URBAN STORMWATER POLLUTION

RESEARCH REPORT 8:
HEAVY METAL SPECIATION STUDIES OF NATURAL WATERS: A REVIEW

Gregory M P Morrison
URBAN STORMWATER POLLUTION
RESEARCH REPORT 8:

HEAVY METAL SPECIATION STUDIES
OF NATURAL WATERS: A REVIEW

Gregory M P Morrison
# CONTENTS

<table>
<thead>
<tr>
<th>PREFACE</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FÖRORD</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>v</td>
</tr>
<tr>
<td>SAMMANFATTNING</td>
<td>vii</td>
</tr>
</tbody>
</table>

## CHAPTER 1  INTRODUCTION

1.1 Heavy Metals in Urban Surface Runoff

  1.1.1 Wet and Dry Atmospheric Deposition
  1.1.2 Urban Surfaces
  1.1.3 Sub-Surface Deposition

1.2 Variations of Heavy Metal Loadings during Storm Events.

1.3 The Speciation of Heavy Metals in Natural Waters.

1.4 The Toxicity of Heavy Metals to Aquatic Life.

1.5 The Need for a Study of Heavy Metal Speciation in Urban Runoff and other Polluted Waters.

## CHAPTER 2  INSTRUMENTATION FOR SPECIATION STUDIES

2.1 Ion-Selective Electrodes

2.2 Anodic Stripping Voltammetry

  2.2.1 Theoretical considerations
  2.2.2 Application of the Technique and its problems.
  2.2.3 Comparison of ASV measurements with a Model Cell.

2.3 Electrothermal Atomic Absorption

2.4 Other Analytical Techniques

  2.4.1 Neutron Activation Analysis
  2.4.2 X-ray Fluorescence

2.5 Assessment of the Instrumentation Applicable to Speciation Work.

## CHAPTER 3  PROCEDURES FOR THE PHYSICAL AND CHEMICAL SEPARATION OF HEAVY METALS IN THE DISSOLVED PHASE.

3.1 Ion Exchange Techniques

  3.1.1 Chelex-100 Chelating Resin
3.1.2 Thiol Chelating Resins

3.1.3 Chelating Cellulose Exchangers

3.2 Techniques for the Measurement of Organically Associated Metals.

3.2.1 Ultra-Violet Irradiation

3.2.2 Adsorption of Organics by Resins

3.2.3 Oxidation by Concentrated Acids

3.2.4 Decomposition of Organics by Ozonolysis

3.3 Differentiation of Metal Species by Size Separation.

3.3.1 Centrifugation

3.3.2 Dialysis

3.3.3 Filtration

3.3.4 Ultrafiltration

3.4 Other Separation and Analytical Techniques

3.4.1 Metal Ion Catalysis

3.4.2 Radiotracers

3.4.3 Gel Filtration Chromatography

3.5 The Suitability of Physico-Chemical and Physical Separations for Heavy Metal Speciation Analysis.

CHAPTER 4 PHYSICO-CHEMICAL SPECIATION SCHEMES FOR THE SEPARATION OF HEAVY METAL SPECIES IN THE DISSOLVED PHASE.

4.1 The Speciation Scheme of Batley and Florence (1976b).

4.2 The Ultrafiltration Scheme of Laxen and Harrison (1981b).

4.3 Other Speciation Schemes

4.4 Assessment of the Techniques and Results from Dissolved Heavy Metal Speciation Studies.

CHAPTER 5 THE SEPARATION OF HEAVY METAL FRACTIONS IN SUSPENDED SOLIDS AND SEDIMENTS.

5.1 Chemical Extractants used in sequential Extraction Schemes.

5.1.1 Exchangeable Fraction

5.1.2 Carbonate Fraction

5.1.3 Hydrous Iron and Manganese Oxide Fraction

5.1.4 Organic Fraction

5.2 Evaluation of Sequential Schemes
5.3 The Importance of determining Heavy Metal Associations in Suspended Solids and Sediments.

CHAPTER 6 ALTERNATIVE APPROACHES TO SPECIATION STUDIES. 47
6.1 Mathematical Models 47
6.2 Theoretical Studies of the Interaction of Heavy Metals with Particulate Fractions. 48
6.2.1 The Theory of Surface Exchange 50
6.2.2 The Role of Hydrous Manganese Oxides 52
6.2.3 Interactions of Heavy Metals with Organic Solids and Coatings. 52
6.3 Laboratory Simulation Studies of Real Systems 55
6.4 The Product Approach 58
6.5 The Relevance of Theoretical Studies of Heavy Metals in Natural Waters. 58

CHAPTER 7 APPARENT HEAVY METAL COMPLEXING CAPACITIES AND CONDITIONAL STABILITY CONSTANTS IN THE AQUEOUS ENVIRONMENT. 61
7.1 Polarography and Voltammetry 62
7.1.1 Determination of Apparent Complexing Capacity 62
7.1.2 Calculation of the Conditional Stability Constant. 64
7.2 Other Methods 66
7.3 Application and Interpretation of Heavy Metal Complexing Measurements. 68

CHAPTER 8 A SPECIATION SCHEME FOR THE ANALYSIS OF HEAVY METALS IN POLLUTED WATERS. 69
8.1 Description of the Speciation Scheme 69
8.2 Chemical Treatment of the Samples 71
8.3 Problems Encountered in the Determinations 73

BIBLIOGRAPHY 75
| FIGURE 1.1 | THE SIZE SPECTRUM AND ASSOCIATIONS OF METAL SPECIES IN NATURAL WATERS. | Page No. 5 |
| 1.2 | THE MOST RELEVANT REACTIONS OF A TOXIC METAL IN A WATER SAMPLES. | 6 |
| 2.1 | A COMPARISON OF THE CONDITIONS DURING THE SENSING OF METAL IONS AT TWO ELECTRODE SYSTEMS AND A MODEL CELL. | 16 |
| 4.1 | SPECIATION SCHEME OF BATLEY AND FLORENCE (1976b) | 31 |
| 4.2 | THE PHYSICAL SEPARATION SCHEME OF LAXEN AND HARRISON (1981a). | 33 |
| 5.1 | SEQUENTIAL EXTRACTION SCHEME APPLIED TO POLLUTED AND UNPOLLUTED RIVER SEDIMENTS. | 45 |
| 6.1 | UPTAKE EXPERIMENTS FOR HYDROUS IRON OXIDES. | 53 |
| 6.2 | UPTAKE EXPERIMENTS OF HYDROUS MANGANESE OXIDES. | 54 |
| 6.3 | PROCEDURE FOR MIXING EXPERIMENTS. | 59 |
| 7.1 | TITRATION OF A WATER SAMPLE WITH COPPER. | 63 |
| 8.1 | A SPECIATION SCHEME FOR THE ANALYSIS OF HEAVY METALS IN POLLUTED WATERS. | 70 |
### TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>ANALYSIS OF SEQUENTIAL SCHEME EXTRACTANTS FOR THE DECOMPOSITION OR REMOVAL OR ORGANIC MATTER IN A ROAD SEDIMENT.</td>
<td>41</td>
</tr>
<tr>
<td>5.2</td>
<td>SEQUENTIAL EXTRACTION SCHEME OF FORSTNER AND PATCHINEELAM (1980).</td>
<td>42</td>
</tr>
<tr>
<td>5.3</td>
<td>SEQUENTIAL EXTRACTION SCHEME FOR SEDIMENTS AND SUSPENDED SOLIDS (TESSIER ET AL, 1979).</td>
<td>44</td>
</tr>
<tr>
<td>5.4</td>
<td>SEQUENTIAL SCHEME OF EISENREICH ET AL (1980).</td>
<td>46</td>
</tr>
<tr>
<td>6.1</td>
<td>CONTROL MECHANISMS FOR HEAVY METALS IN AQUATIC SAMPLES.</td>
<td>49</td>
</tr>
<tr>
<td>6.2</td>
<td>HYDROLYSIS CONSTANTS FOR HEAVY METAL IONS.</td>
<td>51</td>
</tr>
<tr>
<td>6.3</td>
<td>CONDITIONAL STABILITY CONSTANTS FOR METAL COMPLEXATION OF AMORPHOUS Fe(OH)₃ AND ORGANIC MATERIAL AT pH 8.0.</td>
<td>56</td>
</tr>
<tr>
<td>6.4</td>
<td>SORPTION CHARACTERISTICS OF HUMIC ACID, MnO₂ AND BENTONITE.</td>
<td>57</td>
</tr>
</tbody>
</table>
1. The Urban Pollution Research Centre was established in 1976 with the purpose of investigating problems of urban stormwater pollution within catchments in North West London. This aim has been extended to the investigation of water and atmospheric quality problems and management within urban areas generally and to educational objectives of postgraduate student training.

2. Existing members of the Research Centre are:

**Academic Staff**
- Mr J B Ellis
- Dr R Hamilton
- Mr D Jarrett
- Dr D M Revitt
- Mr B S Shutes

**Research Assistants/Students**
- Mr I Davies
- Mrs A Gavens (Part-time)
- Mrs M A House
- Ms H Roberts
- Mr G Morrison
- Mr P Beckwith
- Mr R Warren
- Mr O Harrop

**Technical Staff**
- Chief Technician: Mr G S Morris
- Research Technicians: Mr A J LaGrue, Mr G Patel, Mr G Adie

3. Current research projects being undertaken by the Research Centre, with source of support and operating dates, include:

- **Anglers' response to changes in water quality.** I Davies, D J Parker, Science Research Council, 1979-82.


Magnetic methods for tracing and monitoring pollution sources in urban catchments. J B Ellis, D M Revitt, Professor F Oldfield (University of Liverpool), P Beckwith. 1980-84.


4. Research Reports in the current series include:


No.8 Heavy Metal Speciation Studies of Natural Waters: A Review. G Morrison. September 1983.
FÖRORD

Samarbete mellan Middlesex Polytechnic, Centre for Urban Stormwater Pollution Research i London och institutionen för Vatten- försörjnings- och Avloppsteknik, Chalmers tekniska högskola har lett fram till ett gemensamt forskningsprojekt med titeln: "Heavy Metal Speciation in Urban Runoff".


Medel till projektet har erhållits dels genom ett doktorandstipendium till Middlesex Polytechnic dels genom ett forskningsanslag från Forskningsnämnden, Statens Naturvårdsverk.

