbon (DOC). This water was collected from the Nicomekl River, a meandering stream that receives runoff from peat bogs and agricultural lands in southern Greater Vancouver. Nicomekl River waters have been previously shown to complex Cu (Lorax, unpublished data). In addition, bioassays using the copepod *Daphnia magna* were conducted on the experimental solutions in which the dissolved, DGT-detectable and ASV-detectable concentrations had been determined.

2 Copper

2. Copper

Copper concentrations in surface waters are subject to considerable regulatory scrutiny due to the known toxicity of the metal to aquatic organisms (Sunda *et al*., 1987; Kessler, 1986). Copper speciation, and hence toxicity, is strongly influenced by the presence of a variety of ligands in natural waters. For example, natural organic molecules may form strong complexes with copper, significantly reducing the concentration of labile Cu species in natural waters (Xue and Sunda, 1997; Sunda and Huntsman, 1998; Coale and Bruland, 1988; Playle, 1998; Croot *et al*., 2000).

Previous work has demonstrated that copper concentrations determined by DGT have the potential to provide a valuable indication of Cu complexation by both EDTA and DOC-rich waters (Lorax, unpublished data). Specifically, DGT-Cu concentrations were observed to be significantly lower in the presence of these ligands as compared to dissolved concentrations (*i.e.,* as determined following 0.45 μm filtration). The present study expands on the previous work through a series of laboratory speciation and toxicity studies. This chapter summarizes the data from the copper speciation intercomparison experiments, as well as from a toxicity experiment. In brief, speciation intercomparisons were carried out in the presence of: 1) EDTA, a strong, well-characterized ligand; 2) NTA, a moderately strong (weaker than EDTA), well-characterized ligand; and 3) Nicomekl River water, a DOC-rich freshwater stream previously shown to have a high complexing affinity for copper. Experimental and analytical conditions for the experiments are listed in Appendix A.

2.1 Experiments with EDTA

The aim of the experiments using EDTA as a ligand was to assess the effect of a known strong ligand on the speciation of copper as measured by both ASV and DGT. In this experiment, multiple solutions containing 1.47 mg/L EDTA (3.94 μM) had varying amounts of Cu added to generate dissolved Cu concentrations ranging from 0-500 μg/L (0 to 7.7 μM). These solutions were prepared using "moderately hard water" (Appendix A).

Labile Cu concentrations as determined by DGT and ASV are plotted against dissolved concentration in Figure 2-1. A theoretical 1:1 slope in Figure 2-1 represents the relationship that would be expected in the absence of a ligand (*i.e.,* labile Cu = dissolved Cu). The line that forms the boundary between the shaded and un-shaded areas in Figure 2-1 represents the concentration of Cu that equals the complexing capacity of the EDTA. The shaded area therefore represents concentrations of Cu in excess of the ligand, where

added Cu would be expected to be in labile form. The theoretical distribution of labile Cu (calculated as the sum of inorganic species) versus dissolved Cu in the presence of EDTA, as determined by the geochemical speciation model MINEQL+ (version 4.5; Appendix A), is also presented in Figure 2-1.

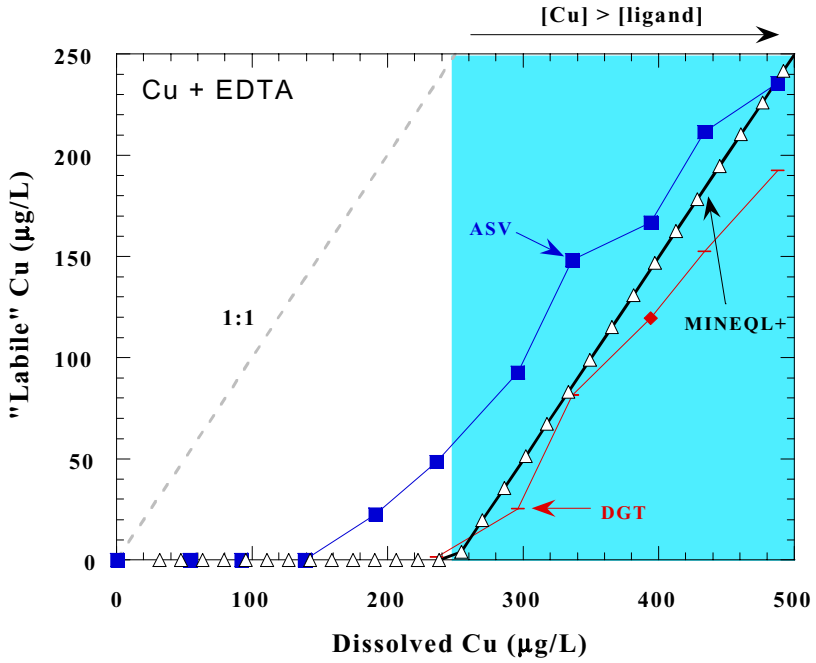


Figure 2-1: DGT-Cu and ASV-Cu concentrations versus dissolved Cu in the presence of EDTA. Also shown is the theoretical 1:1 slope for labile versus dissolved Cu in the absence of a ligand and the labile Cu concentration in the presence of EDTA as predicted by MINEQL+ (version 4.5).

DGT-Cu concentrations were below or close to detection limits (0.05 µg/L) in the presence of excess EDTA (un-shaded area in Figure 2-1). Once the complexing capacity of the EDTA was exceeded (shaded area in Figure 2-1), the concentration of labile and dissolved Cu approximates a 1:1 slope, suggesting that all Cu was present in labile form. The DGT data are in good agreement with the theoretical distribution of labile Cu as calculated by MINEQL+ (Figure 2-1), although high DGT Cu blanks prevented a rigorous intercomparison in this concentration range. These data indicate that DGT-Cu concentrations provide a realistic measure of labile Cu concentrations in the presence of a strong ligand.

ASV-Cu concentrations are higher than both DGT-Cu concentrations and those predicted by MINEQL+ in solutions where the dissolved Cu content approaches and exceeds the complexing capacity of EDTA (Figure 2-1). Once the complexing capacity of EDTA is exceeded (shaded area in Figure 2-1), dissolved versus labile Cu concentrations as

determined by ASV approximate a 1:1 slope, indicating that Cu added beyond the complexing-capacity threshold was in labile form.

Generally, both ASV-Cu and DGT-Cu concentrations were significantly lower than dissolved Cu concentrations, as would be expected in the presence of a strong ligand such as EDTA. Both methods demonstrate that once the complexing capacity of EDTA has been exceeded, all Cu added is labile. The main difference in assays of labile Cu by the two methods occurred in solutions where the complexing capacity of EDTA was approached (ligand > Cu). When this condition is established, measured ASV-Cu concentrations were significantly higher than those determined by DGT-Cu or predicted by MINEQL+. The reason for the "overestimation" of labile Cu as determined by ASV in the presence of EDTA is not obvious; it is unlikely to be due to kinetic effects, as the solutions were prepared at least 15 hours before the ASV determinations commenced.

Note that in all model runs, the "labile" fraction predicted by MINEQL+ in all model runs is assumed to include all inorganically complexed Cu, not just Cu^{2+}. Furthermore, in all MINEQL+ model runs, Cu mineral precipitation was turned off (see Appendix A). MINEQL+ predicted precipitation of Cu-containing minerals at high Cu concentrations, but such precipitation was not observed, hence it was not permitted in the model runs. This observation can be explained either by slow precipitation kinetics or inaccuracies in the MINEQL+ database.

2.2 Experiments with NTA

The aim of the NTA experiment was to determine Cu speciation in the presence of a well-characterized ligand that is substantially weaker than EDTA. In this experiment, NTA was added to the solutions at a concentration of 2.17 mg/L (7.88 μM), while Cu was added to yield dissolved concentrations between 0 and ~900 μg/L (0 to 14 μM). DGT-Cu and ASV-Cu concentrations, as well as the theoretical distribution of labile Cu in the presence of NTA, as predicted by MINEQL+, are presented in Figure 2-2.

ASV-Cu concentrations were below the detection limit for solutions in which NTA was well in excess of Cu concentrations (Figure 2-2). ASV-Cu increased in solutions approaching the complexing capacity of NTA, although this increase was observed when there was still an excess of NTA. Labile Cu concentrations predicted by MINEQL+ were lower than the ASV results and much closer to the ISE results (Figure 2-2).

The DGT-Cu concentrations in the presence of NTA are consistently higher than ASV and predicted (MINEQL+) concentrations. At Cu levels >~300 μg/L, the trend of the DGT-Cu versus dissolved Cu data is near linear and approximates a 1:1 slope. DGT-Cu values are however approximately 30% lower than dissolved concentrations (Figure 2-2).

The most likely explanation for the higher DGT-Cu, relative to the ASV-Cu, is that DGT assays have a much longer time constant. Dissociation of relatively weak (and thus "labile") NTA-Cu complexes would occur at the Chelex-solution interface where Cu was adsorbed. Given the relatively long deployment time of the DGT sampler, and ongoing diffusion of NTA-Cu through the gel, this would permit a higher proportion of Cu to be sequestered by the resin than would be the case for an "instantaneous assay", such as that provided by ASV. In the extreme case, therefore, DGT could potentially identify all of the Cu as labile in the various NTA-bearing solutions.

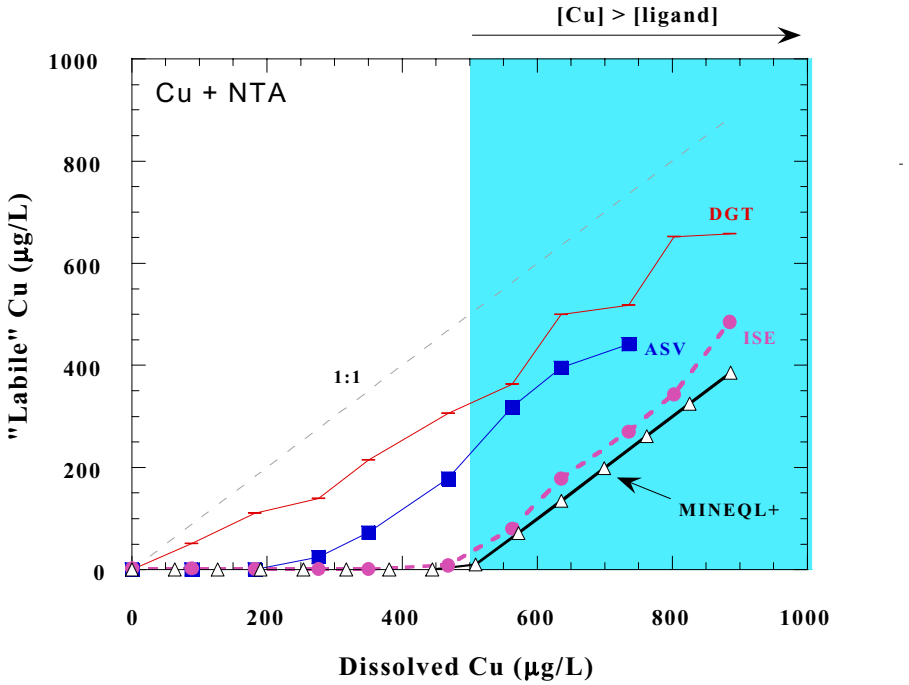


Figure 2-2: DGT-Cu and ASV-Cu concentrations versus dissolved Cu in the presence of NTA. Also shown are dissolved copper measurements made by ion selective electrode, and the theoretical 1:1 slope for labile versus dissolved Cu in the absence of a ligand and the labile Cu concentration in the presence of NTA as predicted by MINEQL+.

If this postulate is correct, the DGT-determined Cu concentrations could be expected to be the same as those for dissolved Cu, and the analytical data should fall on the 1:1 slope shown in Figure 2-2. Instead, the DGT-Cu values are approximately 30% lower than dissolved concentrations resulting in an offset from the 1:1 line (Figure 2-2).

This contrast between the proposed and the actual behaviour likely relates to the method used to calculate the DGT-Cu concentrations. Because Fick's First Law of diffusion is used to calculate Cu concentrations, the calculated DGT-labile concentration is inversely proportional to the diffusion coefficient of the diffusing species (Part I, Chapter 4). For the DGT-Cu values calculated in this study, the molecular diffusion coefficient for Cu^{2+}

was used. Given that the molecular weight of Cu is approximately 64 and that for the Cu_3NTA_2 complex is 572, the diffusion coefficient for Cu_3NTA_2 (which is the actual diffusing species) would be lower than the value used in the calculations for Cu^{2+}. The actual diffusion coefficient for Cu would therefore be overestimated, and this could explain the underestimation of Cu concentrations by DGT in NTA solutions. This hypothesis is supported by the linear trend in the DGT-Cu versus dissolved Cu data, which implies that the lower than postulated DGT-Cu concentrations result from an overestimated diffusion coefficient. If the DGT-determined Cu values instead reflected ligand strength, a non-linear relationship would have been observed (contrast Figure 1-2).

2.3 Experiments with DOC-rich Water

An experiment was carried out using water from the Nicomekl River as a Cu-complexing ligand. Nicomekl waters are DOC-rich, and have previously been shown to complex Cu (Lorax, unpublished data). The experiment was conducted using 10% Nicomekl River water and 90% "moderately hard water" (Appendix A), in order to keep conditions as comparable as possible to those for the other experiments, and to minimize complications due to the high-DOC matrix. Added Cu concentrations in the experiments varied between 0 and 130 µg/L (0 and 2 µM). DGT-Cu, ASV-Cu, and ion-selective electrode Cu values plotted versus total dissolved Cu concentrations are presented in Figure 2-3.