Peter Balmér                         Gilbert Svensson
professor                             projektledare
ACKNOWLEDGEMENTS

Dr D M Revitt and Mr J B Ellis, both in the Urban Pollution Research Centre, have given helpful criticism of the draft copies.

Professor P Balmér and Mr G Svensson, both in the Department of Sanitary Engineering, Chalmers Tekniska Högskola provided helpful discussion.

Mrs V Norman typed the final report.

The U.K. Natural Environment Research Council and the Swedish National Environment Research Council are providing financial assistance for the project.
SUMMARY

The aim of this report is to discuss physico-chemical speciation techniques which allow the separation of bioavailable i.e. toxic or geochemically mobile heavy metal fractions in aqueous systems. It is well known that there are high total metal concentrations and loadings in urban runoff but the nature and amount of the toxic fractions have not been determined. The identification of the potentially toxic fractions is aided by a preliminary discussion of the complex interactions between the dissolved, colloidal and suspended solid phases and the chemical form of the metal.

An instrumental technique which readily lends itself to routine speciation studies is Anodic Stripping Voltammetry (ASV). Direct ASV measurement is compared to Ion Selective Electrodes and Atomic Absorption with pre-concentration as alternative direct measurement techniques for potential toxicity assessment.

Ion exchange chelating resins, such as Chelex-100 and a thiol chelating resin, allow a useful preliminary separation of metal species in the dissolved phase. Organically associated metal can be separated by either adsorption onto resins, oxidation by concentrated acids, u.v. irradiation or ozonation. Physical separation, on the basis of size or molecular weight, can be accomplished by centrifugation, dialysis, filtration or ultra filtration.

Two major schemes have been proposed for the speciation of the dissolved phase. One scheme separates metals using ASV lability, Chelex-100 separation and u.v. irradiation and has been applied successfully to the analysis of seawater. An alternative scheme based on size separation has been used with limited success in wastewater analysis. The results from these and other schemes indicate the importance of dissolved
and colloidal organic associations, especially for copper and lead, while zinc and cadmium are normally found as free metal or weakly complexed forms.

Although the dissolved phase is the most bioavailable, suspended solid associated metal may be weakly bound and therefore relatively easily remobilised. Sediments can be fractionated by sequential chemical extraction schemes and although the extractants are not strictly selective, exchangeable, carbonate, hydrous metal oxide, organic and residual fractions can be differentiated.

Alternative approaches to speciation studies have included mathematical models, theoretical and laboratory studies of surface exchange theory, laboratory simulation studies and mixing experiments. These studies have provided some insight into the mechanisms for heavy metal transport between the different phases.

An understanding of the importance of metal-organic associations can be obtained by determining the complexing capacity and stability constants of organic ligands in natural waters. Most methods have relied on a complexometric titration of free metal ion, typically copper, with the sample. The uncomplexed metal is analysed by ASV and the complexation capacity and conditional stability constant are estimated from the resulting titration curve.

On the basis of this previous work, a speciation scheme for the routine analysis of urban runoff and similar polluted waters is proposed. The dissolved phase is separated into three fractions, viz. Electrochemically available, Chelex Removeable and Colloidal. The concentration of metal in each of the fractions is dependent on the complexation. The suspended solid phase is also separated into three fractions, viz. Exchangeable, Carbonate and hydrous metal oxide and Organic. The metal level in these fractions control the ease of metal release to the soluble phase. The advantages and problems of this scheme are discussed.
SAMMANFATTNING

Avsikten med denna rapport är att diskutera olika fysikaliskt-kemiska specieringstekniker (eng: speciation techniques) av för biologiskt liv tillgängliga och geokemiskt rörliga tunga metaller i vattensystem. Det är väl känt att metallkonzentrationen och belastningen i dagvattenavrinning är höga men egenskaperna och mängden av toxiska fraktioner har inte bestämts. Identifieringen av de potentiellt toxiska fraktionerna förstås bättre om komplexbindningar mellan lösta, kolloidala och suspenderade ämnen samt metallens kemiska egenskaper är kända.

En instrumentell teknik som lämpar sig för specieringsstudier är s k anodisk stripping (ASV). Direkt mätning med ASV kan jämföras med jonselektiva elektroder och atomabsorption på uppslutna prov som en alternativ teknik för direkt mätning av potentiell giftighet.

Jonbytarmassor som Chelex-100 och av thioltyp ger möjlighet för en preliminär separering av metallspecier i löst fas. Organiskt bundna metaller kan separeras antingen genom adsorption, oxidation med koncentrerade syror, UV-strålning eller med ozon. Fysikalisk separering, baserad på storlek eller molekylvikt kan utföras genom centrifugering, dialys, filtrering eller ultrafiltrering.

Två huvudmetoder har föreslagits för speciering av den lösta fasen. En metod som separerar metaller med hjälp av ASV, Chelex-100 och UV-strålning och har använts med framgång på havsvatten. En alternativ metod baserad på separering av olika storlekar har använts med begränsad framgång på avloppsvatten. Resultaten från dessa och andra metoder tyder på att koppar och zink är associerade med lösta och kolloidala organiska föreningar medan zink och kadmium vanligtvis förekommer som fria metaller eller är svagt komplexbundna.
Fastän den lösta fasen är den mest tillgängliga för biologiskt liv, kan metaller bundna till suspenderade ämnen vara svagt bundna och därför relativt lätt bli lösgjorda. Sediment kan fraktioneras genom sekventiell kemisk extraktion och trots att extraktionsmedlen inte är fullständigt selektiva kan jonutbytbar metall, karbonater, metallhydroxider, organiskt bunden metall och restfraktioner separeras.

Alternativ till specieringsstudier har omfattat matematiska modeller, teoretiska och laboratoriestudier av ytfenomen, laboratoriesimuleringar och blandningsexperiment. Dessa studier har givit en del kunskap om mekanismer för tungmetalltransport mellan de olika faserna.

En förståelse för betydelsen av metallorganiska bindningar kan uppnås genom att bestämma komplexbindningskapaciteter och stabilitetskonstanter mellan organiska ligander och metaller i naturliga vatten. Flertalet metoder har baserats på komplexometrisk titrering av fria metalljoner, typexempel koppar, tillsammans med provet. Den icke komplexbundna metallen analyseras med ASV och komplexbindningskapaciteten och den aktuella stabilitetskonstanten beräknas från den resulterande tittrengskurvan.

CHAPTER 1

INTRODUCTION

The evaluation of metal toxicity in aquatic systems is of prime concern particularly where receiving water standards or quality objectives might be endangered from chronic accumulative emissions. It is now generally accepted that total levels of heavy metals do not provide a sufficient perspective of their likely toxic effects to organisms and that a better knowledge is required of the different metal forms associated with specific discharges. The objective of this report is to provide a review and assessment of approaches that have been adopted to the determination of the physico-chemical speciation of heavy metals. Speciation schemes are intended to differentiate the heavy metals into fractions dependent upon their physico-chemical properties and a satisfactory speciation scheme should allow identification and evaluation of both toxic and geochemically mobile heavy metals. Most schemes that have been developed have been applied to relatively clean, unpolluted waters and are often too elaborate for routine analysis. A more rapid, but still meaningful, scheme will be presented at the end of the review following an assessment of a number of differing approaches to speciation analysis.

1.1 Heavy Metals in Urban Surface Runoff

The impact of polluted waters from non point sources, including urban surface runoff, on receiving waters is considered by many workers to be a serious cause for concern. Considerable variation and inconsistency in the reported concentrations and loadings of heavy metals in storm runoff discharges present considerable difficulty in evaluating stream impacts as well as in pipe heavy metal kinetics.

Urban surface runoff may include any pollutant or natural material
that is available for transport within the catchment area. Increased urban surface impermeability results in both increased runoff and heavy metal loadings. The degree of industrialisation, vegetation and animal populations within the urban catchment will also affect pollutant contributions.

In spite of the potential variety of contributing sources within the urban catchment three main inputs can be identified.

1.1.1 Wet and Dry Atmospheric Deposition

Significant quantities of heavy metals have been reported in urban rainwater (Malmqvist and Svensson 1977, Cawse 1974) and certainly urban traffic contributes large quantities of lead and other heavy metals to the immediate environment (Harrison et al., 1981). It has been shown that even in relatively unpolluted areas, rainfall and dry dustfall may well be a major contributing source of heavy metals in stormwater runoff (Randall et al., 1978).

1.1.2 Urban surfaces

This non-point source makes an important contribution of heavy metals to urban runoff. Automobiles have been conclusively identified as one of the major contributors (Newton et al. 1974, Bryan 1974). Christensen and Guinn (1979) reported the average deposition rates of metals on road surfaces to be 0.003 g Zn/vehicle km. and 0.0049 g Pb/vehicle km. An accumulation on the road surface between storms is to be expected with the rate of metal build-up being related to traffic density and type, duration of the antecedent dry period as well as the season of the year. In Scandinavia copper and zinc are commonly used in roofing and guttering and their corrosion may generate large amounts of these metals (Malmqvist and Svensson, 1977).

1.1.3 Sub-Surface Deposition

This category includes in pipe deposition and gully pot accumulations. According to Mance (1981) in pipe sediments generally accumulate at a mean rate of 17.15 g sediment m$^{-1}$ day$^{-1}$ and
metals may accumulate at a proportional rate. Harrop et al (1983) have reported greatly enhanced levels of heavy metals in gullypot sediments, particularly in the finer fractions. There is evidence to suggest that elevated levels of metals occur in gully pot sediments over and above those which are characteristic of the road surface, this is particularly noticeable in the finer size fractions (Harrop et al 1983).

1.2 Variations of Heavy Metal Loadings during Storm Events

It is not fully understood how urban heavy metal source loadings are reflected in the stormwater discharges at the outfall of the catchment pipe system. Certainly a first flush of highly turbid water, with a duration of some twenty to thirty minutes, has been observed at the beginning of storm events (Wilkinson 1956). Ellis (1982) has discussed the importance of the time of concentration for the catchment which he considers explains the lack of first flush noted in some storm events. From these observations heavy metals might be expected to be concentrated in the first part of the storm event (Mance 1981). This assumption is also based on the strong affinity of metals for stormwater suspended solids (Bourcier and Sharma 1980, Urbonas and Tucker 1980, Wilber and Hunter 1980) which often parallel the storm hydrograph. However, this assumption has largely been refuted by direct measurements throughout individual storms (Revitt et al 1981). This apparent discrepancy in views would suggest that metal inputs are being delivered from a variety of sources and are being mobilised at different rates and at different times during the storm event and are thus loaded on to the hydrograph at different times.