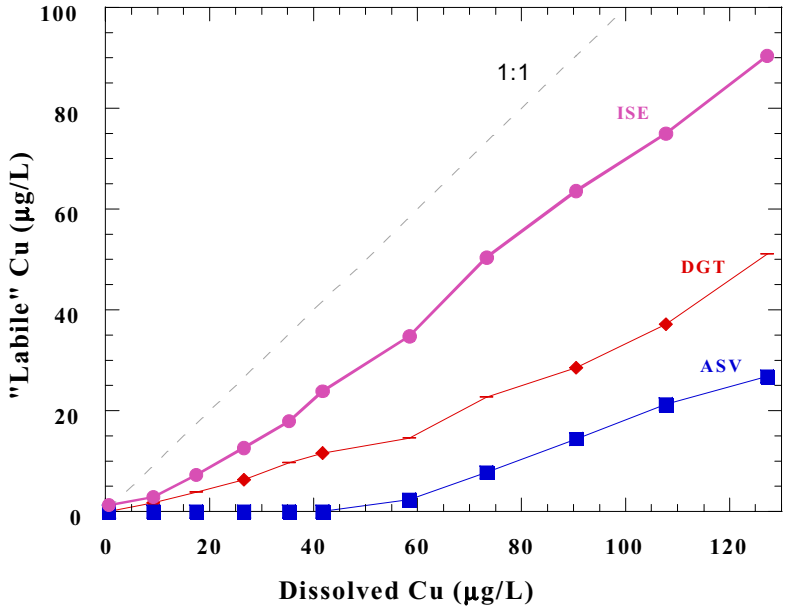


Figure 2-3: DGT-Cu, ASV-Cu, and ion-selective electrode Cu concentrations versus total dissolved Cu in the presence of the 10% DOC-rich Nicomekl and 90% moderately hard water mixture. Also shown is the theoretical 1:1 slope for labile versus dissolved Cu in the absence of a ligand.

At dissolved Cu concentrations less than approximately 50 µg/L, the ASV-determined Cu values were below the detection limit (Figure 2-3), suggesting the presence of a strong Cu-complexing ligand. Based on the break in slope of the ASV-Cu data, the copper-complexing capacity of the dominant ligand in the DOC-rich waters of the experimental diluted Nicomekl River water is on the order of 50-60 µg/L. Above a dissolved concentration of approximately 50 µg/L, the ASV-Cu concentration increased with increasing Cu addition, suggesting that the complexing capacity of the primary Cu-complexing ligand in the diluted Nicomekl water was exceeded. However, above a dissolved Cu concentration of 60 µg/L, the ASV-Cu concentration increased more slowly than the dissolved concentration, showing that not all of the Cu added to the solutions was in labile form. One possible explanation for this trend is that a weaker Cu-complexing ligand is present in the water in addition to a more dominant (stronger) ligand. This would be expected in natural waters such as those from the Nicomekl; a number of ligands of varying strengths would be common in such waters. Another possibility is that the sensitivity of the ASV method was reduced in the presence of the high-DOC matrix. This phenomenon is well documented in the ASV literature (Florence, 1982).

DGT-Cu concentrations show a similar trend to ASV-Cu, with the exception that they are consistently higher (Figure 2-3). The main difference in the DGT and ASV data lies in the 0-50 µg/L dissolved Cu concentration range, where ASV-Cu values are all below detection limits while DGT shows a near-linear increase in labile Cu. However, the break in slope of the DGT-Cu curve (which suggests a complexing capacity of 50-60 µg/L for the dominant ligand in the dilute river water) and the steeper subsequent slope are similar to those seen in the ASV data and support the notion that at least one secondary weaker ligand is present in the Nicomekl River waters. The ISE results suggest a much higher concentration of labile Cu than either the ASV or DGT technique. The reasons for the higher ISE concentrations are not clear, but may be due to matrix-dependent differences in instrument sensitivity. Further comparative experiments are necessary to determine is such influences are key to the observed differences.

The consistently higher DGT-Cu as compared to ASV-Cu concentrations may be explained by either or a combination of the following: 1) the sensitivity of the ASV technique is reduced in the presence of DOC (Florence, 1982) or, 2) the DGT method measures species that are not detected by ASV. The latter explanation relates to differences in the timescale of measurement for DGT and ASV methods, as elaborated immediately below.

The effective analytical time of speciation measurements is proportional to the square of the distance that the analyte must diffuse between the bulk solution and the measurement interface. The diffusion distance for ASV is on the order of 10 μm in a typical well-stirred solution (Zhang and Davison, 2000), while it is on the order of 1 mm (1000 μm) for a DGT device. For ASV, the effective measurement time translates to approximately 0.1 seconds, while for DGT it is closer to 13.5 minutes, roughly 10^4 times as long (Zhang and Davison, 2000). Thus, the ASV technique will only detect species with rapid dissociation kinetics (*i.e*. those that can dissociate within 0.1 seconds). Such dissociation rates are limited to weak inorganic complexes. In contrast, the DGT technique will detect both rapidly dissociating species and those with much slower dissociation kinetics (*i.e.* those that dissociate within ~10-15 minutes). The DGT approach would thus be expected to detect less labile species, because there is considerably more time for such complexes to dissociate during diffusion. Based on this discussion, the labile fraction as determined by DGT would include complexes that dissociate more slowly than those measured by ASV. This suggestion is consistent with the higher DGT-Cu concentrations (compared to ASV-Cu) observed in the NTA and DOC speciation experiments. DGT therefore provides a more liberal estimate of labile metal species in natural waters than ASV.

2.3.1 Speciation and Toxicity Comparisons

The same diluted Nicomekl River waters that were analyzed by all three speciation methods were also used in a 48-hour acute lethality bioassay on the copepod *D. magna*. Thus, direct comparisons can be made between the speciation and mortality data to assess which speciation technique provides the best indication of the bioavailability of copper to this organism. ASV-Cu, DGT-Cu and ISE-Cu concentrations plotted against dissolved Cu concentrations and toxicity data from D. *magna* bioassays are presented in Figure 2-4.

The onset of mortality for D. *magna* occurred at dissolved (0.45 μm filtered) Cu concentrations of approximately 30 μg/L, with an abrupt increase in mortality being observed at a dissolved Cu concentration of ~60 μg/L (Figure 2-4). These toxicity data are consistent with DGT and ASV results which indicated that the complexing capacity of the dominant ligand was 50-60 μg/L.

ASV-Cu concentrations were below detection limits at concentrations in which a 10% mortality rate for *D. magna* was observed (Figure 2-4). Over the 10 to 70% mortality range, ASV-Cu increased from 0 to only ~ 3 μg/L. The low ASV-Cu concentration measured in a solution with 70% mortality demonstrates that the ASV technique underestimates toxicity to D. magna. Underestimation of Cu toxicity by the ASV technique has been observed elsewhere (Florence, 1982). By contrast, DGT-Cu values of approximately 10 μg/L were observed in the lower range of *D. magna* mortality (10%).

These increased to approximately 15 µg/L as mortality increased to 70% (Figure 2-4). The DGT-determined Cu values therefore demonstrated better correlation with the observed toxicological effects than did the ASV-Cu concentrations. However, only 10% mortality was observed at DGT-Cu concentrations as high as 10.5 µg/L Cu, a concentration essentially equivalent to the dissolved Cu LC_{50} (10.8 µg/L) in the absence of any ligand. This suggests that the DGT approach may overestimate Cu toxicity to *D. magna*.

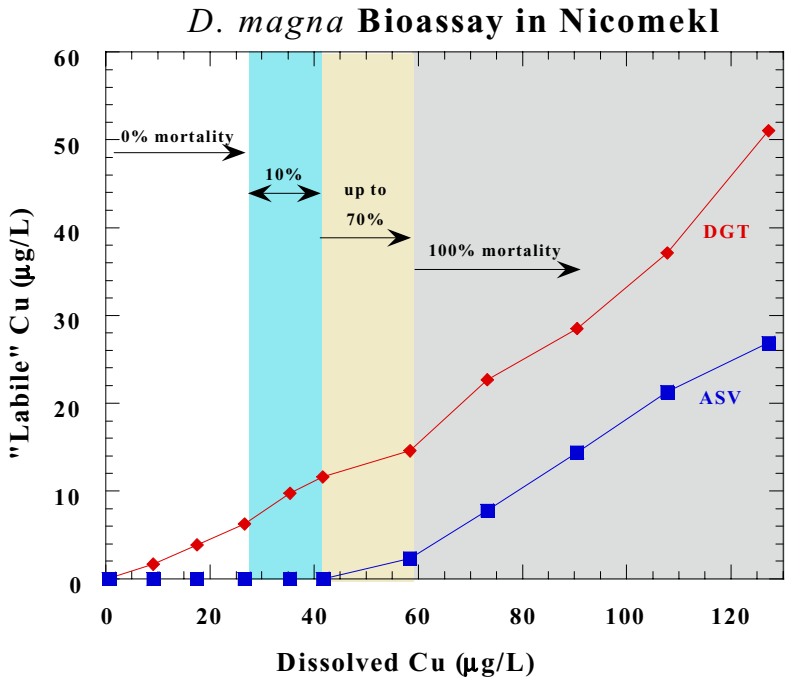


Figure 2-4: DGT-Cu, ASV-Cu, and ISE-Cu concentrations versus dissolved Cu and *D. magna* toxicity data in the presence of the 10% DOC-rich Nicomekl and 90% moderately hard water mixture.

LC_{50} values for dissolved Cu in moderately hard water (no ligand) and for DGT, ASV and dissolved Cu in the Nicomekl and moderately hard water mixture (with ligand) are presented in Figure 2-5. The dissolved Cu LC_{50} was 49 µg/L in the presence of the Nicomekl/moderately hard water mixture, considerably higher than the dissolved Cu LC_{50} of 10.8 µg/L in moderately hard water with no added DOC, demonstrating the effect of Cu complexation on the toxicity of the Nicomekl water mixture. These data reinforce the now well-known limitations of "total dissolved Cu" concentrations as an indicator of toxicity to aquatic organisms. By contrast, the DGT-Cu LC_{50} of 13.3 µg/L was much closer to the dissolved Cu determined in the absence of added ligand (Cu LC_{50} of 10.8 µg/L). The close agreement between the LC_{50} for labile Cu in the test solution with no ligand and that for the labile Cu as determined by DGT in the presence of at least

one ligand suggests that DGT provides a reasonable measure of the bioavailable copper. By contrast, the ASV-Cu LC_{50} is 2.4 µg/L, considerably lower than the dissolved (labile) value determined in the absence of any ligand. As discussed previously, the reasons for the low ASV values may relate to analytical artifacts related to the lower sensitivity of the method to less labile species as compared to DGT.

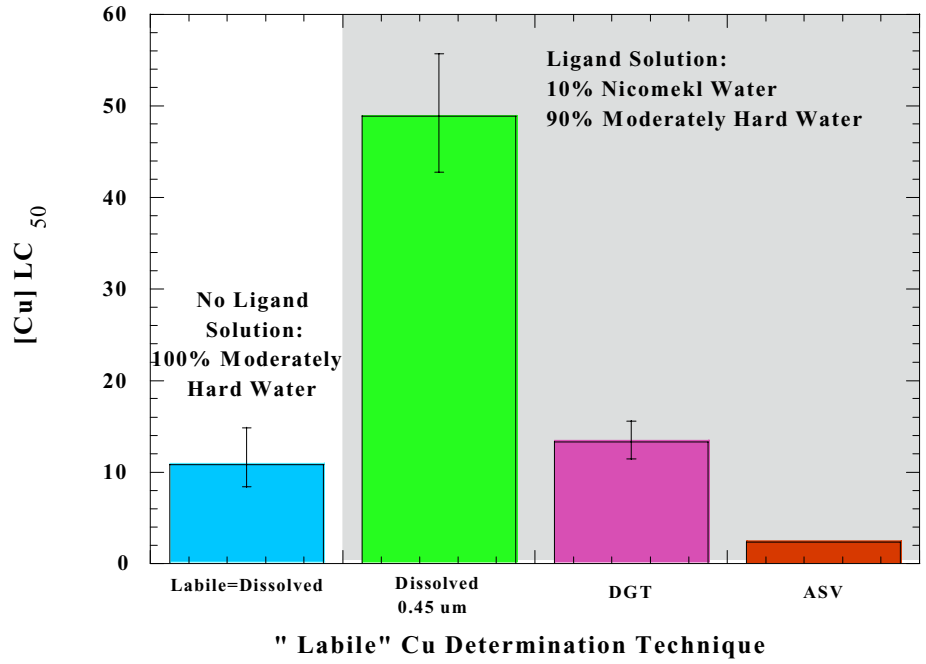


Figure 2-5: LC_{50} for DGT, ASV and dissolved concentrations in the Nicomekl and moderately hard water mixture. LC_{50} for dissolved Cu in the absence of Nicomekl water (*i.e.*, no ligand) is also presented.

3 Cadmium

3. Cadmium

Although cadmium speciation in natural waters is not as well understood as that of copper, a variety of studies have demonstrated that complexation of Cd can be significant. For example, natural organic molecules have been shown in seawater to form strong complexes with cadmium, strongly reducing the concentration of "labile" Cd species (Kozelka and Bruland, 1998; Wells et al., 1998).

This chapter summarizes the data from the speciation intercomparision experiments carried out with cadmium in the presence of: 1) EDTA, a strong, well-characterized ligand, and 2) NTA (nitrilotriacetic acid), a moderately strong, well-characterized ligand. Experimental and analytical conditions for the experiments are listed in Appendix A.

3.1 Experiments with EDTA

As for copper, the intent of using EDTA was to assess experimentally the impact of a known "strong" ligand on Cd speciation. In the experiment, multiple solutions containing 3.31 mg/L EDTA (8.89 μM) had varying amounts of Cd added, generating solutions with concentrations ranging from 0-1600 μg/L (0 to 14 μM). The solutions were prepared using "moderately hard water" (Appendix A).