Many of the heavy metal studies which have been carried out do
not differentiate between particulate and dissolved metals or measure only the particulate associated metal. When relating metal levels to toxic effects it is important to recognise that there is often a considerable loading of dissolved metal in storm discharges. In addition, metals may be mobilised from the particulate phase to the dissolved phase and cadmium, in particular, has been shown to be readily solubilised from road sediments (Revitt and Ellis 1980). Thus a knowledge of heavy metal kinetics and speciation is important if advances are to be made in evaluating the relative toxicity of urban non-point storm discharges.

1.3 The Speciation of Heavy Metals in Natural Waters

Heavy metals in aqueous systems may occur as organic and inorganic complexes of varying sizes or be associated with particulate materials of a heterogeneous nature. Stumm and Brauner (1975) have outlined the various forms in which metals are thought to exist in seawater (Fig. 1.1). An important problem, which relates to most natural aquatic systems, is the difficulty of distinguishing between dissolved and colloidal species using physical methods.

Theoretical considerations of metal speciation have been further developed by Buffle (1981). Dissolved ligands, living cells and suspended solids compete for metals under certain thermodynamic and kinetic constraints. The complexity of this system is partly illustrated in Fig. 1.2. Metal ligand complexes have a metastable relationship with the free metal ion, living cells and suspended sediment particles and, although the different constants are not known with any certainty, progress is being made in their measurement. This approach indicates that the free metal ion only competes for the living cell, whilst both free metal ion and metal complex can attach to the suspended solid. The ligands that interact with the metal ion can be either organic or inorganic.

These theoretical considerations are a useful basis for the
<table>
<thead>
<tr>
<th>Phase</th>
<th>Species Present</th>
<th>Diameter range (and MWT.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved</td>
<td>Inorganically complexed metals. Metals complexed with small organic molecules. Free metal ions.</td>
<td>0-0.8 nm. (0-150)</td>
</tr>
<tr>
<td>Colloidal</td>
<td>Metals associated with Organic/Iron oxide colloids Metals complexed with organic fibrils.</td>
<td>0.8-400 nm. (150-10^7)</td>
</tr>
<tr>
<td>Suspended Solids and Particulates</td>
<td>Carbonate bound metals. Metals adsorbed onto or bound to iron and manganese hydrous oxides. Organically complexed metal species. Metals adsorbed onto, or contained within the lattice of, clays, sands and silts.</td>
<td>&gt;400 nm. (&gt;10^7)</td>
</tr>
</tbody>
</table>

Figure 1.1: The Size Spectrum and Associations of Metal Species in Natural Waters. (From Stumm and Brauner 1975, Steinnes 1983).
FIGURE 1.2: The most relevant reactions of a Toxic metal in a Water Sample (After Buffle 1981). Diffusion Rates are not included.

\[
\begin{align*}
M & = \text{Metal} \\
L & = \text{Ligand} \\
K & = \text{Dissociation Constant} \\
K_{\text{ML}} & = \text{Adsorption of ML on solid} \\
K_{\text{L}} & = \text{Adsorption of L on solid} \\
K_{\text{M}} & = \text{Adsorption of M on solid} \\
K_{\text{M}} & = \text{Adsorption of M on cell Membrane} \\
K_{\text{ML}}' & = \text{Stability constant of M and L in solution.}
\end{align*}
\]
determination of toxic or bioavailable metals. An exact separation of all the chemical species would be beyond the reach of present speciation techniques, but a determination of toxic metal could be of considerable value for the screening of wastewaters and storm effluents and in developing in-stream standards for water quality objectives.

1.4 The Toxicity of Heavy Metals to Aquatic Life

In order to determine the toxic or bioavailable fractions of heavy metals a basic understanding of their effects on aquatic life is required. The actual toxicity to a particular organism will depend on many factors such as age, surface area, population structure and competing metal ion concentrations. However, certain heavy metal forms are potentially more toxic and are likely to be hazardous to the biota of a receiving water.

Initial heavy metal toxicant studies have been concerned with the relationship between total metal concentrations and their toxicity to organisms. However, recent research points to the aquo ion as being the toxic form of the metal. This is experimentally determined through the observation of the interactions between the chemical form of a heavy metal and a suitable organism.

The toxicity of copper to the freshwater alga, *Scenedesmus quadricauda*, was demonstrated by Petersen (1982). Using EDTA as the complexing ligand, free copper ion was found to be toxic in the range $10^{-10}$ to $10^{-12}$M. Zevenhuisen et al (1979) found that the bacteria, *Klebsiella aerogenes*, was inhibited by $10^{-6}$ to $10^{-10}$M free copper ion. Extracts of the bacteria were found to be highly complexing towards copper, suggesting that biota might modify copper toxicity through their excretion products, exudates, munchates and soluble organic excretions. This extracellular pool, together with soil derived fulvic acids, are possible regulators of copper toxicity to the biota (Saar and Weber 1982). Similar results have been obtained by
Sunda and Gillespie (1979) in a study of bacterial growth in the marine environment.

Weakly complexed copper may also prove to be toxic. Theis and Dodge (1979) reported that Cu$^{2+}$ and CuOH$^+$ were taken up by the midge larva, *Chironomus tentans*, while no uptake was noted for Cu-NTA or Cu-glycine. Magnusson et al (1979) came to the same conclusions for *Daphnia magna* and also found that carbonate complexes were not toxic. On the other hand, Guy and Kean (1980) reported that copper bound to ligands with low stability constants, such as citric acid and ethylenediamine, were toxic to the alga, *Selenastrum capricornutum*, whereas copper bound to ligands with relatively high stability constants such as EDTA were non toxic.

The relationship between free metal ion and toxicity is particularly relevant to soluble elements such as zinc (Spear 1981) and cadmium (Ellis and Revitt 1982). Using EDTA as the complexing ligand, Allen et al (1980) found that Zn$^{2+}$ and ZnOH$^+$ were the toxic species to *Microcystis aeruginosa*. The organic ligands of high molecular weight in pond water were found to reduce cadmium toxicity for *Simocephalus serrulatus* but not for *Gambusia affinis* (Giesy et al 1977).

Mortality rates for the amphipod *Hyalalela azteca* correlate strongly with free lead ion concentrations (Freedman et al 1980). However, both the carbonate and free ionic forms of lead have been reported as toxic to the rainbow trout (Davies et al 1976).

It is thus generally accepted that the free metal ion is most toxic to aquatic life although the exact mechanism of toxicity is not fully understood. Gatcher et al (1978) assumes that for algae, metal toxicity is the result of the formation of metal complexes at important physiologically active sites. These complexes (MA) are related to metal aquo species (M$^{2+}$aq.) in the medium such that:

$$[MA] = \frac{K[Maq^{2+}][Atot]}{1 + K [Maq^{2+}]}. $$
In other words, the algal surface may be treated as a ligand having a certain complex formation capacity ($A_{tot}$) and a certain complex formation constant ($K$). The ligand only reacts with free metal ion or more weakly bound complexed metal. By this mechanism the amount of toxic metal will directly relate to the number of organisms and their surface area. In polluted water bacteria, despite their small biomass, will contribute the greatest surface area relative to other organisms. A comparison of bacterial numbers and heavy metal speciation might well further ideas on toxic metal uptake.

A mechanism of heavy metal toxicity has recently been proposed (Brown et al 1983) based on the ability of certain heavy metals to cause the cleavage of nucleic acids. Lead and copper are particularly toxic because their respective acid dissociation constants as bound water molecules are similar to the physiological pH of approximately 7.4.

1.5 The need for a Study of Heavy Metal Speciation in Urban Runoff and other Polluted Waters.

Whilst the contributing sources of heavy metals within the urban catchment are still somewhat speculative, it is now widely accepted that the levels of these priority pollutants in storm runoff can be substantial. However, very little is known of the form in which these metals are transported or of their potential toxicity to receiving stream habitats.

Progress in the study of speciation in natural water systems is now sufficient to enable a differentiation of toxic heavy metal species in urban stormwater. The dissolved phase is the most acutely toxic, while the suspended solid phase may represent a transport phase and a chronic exposure source for organisms following deposition and accumulation in the receiving water.

In order to interpret toxicity, speciation methods need to isolate the most bioavailable metal, which in the dissolved
phase is the free or weakly complexed metal and in the suspended solid phase is the readily exchangeable metal. Large fluxes of these toxic metals from the stormwater discharges may well be hazardous to both fauna and flora of a receiving stream. Studies must therefore identify and monitor toxic and non-toxic heavy metal species entering receiving waters from polluted sources. The results of Gatcher and Urech (1983) have shown that 10 µg/l Cu and 50 µg/l Zn can cause drastic reductions of phytoplankton and zooplankton in environmental systems. Since many urban storm discharges greatly exceed these dissolved heavy metal concentrations the toxicity may be well above 'safety limits' and certainly above acceptable emission standards.
CHAPTER 2

INSTRUMENTATION FOR SPECIATION STUDIES

The development of sensitive analytical techniques suitable for the measurement of heavy metals at trace levels (10^{-6} to 10^{-10} M) has been of obvious importance in the determination of heavy metal species. Some techniques are particularly suitable for speciation studies. A differentiation of chemical species, such as free metal ions, from total metal can be achieved by Ion Selective Electrodes or Anodic Stripping Voltammetry. A preliminary separation technique, followed by instrumental analysis is also satisfactory and Electrothermal Atomic Absorption can be used as a sensitive detector.

2.1 Ion Selective Electrodes

The species-specific nature of metal ion selective electrodes which respond only to the activity of the free (hydrated) metal ion, has attracted widespread interest (Florence and Batley, 1980). Early work using ion selective electrode potentiometry reported that, despite a non-Nernstian response, concentrations as low as 10^{-9} M could be precisely measured. However, zinc, cadmium and lead ion-selective electrodes are not suitable for natural water analysis (Florence and Batley, 1977).

Some early studies have applied the technique to copper in seawater (Blaedel and Dinwiddie, 1974; Jasinski et al, 1974). There is some uncertainty about the nature of the non-Nernstian response at low concentrations, between 10^{-6} and 10^{-9} M, because equilibrium is not established between the electrode system and the trace metal. Wagemann (1980) reported that the copper electrode responded to the hydroxo- and carbonato-complexes of copper in addition to the free ion.