Labile Cd concentrations as determined by DGT and ASV are plotted against dissolved concentration in Figure 3-1. The theoretical 1:1 slope in Figure 3-1 represents the relationship that would be expected in the absence of a ligand (*i.e.,* labile Cd = dissolved Cd). The boundary between the shaded and un-shaded areas in Figure 3-1 represents the concentration of Cd that equals the complexing capacity of the EDTA. The shaded area therefore represents concentrations of Cd in excess of the ligand, where added Cd would be expected to be in labile form. The theoretical distribution of labile Cd versus dissolved Cd as determined by MINEQL+ (version 4.5; Appendix A), is also shown. Over the full range of total dissolved Cd concentrations in this experiment, the labile Cd levels as determined by ASV are in reasonable agreement with those predicted by MINEQL+ (version 4.5; Figure 3-1). These data suggest that ASV-Cu concentrations provide a realistic measure of labile Cd concentrations in the presence of a strong ligand. However, it must be noted that the correlation between the MINEQL+-predicted and the measured "labile" Cd values was achieved only by suppressing the precipitation of otavite ($CdCO_3$) during the MINEQL+ model runs. Without such suppression, no significant concentration of labile Cd species would have been predicted by MINEQL+ at high concentrations of added Cd. Precipitation of otavite was also predicted by PHREEQC

(another chemical equilibrium mode) using the WATEQ4f database. The fact that precipitation was not observed, despite the prediction by the two equilibrium models, suggests that either precipitation of otavite is hindered by reaction kinetics, or by a lack of nucleation sites, or that the database for this mineral is inaccurate.

With increasing total dissolved Cd levels below 800 µg/L in the presence of EDTA (the unshaded section of Figure 3-1), DGT-Cd concentrations increased slowly, reaching about 50 µg/L at 800 µg/L total Cd. Once the complexing capacity of the EDTA was exceeded (the shaded area in Figure 3-1), the concentrations of labile vs. dissolved Cd approximately define a 1:1 slope, suggesting that all added Cd was present in labile form. When Cd concentrations were less than the EDTA concentration, the labile Cd levels as determined by DGT were higher than those predicted by MINEQL+ (version 4.5; Figure 3-1). These data suggest that DGT-Cd concentrations overestimate labile Cd concentrations in the presence of a strong ligand, and thus provide a liberal measure of labile Cd concentrations under such conditions.

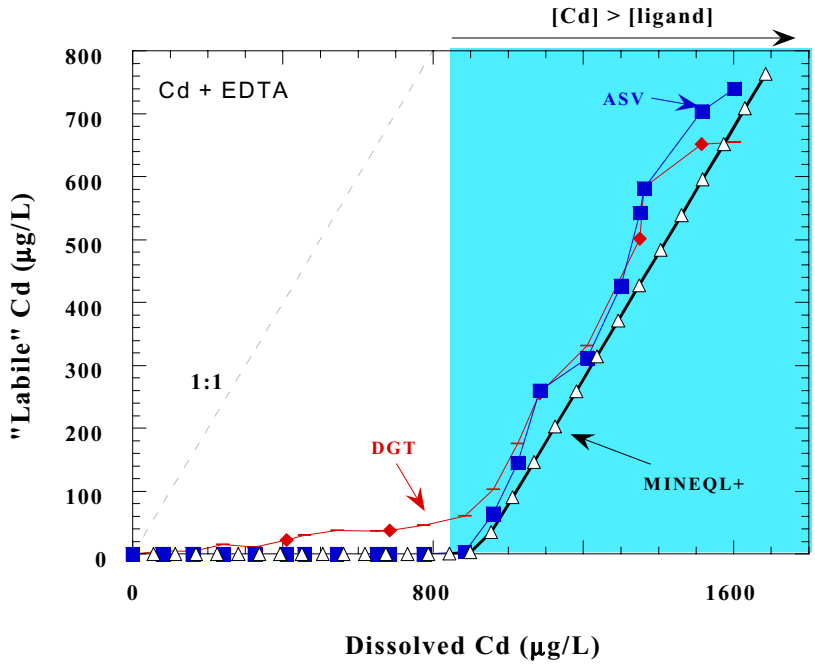


Figure 3-1: Labile Cd concentration as inferred by ASV and DGT approaches plotted versus the corresponding dissolved Cd concentration for the EDTA experiment. Also shown is the labile Cd concentration predicted by the geochemical speciation program, MINEQL+. Precipitation of otavite ($CdCO_3$) was suppressed for these runs.

Bioassays were carried out in solutions containing 0.034 mg/L EDTA (0.09 μM) and 0-21 μg/L (0-0.19 μM) total dissolved Cd. However, no mortality was observed in any of the solutions; hence none of these results are presented. The results do strongly imply, however, that the acute toxicity threshold for *Daphnia magna* with respect to cadmium is higher than 21 μg/L total Cd in "moderately hard water".

3.2 Experiments with NTA

The intent of using NTA as a ligand was to determine Cd speciation in the presence of a well-characterized ligand that is substantially weaker than EDTA. In the experiment NTA was added to the solutions at a concentration of 2.04 mg/L (7.5 μM), while Cd was added at concentrations ranging from 0 to ~930 μg/L (0 to 8.3 μM).

ASV-Cd concentrations were less than half those determined by DGT in solutions in which NTA was well in excess of total dissolved Cd contents (Figure 3-2). ASV-Cd increased quasi-exponentially in solutions as the complexing capacity of NTA was approached, while the DGT-Cd vs. dissolved Cd relationship remained linear across the analytical range (Figure 3-2). The ASV-Cd concentrations are within a factor of two of the MINEQL+ predictions, which is reasonable agreement. However, the DGT-Cd concentrations are much higher.

The most likely explanation for the higher DGT-Cd, relative to the ASV-Cd, is that DGT assays have a much longer time constant. Dissociation of relatively weak (and thus "labile") NTA-Cd complexes would occur at the Chelex-solution interface where Cd was adsorbed. Given the relatively long deployment time of the DGT sampler, and ongoing diffusion of NTA-Cd through the gel, this would permit a higher proportion of Cd to be sequestered by the resin than would be the case for an "instantaneous assay", such as that provided by ASV. In the extreme case, therefore, DGT could potentially identify all of the Cd as labile in the various NTA-bearing solutions. A similar suggestion was made for copper in the preceding chapter. If this postulate is correct, the DGT-determined Cd concentrations should be expected to be the same as those for total dissolved Cd, and the analytical data should fall on the 1:1 slope shown in Figure 3-2. Instead, and as was observed for Cu (Figure 2-2), the DGT-Cd values are approximately 30% lower than dissolved concentrations resulting in an offset from the 1:1 line (Figure 3-2).

As discussed for copper in section 2.2, the difference between the proposed and the actual behaviour likely relates to the use of Fick's First Law in calculating the DGT-labile concentration, which is inversely proportional to the diffusion coefficient of the diffusing

species (Part I, Chapter 4). For the DGT-Cd values calculated in this study, the molecular diffusion coefficient for Cd^{2+} was used. Given that the molecular weight of Cd is approximately 112 and that for the $Cd_3(NTA)_2$ complex is 713, $Cd_3(NTA)_2$, the actual diffusing species, would diffuse more slowly than Cd^{2+}. This could explain the underestimation of Cd concentrations by DGT in NTA solutions. This hypothesis is supported by the linear trend in the DGT-Cd versus dissolved Cd data, which implies that the lower than postulated calculated DGT-Cd concentrations result from an overestimated diffusion coefficient.