The technique has been found to be unreliable for coastal water samples due probably to interference by chelating agents (Jasinski et al 1974) and the same problems might be expected in polluted waters with a high organic content.
2.2 Anodic Stripping Voltammetry

Anodic Stripping Voltammetry (ASV) in the differential pulse mode is sufficiently sensitive, with a typical detection limit of about \(10^{-9}\) M, for the direct determination of heavy metals in natural waters (Florence 1982). This analytical technique has an intrinsic capability for speciation work due to the distinction of the electrochemically available fraction, which may be toxic, and the bound or electrochemically inert fraction which is less likely to demonstrate toxic properties.

2.2.1 Theoretical Considerations

ASV initially involves a bulk electrolysis step (pre-electrolysis) to concentrate the desired metals into the small volume of a mercury electrode. The mercury electrode may be either a hanging drop or thin film type. Following this electrodeposition step, the material is redissolved or stripped from the electrode using differential pulse or linear sweep voltammetry and the current recorded. If the conditions during pre-electrolysis are constant then exhaustive electrolysis of the solution is not necessary and, by proper calibration utilising fixed electrolysis times, the measured voltammetric response can be employed to find the solution concentration.

The redox procedure allows the differentiation of metal species at low levels and during the plating step the potential can be set so that certain species are not reduced. In the stripping process the peak potential will depend on the metal species present and hence the following equations apply:

\[
\text{Deposition (plating) step}
\]

\[
\begin{align*}
\text{ML} & \xrightleftharpoons[k_f]{k_b} M^{2+} + L^{2-} \\
M^{2+} + \text{Hg} + 2e^- & \longrightarrow M(\text{Hg})
\end{align*}
\]

(1) ML = Species of interest.

(2) \(M^{2+}\) = Aquo metal ion

\(L^{2-}\) = Free ligand
The contribution of a metal complex species (ML) to the ASV peak height or current relative to that of the free metal ion, depends on the rate constant for the dissociation of the complex (kf in equation (1)) and the thickness of the diffusion layer. Diffusion layer thickness depends on the stirring rate and therefore the thicker the diffusion layer, the higher the contribution of a metal complex to the ASV wave height (Florence 1982a). No research work has been reported concerning the determination of diffusion layer thickness (δ) which requires a well defined rotating disc electrode to enable δ to be calculated as a function of rotation speed (Whitfield and Turner 1979).

2.2.2 Application of the Technique and its problems.

ASV has commonly been applied to the primary distinction between "labile" and "bound" metals in filtered water samples (Chau and Lum-Shue-Chan 1974, Duinker and Kramer 1977). The normal procedure for estimating the fraction of labile or electrochemically available metal involves a standard addition analysis of an untreated sample and is therefore dependent on the kinetics of the reactions controlling the assimilation of the metal spike (Whitfield and Turner 1979). Labile metal, as defined by the experimental conditions, therefore includes ionic as well as some weakly complexed metal. Bound metal is identifiable as the non-labile fraction and is typically associated with a variety of organic and inorganic colloidal materials (Batley and Florence 1976a).

Obviously there is a need to replace the "lability" concept with equilibrium and rate constant values (Astruc et al 1981) Whitfield and Turner (1979) have suggested that this is possible with a rotating disc electrode, although most measurements have been made with hanging mercury drop electrodes.
There are a number of problems pertaining to the ASV analytical procedure which must be considered:

i) Surface active compounds may adsorb on the mercury electrode e.g. humic and fulvic acids, long chain amines and alcohols which cause interference with the Cd and Pb peaks (Florence 1982a). This is particularly noticeable when operating in the differential pulse mode.

ii) Tensammetric waves arise as the result of adsorption - desorption processes at the mercury electrode and have been mistaken for Cd, Pb or Cu peaks in seawater analysis. Natural organic compounds in seawater give rise to these tensammetric waves which, at pH 5.0, occur near the Pb and Cd peaks. However, they do not appear if a simple linear voltage scan is used (Florence 1982a).

iii) In practice, the removal of oxygen by bubbling nitrogen through the sample is necessary to prevent interference on the polarogram. In poorly buffered waters this may cause a large pH increase, due to carbon dioxide evolution, and a resulting change in metal speciation. This problem can be overcome by the addition of a suitable buffer or by flushing with a suitable N₂/CO₂ gas mixture. Sodium acetate is probably the most commonly employed buffer but cannot be considered to be completely non-complexing.

iv) As ASV is an electrochemical procedure a base electrolyte must be employed if the sample does not already have an ionic strength of at least 0.02M. Many natural waters do not meet this requirement and, therefore, the addition of KN0₃ or KCl may be considered.

v) Cu and Ni form intermetallic compounds with zinc resulting in an underestimation of the zinc concentration. To overcome this problem zinc is determined separately.

2.2.3 Comparison of ASV Measurements with a Model Cell

Whitfield and Turner (1979) have reported two concepts that have been used to provide chemical analogues of the biological
availability of trace metals.

i) Thermodynamic availability; this relates to the activity of the particular chemical form that is taken up preferentially by an organism.

ii) Electrochemical availability; this is the fraction of the total metal concentration that is available for electrodeposition at an electrode immersed in the solution during the timescale of the plating process.

Figure 2.1 illustrates (a) the reactions occurring at an Ion Selective Electrode, (b) at the ASV hanging mercury drop or mercury film and (c) at the surface of a model biological cell.

The membrane of the Ion Selective Electrode simply senses the free metal ion without accumulation and hence the metal flux (J) is zero. The accumulation of metal ion at the model cell is not comparable to this system. On the other hand, ASV shows a metal flux (J_k) which depends on the conditions employed in analysis. If the diffusion layer thickness (δ) is known then a useful comparison with the metal flux at the model cell can be made. However, it is important to realise that the transport of metal into the cell is dependent on membrane conditions whilst in ASV the limiting condition is the metal flux in the diffusion layer. Consequently uptake by the model cell is rather low.

Although some features of the model cell cannot be reproduced electrochemically, e.g. surface adsorption sites, the work of Whitfield and Turner (1979) suggests that electrochemically available metal may relate to bioavailable metal.

2.3 Electrothermal Atomic Absorption

Although the technique is non-species selective, Electrothermal Atomic Absorption is an automated sensitive technique suitable for measuring total metal concentrations (Astruc et al, 1981). It can be used, in conjunction with a preliminary separation process, to provide information on metal speciation. Gas Chromatography for volatile organo-metallics (Fernandez 1977, Van Loon 1979) and separation on the basis of size or charge (Guy and Chakrobarti 1976) are typical first steps.
FIGURE 2.1: A Comparison of the Conditions During the Sensing of Metal Ions at two Electrode Systems and a Model Cell. (after Whitfield and Turner, 1979)
Inductively Coupled Plasma techniques are now being increasingly used, with the high temperature plasma as a spectral emission source. With comparable detection limits to Electrothermal Atomic Absorption the main advantage is application in simultaneous multi element analysis (Pinta 1978).

2.4 Other Analytical Techniques

Neutron activation analysis and X-ray fluorescence can be used to detect heavy metals, although their applications to speciation are limited.

2.4.1 Neutron Activation Analysis

The radioactive properties of a nuclide can be utilised to detect an element using neutron activation analysis. After bombarding a sample with neutrons in a reactor the induced activity is compared with standards to provide both qualitative and quantitative results. The technique is very sensitive providing all the samples and standards are irradiated in the same particle flux. It is usually necessary with water samples to carry out a preliminary separation step to remove sodium, magnesium and phosphorus, which interfere and decrease the precision of the technique. Total metal concentrations are measured by neutron activation analysis, although de Jong and Wiles (1976) have used the method to determine methylmercury in fish.

2.4.2 X-ray Fluorescence

Total heavy metals can also be determined by X-ray fluorescence. This is a highly sensitive technique which has recently been incorporated into a speciation scheme for heavy metals in river water (Pik et al 1982). Particulate material is analysed directly by thin film X-ray fluorescence, while determination of the dissolved phase requires co-precipitation of the metals before analysis. The low detection limits of this method (Cd = 0.1 µg l⁻¹, Pb = 1.6 µg l⁻¹) suggest a more widespread use of X-ray fluorescence in future speciation schemes.
2.5 **Assessment of the Instrumentation Applicable to Speciation Work**

A direct measurement of bioavailable heavy metals is highly desirable for the rapid screening of wastewaters. Whitfield and Turner (1979) demonstrated that direct ASV analysis is more representative of the bioavailable fraction than Ion Selective Electrodes, although the comparison of an electrochemical cell with a model biological cell can only be a theoretical one. Atomic Absorption, Neutron Activation Analysis and X-ray Fluorescence measure only the total heavy metal and therefore the sample requires suitable preliminary separation techniques before metal analysis.
The preliminary separation of heavy metals according to physico-chemical characteristics has been carried out in a variety of ways. Ion exchange resins which are selective for heavy metals may be used to remove the toxic metal species, whilst organically bound metals can be released by several different oxidation procedures. Separation on the basis of size allows the differentiation of dissolved, colloidal and particulate associated metal species. The different approaches are complementary to each other providing a wide range of information on metal speciation.

3.1 Ion Exchange Techniques

The introduction of heavy metal selective ion exchange resins provided an important step forward in speciation studies. It is thought that these resins may provide some indication of bioavailable metal and if such a resin can be developed then it could find a valuable use in the removal of toxic metal species from wastewaters.

Chelex-100 is known to take up the free metal ion and weakly complexed metals. Thiol resins may more closely resemble the natural uptake of metal ions; certainly more copper is removed by this resin (Florence 1982b). Cellulose chelating exchangers provide an almost quantitative removal of metals from the aqueous environment, although species-specific chelating groups might usefully be attached to the cellulose.

3.1.1 Chelex-100 Chelating Resin

This resin contains an iminodiacetate chelating group which is
highly selective for heavy metals and has been successfully applied to seawater analysis (Riley and Taylor 1968, Florence and Batley 1976). The pore diameter is such that colloidal associated metals are excluded.

 Metals are strongly complexed by the oxygen and nitrogen atoms of the iminoacetate groups, but it is mainly only the ionic form of the metal which is taken up due to the small pore diameter of the resin \((1.5 \text{ to } 3 \text{ nm})\). The stability of iminoacetate groups \(- \log K \approx 13\) for copper - is such that weakly complexed metals may dissociate and preferentially associate with the Chelex - 100. The ionic form retained by the resin represents the more readily bio-available metal, whilst the organically coated colloidal particles which are excluded are considered to be important for metal solubilisation and transport.

 An alternative theory on the mechanism of uptake of metals by Chelex - 100 has been put forward by Figura and McDuffie (1979). The slow dissociation of metal complexes in solution, rather than molecular exclusion, is stated to be the cause of the incomplete retention by Chelex - 100 of some trace metals in natural waters. Hence the non-uptake of highly stable metal - NTA complexes by Chelex - 100 complexes may be due to slow resin kinetics.

 Two experimental approaches to the use of Chelex resin have been tested:

 (1) Resin bed in a column; metal ions are taken up as the water sample passes down the column at a known flow rate, typically 1-2 mls/min (Batley and Florence 1976, Montgomery and Santiago 1978). Blocking of the columns due to the swelling characteristics of Chelex - 100 is a common problem with this technique.

 (2) Batch technique; longer contact times, up to 16-24 hours, are employed enabling full equilibration of resin and
sample metals to be reached (Hart and Davies 1977). The method has proved useful in identifying colloidal associated metals on the basis that these are not kinetically labile during the batch experiment (Figura and McDuffie 1980). A criticism of the method is that displacement of chemical equilibria may occur so that chelates that are sufficiently labile may be displaced (Astruc et al. 1981).

In order to maintain stable pH conditions in the sample and to carry out the experiment over a wide range of pH values the calcium form of Chelex - 100 is now preferred (Figura and McDuffie 1977) compared to the sodium (Hart and Davies 1977) and hydrogen (Florence and Batley 1975) forms.

Despite problems of interpreting the results in terms of toxic metal, the use of Chelex - 100 is a flexible technique which allows the concentration of a variety of metal ions over a wide pH range to be determined. A simple separation by either the Column or Batch technique is rapid, inexpensive and with care presents little opportunity for contamination.

3.1.2 Thiol Chelating Resins
Recently Thiol based materials have been used successfully to remove Zn, Cd, Pb and Cu from natural waters (Florence 1982b). The presence of chelating sulfhydryl groups has been shown to correlate with heavy metal toxicity (Fisher and Price 1981) and on this basis the metal fraction removed by the thiol group is considered to be a realistic estimate of bioavailable metal (Florence 1982b). The transportation of metals across a cell membrane is believed to be dependent on the lipid solubility of metal species. Similarities between metallothioneins and membrane carrier proteins (Cherian and Goyer 1978, Koyima and Kagi 1978, Lerch 1980, Roesijaldi 1980/81) suggests that metals may be complexed and transported in association with sulfhydryl groups. This process may be simulated using a Thiol resin and it has been reported that thiol materials generally have a lower affinity for Pb, Cd and Zn, but a higher affinity for Cu, than Chelex - 100. The order of selectivity
at pH 5.5 is Cu (II) > Pb (II) > Cd (II) > Zn (II). This preference for copper may explain the high toxicity of copper towards aquatic organisms.

Florence (1982b) has reported metal speciation results for a thioglycollate chelating resin which was initially prepared by Phillips and Fritz (1978). The resin contains the thioglycol-oxyloxymethyl chelating group (-CH$_2$OCO-CH$_2$SH) attached to the benzene ring of a polystyrene-divinylbenzene resin (XAD-4). The use of 500 mg resin/50 mls of sample is considered to be suitable for a batch extraction. The sulphur content of the prepared resin is generally 1.87 mmol/g and heavy metals form a 1:2 complex with the mercapto group of the resin (Phillips and Fritz 1978).

3.1.3. Chelating Cellulose Exchangers.

Cellulose exchangers have been used to remove heavy metals from water samples by attaching suitable chelating groups. HYPHAN is a cellulose chelating exchanger which is selective for heavy metals at trace levels. Using this material Burba and Willmer (1982a, 1982b) found that heavy metals were recovered from seawater to the extent of 80-100%. In simulated aqueous samples the metals were stripped off humic acids, but were not removed from EDTA complexes.

3.2 Techniques for the measurement of organically associated metals.

Several methods, both chemical and physical, have been used to decompose organic materials in natural waters. Some organic compounds are highly resistant and require extensive oxidation before their associated heavy metals are released.

3.2.1 Ultra-Violet Irradiation

The decomposition of organic compounds in natural waters by ultra-violet irradiation was introduced by Armstrong et al (1966) for the determination of organic carbon in seawater and was subsequently used by other workers to release organically assoc-
iated heavy metals (Florence and Batley 1977, Laxen and Harrison 1981a). The sample usually 150 to 200 mls, is intro-
duced into quartz tubes and after the addition of a few drops of 30% H₂O₂, is irradiated for 4 to 8 hours with a medium
pressure ultra-violet lamp of between 500 and 1000W.

The method has had most success in seawater analysis because in freshwaters, iron hydroxide is released from an organic
colloidal coating and co-precipitates the heavy metals (Laxen and Harrison 1981b, Florence 1982a). It should be possible to
dissolve the precipitate in 1M HNO₃ prior to analysis although this has not been tested. Heavy metals will rapidly adsorb
onto the surface of the quartz tubes during cooling and therefore immediate analysis is recommended.

3.2.2 Adsorption of Organics by Resins

SM2 and XAD-2 are divinylbenzene resins with a high surface area which can adsorb organic material from aquatic samples. It is
possible to remove lipid soluble metal from a variety of water samples using SM2 or XAD-2 (Florence 1982b). Following adsorp-
tion, the lipid associated metals are either eluted with methanol and decomposed by wet acid oxidation (Florence 1982b) or the
resin and organics are decomposed by a dry ashing procedure (Montgomery and Santiago 1978).

3.2.3 Oxidation by Concentrated Acids

Organic material can be completely oxidised by the addition of suitable concentrated acids. Chau et al (1974) used a persulphate/
sulphuric acid oxidising mixture, while Florence (1977) used a mixture of perchloric and nitric acids. In seawater, a reduction
to pH 2.0 followed by boiling for 15 minutes is usually sufficient to liberate all the heavy metals (Florence and Batley 1977).

In the Middlesex Polytechnic Urban Pollution Research Centre a mixture of concentrated perchloric and nitric acids in a ratio

- 23 -
of 1:9 is used as a standard method for organic degradation (Revitt et al 1981). Concentrated nitric acid (0.18 mls) and concentrated perchloric acid (0.02 mls) are added to a filtered sample (25 mls) and the resulting mixture evaporated to dryness. The liberated metal is taken up in 1M HNO₃ (10 mls) prior to analysis.

3.2.4 Decomposition of Organics by Ozonolysis

The use of ozone to decompose organics in natural waters has been investigated (Laxen and Harrison 1981a) and shown to give an unexpected decrease in the levels of electrochemically available metals. In the case of lead it was suggested that this was due to the precipitation of the metal as lead dioxide after ozone oxidation of Pb (II) to Pb (IV).

3.3 Differentiation of Metal Species by Size Separation

Heavy metals may be associated with dissolved, colloidal or particulate phases. The accepted separation of the dissolved phase by 0.4 μm or 0.45 μm filtration often results in clogging of the filters due to the presence of organic and inorganic colloids (Astruc et al 1981). Another approach for separating metal fractions is therefore to discriminate on the basis of molecular size or molecular weight by either centrifugation, filtration, ultrafiltration or dialysis. Size fractionation can be used to complement other physico-chemical separation methods (de Mora and Harrison 1983).

3.3.1 Centrifugation

The speed and time of centrifugation determines the quantity of particulates removed from suspension, the separation depending not only on size but also on density. This makes an efficient comparison with other physical separation techniques difficult. Centrifugation at 3000 rpm for 30 minutes has been shown to separate particles smaller than 190 nm (Benes and Steinnes 1975)
whilst centrifugation at 40,000 rpm for up to 5 hours may remove humic substances (Buffle et al 1978).

The radius of the centrifuged particles shows the following relationship to centrifugation time (Steinnes 1983):

\[ r^2 = \frac{9\eta \ln \left( \frac{x_2}{x_1} \right)}{2(\rho - \rho_o)\omega^2 t} \]

- \( t = \) centrifugation time
- \( x_1, x_2 = \) initial and final distances of the particles from the axis of rotation.
- \( \rho, \rho_o = \) densities of respectively the particle and the dispersing medium.
- \( \eta = \) viscosity of the dispersing medium.
- \( \omega = \) angular rotation velocity

3.3.2 Dialysis

Dialysis allows the separation of different groups of species on the basis of particle size (Buffle 1981). Typically ultra-pure water in a dialysis bag is allowed to equilibrate with the sample for 24 hours (Guy and Chakrabarti 1976). Under these conditions and with a pore size of 1 to 5 nm (molecular weight cut-off value \( \sim 1000 \)) it is found that free dissolved metal ion concentrations inside and outside the dialysis bag are equal. An alternative approach is to place the bag in situ for 1 to 14 days until the absorption equilibrium is reached. However, the length of time of equilibration has been reduced by Hart and Davies (1981) using a semi-continuous process which has been incorporated into their speciation scheme. A dialysis unit coupled with a Chelex - 100 column was found to reduce the time of equilibration to 5 hours. Metal adsorption and dissociation at the membrane surface are the main problems and probably limit the application of the method to in situ analysis.
3.3.3 Filtration

A single filtration step, through a 0.4 µm or 0.45 µm filter is often employed as a preliminary separation for the dissolved and particulate phases. However, this separation is complicated by the presence of colloids (Stumm and Brauner 1975). Laxen and Harrison (1981a) have introduced a speciation scheme using a series of five nucleopore filters ranging in pore size from 0.015 µm to 12 µm. Nucleopore filters act as barrier rather than depth filters allowing a very effective cut-off value and few adsorption losses (Sheldon 1972). In a recent review of size separation techniques de Moya and Harrison (1983) recommended the use of filtration as a useful comparison to other speciation techniques.

3.3.4 Ultrafiltration

Benes and Steinnes (1974) pioneered the use of ultrafiltration in speciation work. Ultrafiltration can be defined as the separation of metal fractions by filtration below 0.015 µm. However, ultrafiltration cannot be exactly related to molecular weight as the ultrafiltered fractions are separated not only on the basis of size, but also shape and charge characteristics (Guy and Chakrabarti 1976). Nevertheless the sizes for metal species can be compared with typical cut-off values for ultrafilters. The Amicon PM10 filter has a pore size of 1.8 nm and should permit the separation of free metal ions and small organic and inorganic complexes from trace metals associated with humic substances and colloidal species. Laxen and Harrison (1981) incorporated this single ultrafiltration step in their filtration based scheme.