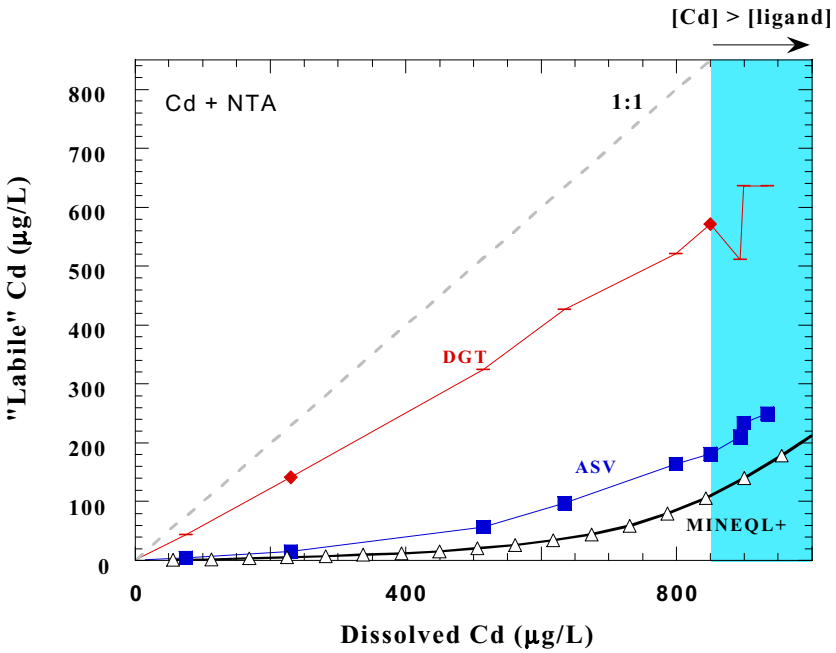


Figure 3-2: Labile Cd concentration as inferred by the ASV and DGT approaches plotted versus the corresponding dissolved Cd concentration for the NTA experiment. Also shown is the labile Cd concentration predicted by the geochemical speciation program, MINEQL+. Precipitation of otavite ($CdCO_3$) was suppressed for these runs.

It is worth noting that the stability constant for the Cu-NTA complex is three orders of magnitude larger than that for the Cd-NTA complex (Table 1-1, and Morel and Hering, 1993). Hence, there should be a significant difference in the trends of labile Cu and Cd versus the dissolved concentrations when NTA is present in excess, as is predicted by MINEQL+. Comparison of the curves for MINEQL+ in Figures 2-2 and 3-2 illustrates well the net result of the difference in the stability constants. However, this contrast did not carry through to the actual DGT-based determinations of labile Cd and Cu. The DGT approach consistently determined concentrations of both metals only one-third or so lower than the dissolved values, while ASV yielded lower values. As noted above, we

attribute this contrast to the longer timescale of the DGT measurement, a function of diffusion through the gel layer. This probably leads to detection of more slowly dissociating species (and hence higher measured concentrations) compared to ASV.

3.3 Nicomekl River Water

An additional experiment was conducted using Nicomekl River water as the metal-complexing ligand. However, unlike the case for copper, zero complexation of Cd was apparent in the ASV determinations. As a result, no DGT units were deployed, and no data are presented.

4 Zinc

4. Zinc

As is the case for a copper and cadmium, a variety of studies have demonstrated that zinc exists largely in complexed form in natural waters. For example, Zn in river water has been shown to be significantly complexed, leading to a ~50% reduction in bioavailability (Jansen et al, 1998). Furthermore, Zn in seawater has been shown to be largely complexed by organic ligands, strongly reducing the concentration of "labile" zinc species (Kozelka and Bruland, 1998; Wells et al., 1998). This chapter summarizes the data from a Zn speciation intercomparison experiment that was carried out in the presence of EDTA, as well as data from a bioassay carried out on *D. magna*. Experimental and analytical conditions for the experiments are listed in Appendix A.

4.1 Experiments with EDTA

As for the other metals discussed in this report, the intent of using EDTA as a ligand was to assess experimentally the impact of a known "strong" ligand on Zn speciation. In this experiment, multiple solutions containing 8.56 mg/L EDTA (23 μM) had varying amounts of Zn added to generate solutions with concentrations ranging from 0-2700 μg/L (0 to 42 μM). The solutions were again prepared using "moderately hard water" (see Appendix A).

Labile Zn concentrations as determined by DGT and ASV are plotted against total dissolved concentration in Figure 4-1. The theoretical 1:1 slope in Figure 4-1 represents the relationship that would be expected in the absence of a ligand. The boundary between the shaded and un-shaded areas in Figure 4-1 represents the zinc concentration (~1500 μg/L) that equals the complexing capacity of the EDTA. The shaded area therefore represents concentrations of Zn in excess of the ligand, where added metal would be expected to be in labile form. The theoretical distribution of labile versus dissolved Zn in the presence of EDTA, as predicted by MINEQL+ (Appendix A), is also shown in the figure.

DGT-Zn concentrations in the presence of excess EDTA (the un-shaded area in Figure 4-1) range from undetectable at zero added Zn^{2+} to ~110 μg/L at 1500 μg/L added total dissolved zinc. Above the complexing capacity of the EDTA (the shaded area in Figure 4-1), the slope of the labile vs. dissolved Zn relationship is ~1, implying that all added zinc was present in labile form. DGT-Zn concentrations significantly exceed those

predicted by MINEQL+ at concentrations below the EDTA complexing-capacity threshold, but agree well above this boundary (Figure 4-1).

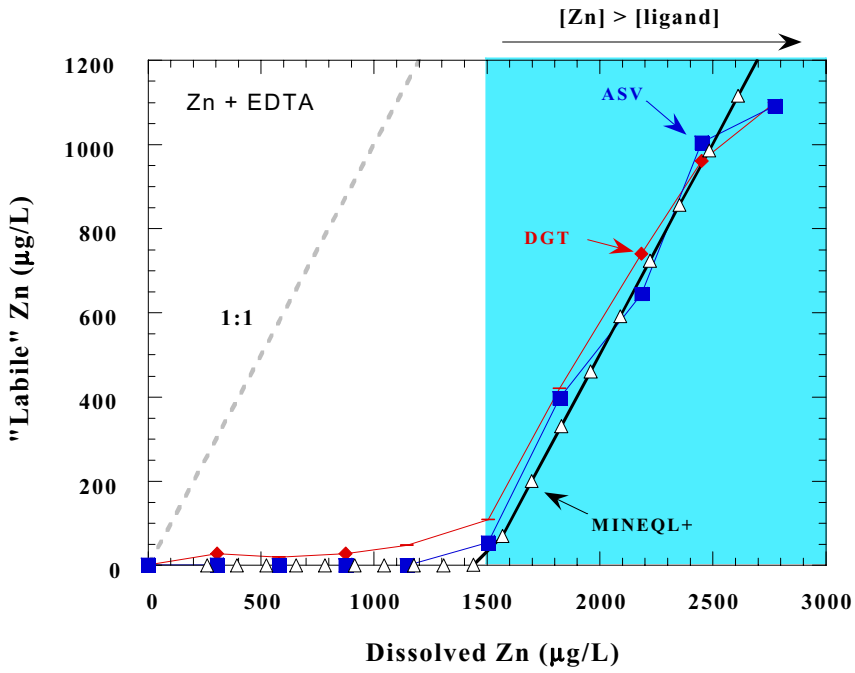


Figure 4-1: Labile Zn concentration as inferred by ASV and DGT approaches plotted versus the corresponding dissolved Zn concentration for the EDTA experiment. Also shown is the labile Zn concentration predicted by the geochemical speciation program, MINEQL+.