A cascade system of ultrafilters, or sequential ultrafiltration, can be used to reduce the adsorption of high molecular weight compounds by preventing their contact with small pore size materials. Hoffman et al (1981) found that large concentration gradients could be avoided by only filtering the first 50% of the sample at each step and hence avoiding the need for repeated washings. To calculate the concentration of metals in each molecular weight range a system of mass balance equations was
The main problems relating to ultrafiltration are as follows:

i) the membranes are expensive

ii) the filters as supplied are often contaminated with metal and soluble organic material.

iii) contamination and adsorption losses in ultrafiltration are especially serious because there is a large surface to volume ratio.

iv) the concentration gradient at the surface of the membrane filter may cause dissociation of colloidal associated metals and changes in ionic strength.

v) the trapping of particles in the pores of filters make the separations difficult to achieve and consequently result in long filtration times.

Despite recent advances in ultrafiltration this technique is still open to criticism. Nominal molecular cut-offs are imprecise making the interpretation and comparison of results difficult. The technique is expensive requiring a new membrane for each filtration and the long filtration times inhibit the handling of large numbers of samples. Because of these problems de Moya and Harrison (1983) prefer dialysis to ultrafiltration on the basis of cost, speed of analysis and efficiency of separation.

3.4 Other Separation and Analytical Techniques

Several alternative methods of separation and analysis have been tested and, although not often used, these techniques can be useful for comparison with the more generally accepted methods. For example metal ion catalysis is very specific to the free ion and should compare with other free ion measurements such as anodic stripping voltammetry or dialysis.

3.4.1 Metal Ion Catalysis

During the metal ion catalysis of oxidation-reduction reactions...
the rate of reaction is directly proportional to the free metal ion concentration. An example of such a system which has been studied is the catalytic effect of copper on the oxidation of L-Ascorbic acid (Mottola et al 1968). Ferrer-Herranz and Perez-Bendito (1981) devised a method, based on this technique, for the determination of copper in water at the 10–90 µg/l level and Nakano et al (1981) used a similar approach for copper in tap water. A method which is free from interferences has been devised for the determination of zinc in water samples (Haraguchi et al 1980). The presence of zinc causes a difference in the rate of the ligand substitution reaction between 1-(2-thiazolylazo)-5-napthol chelates and EDTA.

However, the method has been avoided in natural waters, despite its specificity for free metal ion, as it is thought that catalysts or inhibitors in the sample may affect the accuracy of the determination.

3.4.2 Radiotracers

Radiotracers can be added to a sample as a means of tracing the species which a heavy metal can form. Attempts to use ionic radiotracers have been hindered by the slow equilibration of the labelled ions with the non-ionic species of the heavy metal in the sample (Benes and Steinnes 1976). The equilibration period may be of the order of months or even years (Bowen et al 1979).

3.4.3 Gel Filtration Chromatography

For polluted waters gel filtration chromatography may be an attractive method for separating metals into molecular size fractions (Steinberg 1980).

Sample or solute flow is retarded in relation to eluent on a column of porous polymeric beads. The large molecules elute first followed by a continuous size spectrum of molecules (de Moya and Harrison 1983).
Direct analysis of a sample by this method is limited to waters with high metal concentration because the small sample volume compared to eluent gives rise to high dilution factors and large blank values. An alternative approach is to use pre-concentration procedures prior to gel filtration chromatography. This method has been used to distinguish between copper and lead size associations with organic material in coastal waters (Sugai and Healy 1978), although the procedure has unknown effects on metal speciation.

3.5 The Suitability of Physico-Chemical and Physical Separations for Heavy Metal Speciation Analysis

Several different approaches have been applied to separating fractions of heavy metals in natural waters. Chelating resins are easy to use and relatively free from contamination. By adjusting the properties of the chelating groups attached to a resin it is possible to remove those metals which are predominantly bioavailable. The organically associated metals are best extracted by acid digestion or by U.V. irradiation, as ozonolysis and resin removal are more subject to interferences. Size separation is most satisfactorily achieved by filtration or dialysis techniques, although centrifugation is a viable alternative. Ultrafiltration is a difficult technique requiring experienced operators whilst Gel Filtration Chromatography is an interesting method which still requires further development in relation to metal speciation work.
CHAPTER 4

PHYSICO-CHEMICAL SPECIATION SCHEMES
FOR THE SEPARATION OF HEAVY METAL
SPECIES IN THE DISSOLVED PHASE

The formulation of the analytical procedures described in the previous chapters into detailed speciation schemes was first attempted by Batley and Florence (1976b). The use of size separation was incorporated into a comprehensive scheme by Laxen and Harrison (1981). However, both schemes are detailed and require specialised and lengthy analytical procedures. For polluted waters a simple, fairly rapid evaluation of toxic metal content is required (Florence 1982).

4.1 The Speciation Scheme of Batley and Florence (1976b)

Despite the difficulties of separating heavy metal species in the dissolved phase, a scheme for application to sea water has been introduced by Batley and Florence (1976b). This comprehensive scheme is illustrated in Figure 4.1. Labile (pH 4.8) and bound metals are analysed by Differential Pulse Anodic Stripping Voltammetry both before and after the sample is subjected to a number of separation techniques. Labile and bound measurements are made at the following stages:

i) before treatment
ii) after passage through a Chelex-100 column
iii) after U.V. irradiation
iv) after passage of the U.V. irradiated sample through a Chelex-100 column

Seven metal species are identified from the eight measurements and the scheme has been applied by Batley and Florence (1976a, 1976b) to sea water samples.

The results showed that, in sea water, cadmium is mainly present
FIG. 4.1: Speciation Scheme of Batley and Florence (1976b)
as an ASV labile organic species. Most of the copper is found to be in the form of organic colloidal complexes but lead, which forms weaker complexes with organic materials, is mainly present as inorganic colloids.

Batley and Gardner (1978) applied the same scheme to copper, lead and cadmium speciation in estuarine and coastal waters. Associations of cadmium (15-35%) and lead (45-70%) with inorganic colloids were found to be important, while 40% of copper was present as inorganic and organic colloids.

In river water the colloidal organic and inorganic species together account for 52% of total copper, 24% of total lead and 5% of total cadmium (Batley 1983).

4.2 The Ultrafiltration Scheme of Laxen and Harrison (1981b)

A scheme incorporating filtration and ultrafiltration as the major separation techniques has been introduced by Laxen and Harrison (1981) and is illustrated in Figure 4.2. The metal species were size fractionated using Nucleopore filters of 12, 1.0, 0.4, 0.08 and 0.015 μm rated pore size, followed by a series of Amicon ultrafilters. ASV-labile, Chelex-labile and U.V. irradiated fractionations were also included in the scheme. The separation techniques are complex and only a few of the fractions possessed metal levels above the detection limit. However, the ultrafiltration steps give data on truly dissolved metals which are believed to be highly toxic. In this respect a comparison of the ultrafiltration steps with, for example, bioassays, would be interesting.

The results for the application of this scheme to the effluent from a sewage treatment plant showed that most of the metals were associated with the colloidal and particulate phases (Laxen and Harrison 1981b). However, a high percentage of the cadmium (45%) was present in the ultrafilterable, less than 0.015 μm, fraction. Copper is also important in the ultrafilterable fraction (47%) but, although Chelex-labile, it is not present as the ASV-labile form.
FIG. 4.2: The Physical Separation Scheme of Laxen and Harrison (1981a).
In a later application of the scheme to the effluent from a lead-acid battery manufacturer, the ultrafiltration stage was omitted (Laxen and Harrison 1983), probably because of the problems associated with this technique. The metal levels for the Chelex-labile metal of the <1 μm fraction and the total metal of the <0.015 μm size fraction were found to be similar.

The scheme has, therefore, proved useful in determining the impact of heavy metals from both industrial and sewage effluents on receiving waters.

4.3 Other Speciation Schemes

The schemes described in 4.1 and 4.2 require Class-100 clean room facilities, which are designed for the analysis of sub-ppb levels. Another inhibiting factor is their intrinsically detailed nature, leading to an abundance of data which is difficult to interpret. A more satisfactory situation for polluted waters would be a scheme which provides information on the bioavailability, or geochemical mobility, of metals and yet is relatively easy to apply.

The first attempt to devise a scheme for polluted waters was introduced by Stiff (1971). This scheme was designed to assess the impact of copper in polluted freshwater and sewage effluents. Free copper ion, measured by ion selective electrode, was less than 2 μg/L. CuCO₃ was thought to be an important species on the basis of calculations using uncertain stability constants, total hardness values and free copper ion concentrations. The species present in the greatest concentration was that described as amino acid complexed, although this fraction probably included any strongly bound species.

Chau and Lum-Shue-Chan (1974) distinguished between labile and non-labile forms of metals in lake water by their response to Differential Pulse Anodic Stripping Voltammetry. Labile metal was determined after the addition of acetate buffer, while
bound metals were determined after digestion with persulphate/sulphuric acid or nitric/perchloric acids. A similar differentiation of labile and non labile species for North Sea and River Rhine samples were carried out by Duinker and Kramer (1977). Zinc was found to be labile, while copper and lead were mostly in the bound form. An additional extraction with APDC/MIBK gave the same lability results for zinc, but higher values for lead and copper and lower values for cadmium.

Relative labilities of metals have been assessed by Figura and McDuffie (1980) in a scheme based on calcium Chelex. Very labile refers to metals which are directly measurable by ASV. Moderately labile and slowly labile metals are those which are taken up by calcium Chelex column and batch extraction methods, respectively. The inert fraction contains metal which is not susceptible even to calcium Chelex batch uptake and probably represents extremely stable organo-metallic complexes or metal adsorbed strongly to colloidal material. The separations are not completely satisfactory as the very labile fraction is not always fully retained by the Chelex column or batch methods (Batley 1983). Consequently the results cannot be interpreted in terms of dissociation kinetics.

The scheme was applied to river water and most of the cadmium and zinc (81-90% and 86-100% respectively) was found to be present as very labile or moderately labile species. Copper and lead existed in more strongly bound forms with most of the lead associated with the slowly labile fraction and the copper almost entirely in the moderately labile and slowly labile fractions.

Hart and Davies (1981) used a combination of sodium Chelex (batch method), dialysis and acidification to determine metal speciation in estuarine waters. The determination of other parameters such as chloride, suspended solids and organic carbon, followed by an analysis of this data by a computer simulation, allowed some prediction of the species present.

Cadmium and zinc were fractionated on a column of Chelex by
Pik et al (1982). The remaining metal associations described as colloidal, were determined by coprecipitation of the metals with a molybdenum carrier complex. Metal determinations were carried out using the highly sensitive X-ray fluorescence technique. The results showed that, for estuarine and river waters, zinc was 70-100% Chelex exchangeable, whilst 50-100% of copper was colloidal.

The importance of the colloidal associations has been shown by Sholkovitz et al (1978) to be due to the flocculation under estuarine conditions of the 0.1 um and 0.45 um size fractions. Welte and Montiel (1980) suggested 0.45 um and 0.22 um filtration, to separate these organic colloids, followed by columns of anionic (Cl⁻ form) and cationic (H⁺ form) resins. U.V. irradiation may also be incorporated into the scheme to provide information on organic and inorganic forms.

Sugai and Healy (1978) used Gel filtration chromatography combined with Anodic Stripping Voltammetry to identify metal-organic speciation in an anoxic fjord. Complexation of both copper and lead with organic material was observed over a wide range of molecular weights, typically 500-10,000.

The concepts of lability and physical separation have been combined by Hasle and Abdullah (1981). Ultrafilterable fractions of coastal seawater were determined by Differential Pulse Anodic Stripping Voltammetry for labile, acid soluble (pH 2.8) and total metals. Acidification releases inorganically and organically complexed metals, whilst organo-metallic compounds probably account for most of the total non labile fraction. Cadmium and lead were found to be associated with the low molecular weight fractions with cadmium showing a tendency to be non labile. Copper was largely associated with organic colloids.

4.4 Assessment of the Techniques and Results from Dissolved Heavy Metal Speciation Studies

Speciation schemes for separating dissolved heavy metal fractions
have provided some insight into the toxicity and geochemical mobility of metals.

The lability concept applied to ASV and Chelex resin analysis allows some distinction between free and complexed forms (both inorganically and organically). Physical separation is an additional technique which allows differentiation between colloidal and dissolved metals.

The results for fresh waters generally show that cadmium and zinc exist in relatively free forms, lead is predominantly inorganically complexed, but copper is found mostly as organic complexes (both colloidal and low molecular weight).

Previous work indicates that the procedures described can be adopted for polluted waters. In such waters the suspended solid phase also becomes an important source of heavy metals.
Although the toxicity of the dissolved phase is higher because of its direct contact with organisms, the non lithogenic fractions of suspended solids may subsequently release heavy metals into aqueous systems. The fractions studied must therefore reflect all heavy metal species which might have a direct effect on the aquatic biota.

Municipal, industrial and storm surface runoff are increasingly polluting river, marine and estuarine sediments with heavy metals (Gupta and Chen 1975). For instance, the cadmium concentrations in the sediments of the river Rhine have increased more than 100 fold in eighty years (Salomons and Forstner 1980). Metal contaminants exist in relatively unstable fractions and may be released by engineering operations such as dredging or by resuspension during storm surges.

The concentrations in each fraction will depend on such solid-phase reactions as precipitation, adsorption and ion exchange. From these considerations several schemes have been proposed for the sequential extraction of chemical fractions from sediments and suspended solids.

5.1 Chemical Extractants used in Sequential Extraction Schemes

Because of the importance of the availability of metals to plants most of the early work was carried out on soils (Jackson 1958). In marine pelagic sediment studies (Chester and Hughes 1967, Nissenbaum 1972) it is the interactions of metal fractions with pore water and the sediment/sea water interface which are of more concern.
The separation of sediment associated metal species into fractions is best achieved by a series of sequential chemical extractions. The extraction steps are not species selective and repeated treatment often gives a further release of metals, especially in the reducible fractions. The reactions are also influenced by the ratio of solid material to volume of extractant.

5.1.1. Exchangeable Fraction

The exchangeable fraction is considered to be that which is available primarily and immediately for biological uptake. It may include metals which are weakly attached to the surfaces of clays or hydrous iron and manganese oxides or to organic coatings. The addition of a high concentration of chloride or acetate provides a competing ligand for the heavy metals. Hence MgCl₂ (Tessier et al 1979, Eisenreich et al 1980), BaCl₂- triethanolamine (Forstner and Patchineelam 1980) and NH₄OAc (Salomons and Forstner 1980) have been proposed as extracting agents, usually at 1M concentrations and at pH 7.0. Ammonium acetate is not a suitable extractant due to its tendency to attack carbonates (Tessier et al 1979).

5.1.2 Carbonate Fraction

Carbonates in sediments exist as cements and coatings which co-precipitate with heavy metals. A lowering of pH, such as occurs with acidified rain, would dissolve carbonates and release the associated heavy metals. The most accepted extraction method is a sodium acetate/acetic acid (pH 5.0) treatment although some attack on metals weakly bound to hydrous iron and manganese oxides may occur (Tessier et al 1979). Forstner and Patchineelam (1980) claim that their acidic cation exchange method is very specific for carbonate associated metals.

5.1.3 Hydrous Iron and Manganese Oxide Fraction

Hydrous Fe and Mn oxides are thought to exist as coatings on particulate surfaces (Davis and Leckie 1978). These coatings have a high capacity for metal adsorption (Gadde and Laitinen 1974).
In order to reduce this fraction (Fe (III) → Fe (II), 
Mn (IV) → Mn (II)), several extractants have been proposed.
Dithionite/citrate (Salomons and Forstner 1980), hydroxy-
lamine hydrochloride/acetic acid (Chao 1972, Tessier et al 1979) 
and 0.3M HCl (Eisenreich et al 1980), all appear to have been 
used successfully.

5.1.4 Organic Fraction

Much of the organic fraction will be particle coatings of plant 
derived organic material. In the Middlesex Polytechnic Urban 
Pollution Research Centre different extractants for the organic 
fraction have been compared for efficiency against dichromate 
oxidation (see Table 5.1). Humic and fulvic acid associated 
metals can be successfully removed using 1M NaOH. However, the 
use of sodium hydroxide remobilises metals from phosphates and 
silicates (Forstner and Patchineelam 1980) and more importantly 
only releases about 30% of the organic material, as shown in 
Table 5.1. Strong oxidising agents, such as 0.4N Na₄P₂O₇ 
(Eisenreich et al 1980) may also attack the crystalline phase and 
so Tessier et al (1979) compromised by using an extraction mixture 
of hydrogen peroxide and 0.02M HNO₃. However, metals released 
from organics by peroxide oxidation are readily adsorbed on 
clays (Eisenreich et al 1980) and the procedure only digests 
50 to 60% of the organic material (Table 5.1). In polluted 
waters a much greater percentage of heavy metals are non-lithogenic 
and therefore it is more realistic to risk alteration of the 
silicate phase. In this respect concentrated nitric/perchloric 
acids in a ratio of 9:1 can be used to provide a 92% digestion 
of organic carbon (Table 5.1). An alternative and promising 
approach would be an investigation into various combinations of 
organic solvents as extractants of the organic fraction followed 
by digestion of the released metals.

5.2 Evaluation of Sequential Schemes

Gibbs (1973) distinguished between exchangeable metals, metals 
attached to metal hydroxide coatings, organic solids and 
crystalline phases in a study of sediments from the Amazon and 
Yukon rivers. Forstner and Patchineelam (1980) (Table 5.2)
<table>
<thead>
<tr>
<th>EXTRACTANT</th>
<th>% ORGANIC CARBON EXTRACTED OR DIGESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 1M MgCl₂, pH 7.0</td>
<td>5.4</td>
</tr>
<tr>
<td>2) 0.04M NH₄OH.HCl in Glacial Acetic Acid (25% v/v)</td>
<td>0.0</td>
</tr>
<tr>
<td>3) 30% H₂O₂ at pH 2.0 and 0.02M HNO₃</td>
<td>56.2</td>
</tr>
<tr>
<td>4) 0.1M NaOH</td>
<td>33.0</td>
</tr>
<tr>
<td>5) Concentrated HNO₃</td>
<td>55.9</td>
</tr>
<tr>
<td>6) Concentrated HNO₃ and HClO₄ (9:1)</td>
<td>92.3</td>
</tr>
</tbody>
</table>

**TABLE 5.1:** Analysis of Sequential Scheme Extractants for the Decomposition or Removal of Organic Matter in a Road Sediment.
<table>
<thead>
<tr>
<th>FRACTIONS</th>
<th>EXTRACTION MEDIUM</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Soluble</td>
<td>H₂O</td>
<td>Easily soluble metals</td>
</tr>
<tr>
<td>2. Exchangeable</td>
<td>0.2M BaCl₂ - triethanolamine pH 8.1</td>
<td>Easily extractable fraction (e.g. exchangeable) cations.</td>
</tr>
<tr>
<td>3. Humic and fulvic acid.</td>
<td>0.1N NaOH</td>
<td>Metals attached to humic and fulvic acids.</td>
</tr>
<tr>
<td>5. Easily reducible</td>
<td>0.1N NH₄OH.HCl + 0.01N HNO₃</td>
<td>Metals bound to Mn-oxides, amorphous Fe-oxides.</td>
</tr>
<tr>
<td>6. Strongly bound organic</td>
<td>30% H₂O₂ + 1N NH₄OAc</td>
<td>Organic residues and sulphides.</td>
</tr>
<tr>
<td>7. Moderately reducible</td>
<td>1N NH₂OH.HCl + 25% acetic acid</td>
<td>Hydrous Fe-oxide associated metals.</td>
</tr>
<tr>
<td>8. Residual</td>
<td>HF/HClO₄</td>
<td>Inorganic residues</td>
</tr>
</tbody>
</table>

**TABLE 5.2:** Sequential Extraction Scheme of Forstner and Patchineelam (1980)
and Tessier et al (1979) (Table 5.3) also used sequential schemes to analyse heavy metal fractions in river and estuarine sediments.

The information which these schemes provide is relevant to the ease of metal release to the aqueous phase as well as to processes such as geochemical mobility. There is a certain amount of overlap between the chemical fractions and a scheme with five or six fractions is analytically demanding. It is preferable to devise a scheme, based on established speciation schemes, which will still identify the relevant processes. Hence a less complex sequential scheme has been applied by Salomons and Forstner (1980) to 18 rivers throughout the world. The data, shown in Figure 5.1, shows that metals in polluted waters are mostly associated with the relatively bioavailable fractions (non-lithogeneus) while in unpolluted waters most of the metals tend to be associated with the crystalline fraction. The extraction scheme of Eisenreich et al (1980), illustrated in Table 5.4, showed similar results in a study of the Mississippi River sediments.