Unlike DGT-determined values, ASV-Zn concentrations are essentially undetectable below 1100 µg/L (Figure 4-1), but as for DGT, they increase on a 1:1 basis at levels above the complexing capacity of the added EDTA. The ASV data agree quite well with concentrations predicted by MINEQL+ (Figure 4-1) across the entire concentration range of the experiment, implying that assays of labile zinc using ASV provide a realistic measure of labile Zn^{2+} in the presence of a strong ligand.

The contrast between the DGT- and ASV-determined labile zinc concentrations at levels below 1500 µg/L total dissolved Zn, and the disagreement between theory (MINEQL+) and DGT-Zn in the same concentration range suggests that ASV provides a better indication of labile zinc content in the presence of a strong ligand than does DGT. This contention is of course based partly on the assumption that MINEQL+ offers a reasonable estimation of the true labile Zn content.

Bioassay experiments support the conclusion that ASV and DGT assays provide a reasonable estimation of bioavailable zinc in the presence of EDTA, a strong ligand. As for copper (Chapter 2) the toxicity of zinc to *Daphnia magna* was determined, via standard bioassays that were carried out in the same solutions in which the DGT units were deployed. An aliquot from each solution was also taken for ASV determinations. The bioassay results support the observation that Zn is significantly complexed by EDTA. No mortality was observed until the dissolved Zn concentration exceeded the EDTA concentration. Indeed, total dissolved zinc values on the order of 2000 µg/L were required to produce significant mortality in the presence of 8.56 µM EDTA (LC_{50} = 2020 µg/L), as summarized in Figure 4-2. DGT assays and ASV analyses of the same solutions yielded LC_{50} concentrations some four-fold lower (~500 µg/L; Figure 4-2). These operationally-defined values for "labile metal" illustrate the effectiveness of EDTA as a chelating agent. More importantly, bioassays carried out by B.C. Research using *D. magna* and EDTA-free "moderately hard water" yielded an LC_{50} of 575±200 µg/L dissolved zinc. This result is statistically indistinguishable from the LC_{50} values determined in the presence of EDTA by both DGT and ASV (Figure 4-2). Such common behaviour suggests that both the ASV and the DGT methods yield reasonable determinations of "labile" Zn when Zn concentrations exceed the complexing capacity of a "strong" ligand.

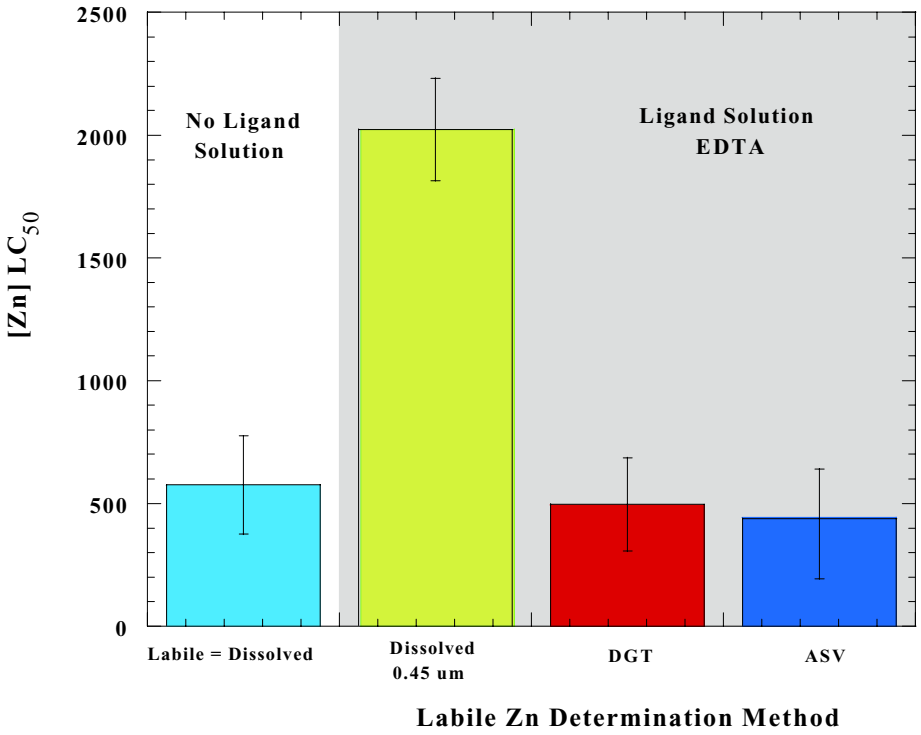


Figure 4-2: Determinations of [Zn] LC_{50} as determined from: 1) the ASV; 2) DGT and 3) dissolved Zn concentrations, all in the presence of EDTA, and 4) the dissolved Zn concentrations with no added EDTA present. The error bars represent one sigma uncertainties.

5 Summary and Conclusions

5. Summary and Conclusions

The experiments discussed in this report have illustrated that both Diffusive Gels in Thin Films (DGT) and Anodic Stripping Voltammetry (ASV) can provide estimates of labile Cu, Cd and Zn in natural waters in which both strong and weak complexing agents are present. Furthermore, bioassays show that the DGT technique provides an accurate measure of the Cu and Zn available to the water flea *Daphnia magna*. A number of caveats exist, however, and these are enumerated in the following key conclusions.

1. In the presence of an excess of the strong ligand EDTA (ethylenediaminetetraacetic acid), labile Cu, Cd and Zn concentrations as measured by both DGT and ASV approaches are substantially lower than total dissolved metal concentrations. However, DGT-labile concentrations are consistently higher than those assayed using ASV. Chemical equilibrium modeling (using MINEQL+) of Cu- and Zn-containing solutions suggests the DGT technique overestimates the labile concentration while the ASV approach provides a more reasonable estimate.

2. When total dissolved metal concentrations exceed the complexing capacity of the EDTA in solution, both methods provide accurate determinations of the labile concentration, suggesting that both approaches can quantify metal complexing capacity in the presence of strong ligands.

3. In the presence of an excess of the moderately strong ligand NTA (nitrilotriacetic acid), labile Cu and Cd concentrations as determined by ASV are substantially lower than the dissolved metal concentrations. DGT, by contrast, measures concentrations only ~25% lower than total dissolved levels. The difference is attributed to two factors: (a) the Chelex resin used in the DGT samplers is a strong complexing agent, and readily adsorbs free metal ions, maintaining a concentration gradient between the resin-gel interface and the bulk solution; (b) ASV samples the solution on a timescale of a fraction of a second, whereas DGT samples over ten or more minutes. Thus, the much greater time required for the metal to diffuse from solution to detection surface in the DGT approach permits dissociation of more slowly dissociating complexes and uptake of freed metal ions and thus leads to detection of a larger "labile" concentration by the DGT technique.

4. As for the strong ligand, EDTA, when the metal concentrations exceed the complexing capacity of the added weak ligand NTA, both ASV and DGT provide accurate determinations of the labile metal concentration.

5. Data obtained from experiments using natural waters suggest the presence of both strong and weak ligands. Under such conditions DGT and ASV delivered similar results. In a dilute solution of DOC-rich Nicomekl River water (90% "moderately hard water" and 10% river water), ASV-labile Cu concentrations were undetectable (<0.05 µg/L) when the dissolved Cu concentration was below ~50 µg/L (0.8 µM), indicating the presence of a ligand with a high affinity for Cu. At higher dissolved concentrations the ASV-labile concentration increased, but not as quickly as the dissolved concentration, suggesting the presence of a weaker ligand at a concentration above 0.8 µM. The DGT-labile concentrations were consistently ~30% of the dissolved concentrations, considerably higher than the ASV-labile values, probably reflecting the two factors summarized in point 3 above.

6. Bioassays for Cu and Zn using *Daphnia magna* offer insight as to which method provides a better measure of metal availability to biota. In the Cu-amended mixture of "moderately hard water" and Nicomekl River water, ASV severely underestimated availability to *D. magna* while DGT predicted within 30% the LC_{50} concentration of copper. In Zn-amended EDTA-containing solution, both the ASV and DGT approaches yielded reasonable estimates of metal availability. These data are consistent with the suggestion that the ASV approach provides a measure of “free” metal ion, but fails to detect an additional fraction of “labile” metal that is available to biota. The DGT approach detects this additional “labile” fraction, although it appears to slightly overestimate the concentration.

Appendix A: Experimental Design

Appendix A: Experimental Design

This chapter provides a brief summary of the design for each of the experiments. In each case, solution composition (other than the metal and ligand concentrations) was kept constant because of the large influence of solution conditions on metal speciation. The medium chosen to achieve this was "moderately hard water" (MHW) (as defined in Eaton *et al*., 1995; Standard Method 8010E). Characteristics of this solution are summarized in Table A-1. This medium satisfies four primary objectives: 1) it permits analyses by ASV as the ionic strength is higher than the minimum required of 1 mm; 2) it permits determinations using DGT since the concentration of cations (~2 x 10^{-3} M) is an order of magnitude higher than the minimum required for DGT determination (Alfaro-De la Torre *et al*., 2000); 3) it maintains pH at a constant value, despite additions of very weakly acidic metal solutions due to the buffering effect of the bicarbonate ion; and, 4) it maintains solutions at a constant hardness (Hardness is known to have a large impact on metal toxicity; Meyer, 1999; Meyer *et al*., 1999).

Table A-1:
Solution conditions for speciation measurements.
Concentrations are given in units of mmol/kg, except where noted.

Parameter	Value	Parameter	Value
Temperature(ºC)	20-21	Na	1.14
pH	7.4-7.8	K	0.05
Hardness (mg/L as $CaCO_3$)	80-100	HCO_3^-	1.14
Ca	0.35	Cl^-	0.05
Mg	0.5	SO_4^{2-}	0.85

In each experiment, ligand concentrations were kept constant while the metal concentrations were varied. Metals were added by diluting 10,000 mg/L metal-sulphate stock solutions (preserved in 0.5% HNO_3) to achieve actual concentrations of 0-2000 µg/L. Such a high degree of dilution ensured that the pH was buffered at a constant value by the bicarbonate ion present in the "moderately hard water", despite additions of varying amounts of metal. The artificial ligands (EDTA and NTA) were added from unacidified, ~1000-fold concentrated stock solutions of their sodium salts. The Nicomekl River water was filtered through a 0.45 µm filter and stored in the dark at 4 °C for 1-2 days prior to its use. Metal and ligand solutions were prepared and mixed 6-24 hours in advance in order to ensure equilibrium between ligand and metal.

Speciation determinations were carried out as follows. Between one and three DGT units were deployed for 1 to 24 hours in 250-400 mL solutions, contained in acid-washed HDPE containers. Solutions were shaken on an orbital shaker table to minimize diffusive boundary layer effects (see Zhang and Davison, 1995). The deployment time and solution temperature were recorded for each experiment. All processing of the DGT samplers was conducted within the confines of a Class 100 laminar flow hood. Metals were extracted from the DGT samplers using 1 mL of Environmental Grade 1 N HNO_3 in a PP (Polypropylene) centrifuge vial. Differential pulse anodic stripping voltammograms were measured using a static hanging mercury drop electrode (EG&G Model 303A) coupled to an EG&G Model 394 Electrochemical Trace Analyzer. Solutions were bubbled with H_2O-saturated ultrahigh-purity N_2 gas. "ASV-labile" concentrations were determined from the measured electrical currents observed in the ligand-containing metal solutions and in the standard curve solutions (in the absence of ligand). Details of the instrument conditions are presented in Table A-2.

Table A-2:
Typical deployment conditions for anodic stripping voltammograms

Variables	Cd	Cu	Zn
voltage range (V)	-0.8 to -0.4	-0.3 to +0.1	-1.2 to –0.8
Deposition time (s)	12	0	0
Equilibration time (s)	30	15	15
pulse height (mV)	20	10	7
purge time (s)	120	180	120
sensitivity (nA/ppb)	~0.5	~0.6	~0.16
conc range free metal (ppb)	0-2000	0-250	0-1200
drop size	medium	medium	medium

Determinations of dissolved metal concentrations in gel extracts and all ASV samples and standards were performed using a VG PQ2+ inductively coupled plasma mass spectrometer (ICP-MS). Metal concentrations were calculated using a linear calibration curve derived from certified mixed metal standards. Internal standards (In^{115} and Sc^{45}) were applied to all samples, standards and blanks to correct for sensitivity variations during analysis. SLRS-2 and TM-02 were used as reference materials. DGT (labile) metal concentrations were inferred from Fick's law, as described in Zhang and Davison (1995), assuming the diffusing species was the free metal ion.

Acute lethality tests (48-h) were carried out on *Daphnia magna* to determine the impact of metal-amended water containing a fixed amount of complexing agent. General

procedures for the *Daphnia magna* bioassay were based on methods outlined by Environment Canada (Environment Canada, 1990a, b; and May 1996 amendments).

DGT analyses and *Daphnia magna* biossay determinations (where applicable) were carried out on the same solution, while ASV and ISE determinations were carried out on an aliquot of this solution.

Speciation calculations were carried out using the chemical equilibrium program MINEQL+, version 4.5 (Schecher and McAvoy, 2001). This program was selected because it includes thermodynamic data for the ligands EDTA and NTA and for a large set of Cu-, Cd-, and Zn-containing minerals in its default database. Furthermore, the database has been recently updated (Schecher and McAvoy, 2001). It is important to note that all mineral precipitation was turned off for the MINEQL+ model runs. No precipitation of minerals was observed, nor was it apparent in any of the concentration data. However, at higher concentrations of modeled Cu and Cd (Chapters 2 and 3), precipitation of assorted Cu minerals and otavite ($CdCO_3$) was predicted by MINEQL and by PHREEQC (using the WATEQ4F database). Such precipitation was not observed, reflecting either slow kinetics of precipitation or inaccuracies in the database.