5.3 The Importance of Determining Heavy Metal Associations in Suspended Solids and Sediments

Sequential schemes for the analysis of heavy metal speciation in sediments rely on the ease of release of the solid bound heavy metals. This approach is fundamentally different to the dissolved measurements which relate to the complexation strength or lability of the metals.

Comparison of sequential extraction results with that metal which is most readily bioavailable, is not straightforward. The most important fraction which can be determined is the exchangeable fraction as this represents the easily released and, therefore, most immediately available metal. Previous studies have shown that this fraction contains higher concentrations in polluted waters where most of the heavy metals are associated with non-crystalline forms. The interpretation of these sequential extraction studies can be greatly assisted by a consideration of theoretical studies of heavy metal associations with particulates. Some of these are described in the next Chapter.
<table>
<thead>
<tr>
<th>FRACTIONS</th>
<th>EXTRACTION MEDIUM</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Exchangeable</td>
<td>MgCl$_2$ (1M) pH 7.0 1 hour</td>
<td>Weakly adsorbed on clays, hydrous oxides, humic acids.</td>
</tr>
<tr>
<td>2. Bound to carbonates</td>
<td>NaOAc (1M) pH 5.0 with acetic acid. 5 hours.</td>
<td>Associated with sedimentary carbonates.</td>
</tr>
<tr>
<td>3. Bound to iron and manganese oxides</td>
<td>0.04M NH$_2$OH.HCl in 25% (v/v) HOAc. 5 hours. 96°C. pH 2.0.</td>
<td>Associated with hydrous oxides as nodules, concretions, cement between particles or coatings.</td>
</tr>
<tr>
<td>4. Bound to organic matter</td>
<td>30% H$_2$O$_2$/0.02M HNO$_3$ pH 2.0. 5 hours. 85°C. Subsequently NH$_4$OAc</td>
<td>Complexed or peptized with natural organic matter. Associated with sulphides.</td>
</tr>
<tr>
<td>5. Residual</td>
<td>HF/HClO$_4$(5:1)</td>
<td>Within the crystalline structure of primary and secondary minerals.</td>
</tr>
</tbody>
</table>

**TABLE 5.2:** Sequential Extraction Scheme for Sediments and Suspended Solids (Tessier et al 1979).
FIGURE 5.1: Sequential Extraction Scheme applied to Polluted and Unpolluted River Sediments (from Salomons and Forstner 1980)
<table>
<thead>
<tr>
<th>FRACTIONS</th>
<th>EXTRACTION MEDIUM</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Exchangeable</td>
<td>0.5M MgCl$_2$, 7 hr.</td>
<td>Adsorbed, ion exchangeable</td>
</tr>
<tr>
<td>2. Organic</td>
<td>0.4N Na$_4$P$_2$O$_7$, 10 hr. pH 7.0</td>
<td>Particulate organic coatings.</td>
</tr>
<tr>
<td>3. Bound to hydrous oxides</td>
<td>0.3N HCl 30 min. 90°C</td>
<td>Hydrous metal oxide coatings.</td>
</tr>
</tbody>
</table>

TABLE 5.4: Sequential Scheme of Eisenreich et al (1980)
CHAPTER 6

ALTERNATIVE APPROACHES TO SPECIATION STUDIES

Other approaches to speciation studies have been suggested to complement previously reported results and aid in their interpretation. Mathematical models can only be used satisfactorily if enough is known about the major species and their relative stability constants. Particulate surface interactions with metals can be theoretically explained as a result of laboratory experiments. In addition laboratory simulation studies help to explain experimental data from sequential extractions on particulates. Mixing experiments allow some insight into the fate of heavy metals on contact with receiving waters.

6.1 Mathematical Models

All the mathematical models presented to date are based on thermodynamic considerations only. If the total concentrations and interactions of all the major components of the aqueous system are known and perfect equilibrium conditions prevail, then the concentration of each chemical species of a given element can be calculated. The values of the stability constants and the corresponding dissolved and surface free ligand concentrations must be known. The uncertainty over many stability constants, particularly with organic ligands, makes a comparison with real data difficult.

In seawater organic ligands are generally present at low levels (<1 mg/l) and are therefore thought to be relative unimportant. This allows calculations on the basis of homogeneous chemical reactions and precipitations, particularly as open ocean water is so well mixed (Sillen 1963). Hence mathematical modelling of seawater has compared favourable with analytical work (Millero 1974, Whitfield, 1975).

In freshwaters two other important interactions must be taken into account:
(i) The adsorption of metals onto particulates (Jenne 1968, Davis and Leckie 1978) and interactions at the suspended solid/water interphase (Westall 1980, Hohl et al 1980).

(ii) The presence of organic material. Most of this material is largely uncharacterised, especially fulvic acids which form important complexes with heavy metals. Organic compounds may be dissolved or present as surface coatings on particulate material (Davis and Leckie 1978).

A computer model taking these factors into account would need to be backed up by a considerable amount of analytical data. Hart and Davies (1981) incorporated data from a speciation study, together with general water quality data, into a computer model. In this way computer calculations were used to assist in the interpretation, rather than prediction, of heavy metal speciation.

6.2 Theoretical Studies of the Interaction of Heavy Metals with Particulate Fractions

Interactions between the dissolved, colloidal and particulate phases control the fractionation of heavy metals between each phase. The significant controlling influence of the particulate phase has been recognised for some time (Jenne 1968). However, the heterogeneous nature of particulates in water has slowed down theoretical advances in this field. The distribution of shapes, densities, surface chemical properties and chemical composition may vary widely with size. The sampling process itself may lead to changes in size and shape (Kavanaugh and Leckie 1980).

Surface exchange theories have been found to apply well to the adsorption of metals on hydrous iron oxides and residual materials. A theory for the exact behaviour of hydrous manganese oxide does not appear to have been formulated. Organic materials are also capable of complexing heavy metals. These theories are summarised in Table 6.1. From these principles the partitioning
<table>
<thead>
<tr>
<th>CONTROL MECHANISM</th>
<th>TYPE OF PARTICULATE</th>
<th>EFFECTS ON HEAVY METALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Surface exchange</td>
<td>Fe₂O₃, silica, alumina, clays.</td>
<td>Regulator. A small change in pH may cause significant adsorption or desorption.</td>
</tr>
<tr>
<td>2. Co-precipitation,</td>
<td>MnO₂</td>
<td>Removal. An effective sink unless low pH is encountered.</td>
</tr>
<tr>
<td>3. Complexation</td>
<td>Organic solids and coatings.</td>
<td>Removal, depending on complexation capacity and stability constant.</td>
</tr>
</tbody>
</table>

TABLE 6.1. Control Mechanisms for Heavy Metals in Aquatic Samples.
of heavy metals between different materials may be more easily interpreted, although component abundance must also be considered.

6.2.1 The Theory of Surface Exchange

Surface exchange theory in natural water systems has been the subject of a recent review. (Hohl et al. 1980, Westall 1980, Davis and Leckie 1979). Metal ion uptake can be explained by a combination of surface reactions involving aquo metal ions and their hydrolytic complexes.

\[ \text{SOH} + M^{2+} \rightleftharpoons \text{SO}^- M^{2+} + H^+ \]

\[ \text{SOH} + M^{2+} + H_2O \rightleftharpoons \text{SO}^- MOH^{(z-1)+} + 2H^+ \]

The binding capacity of the surface will be dependent on the specific surface area and the ionic radius of the reacting metal ion (Luoma and Davis 1983). Hence the unhydrated lead binding capacity for amorphous Fe(OH)₃ is 22 mmol g⁻¹ yet only 1.9 mmol g⁻¹ for the crystalline geothite.

The James-Healy hydrolysis adsorption model (James and Healy 1973) predicts that at constant pH fractional adsorption should increase with increasing \( \log \beta^*_1 \). \( \log \beta^*_1 \) is the hydrolysis constant for addition of the first -OH to the metal ion.

\[ M^{2+} + H_2O \rightleftharpoons M(\text{OH})^+ + H^+ \]

\[ \beta^*_1 = \frac{[M(\text{OH})^+]}{[M^{2+}][H^+]} \]

Consideration of the first hydrolysis constants (Table 6.2) shows that the % adsorption should decrease in the order \( \text{Cu}^{2+} > \text{Pb}^{2+} > \text{Zn}^{2+} > \text{Cd}^{2+} \), at a given pH. In fact adsorption of the metal ion begins to occur at a value of \( p \beta^*_1 - 1.5 \) (Gaddes and Laitinen 1974, Davis and Leckie 1978, Lion et al. 1982). The values of \( p \beta^*_1 - 1.5 \), illustrated in Table 6.2, can be compared to the results for metal ion adsorption on hydrous iron oxides.
<table>
<thead>
<tr>
<th></th>
<th>Cu$^{2+}$</th>
<th>Pb$^{2+}$</th>
<th>Zn$^{2+}$</th>
<th>Cd$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Hydrolysis Constant, $p^*_g$</td>
<td>7.0</td>
<td>7.5</td>
<td>8.5</td>
<td>9.5</td>
</tr>
<tr>
<td>$p^*_g - 1.5$</td>
<td>5.5</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

**TABLE 6.2: Hydrolysis Constants for Heavy Metal Ions**

(from Florence and Batley 1980)
Similar results have been obtained for silica (Huang et al. 1977), clays (Farrah and Pickering 1976a, 1976b, 1977, 1979, 1980) and hydrous aluminium oxides (Shuman 1977).

6.2.2. The Role of Hydrous Manganese Oxides

Hydrous manganese oxides show a rather different behaviour to other surfaces, as illustrated in Figure 6.2. Lead is strongly adsorbed except at very low pH values, whilst zinc and cadmium are gradually released with a lowering of pH. Two possible explanations can be given:

(i) In relation to the zero Point of Charge (ZPC) which has been developed from surface exchange theories because of the importance of surface charge. At pH values higher than the ZPC the surface exhibits an average negative charge and so attracts metal ions. At lower pH values than the ZPC the surface exhibits an average positive charge and so metal ions are repelled. The ZPC for hydrous manganese oxide is 2.8 (Gadde and Laitinen 1974) and this corresponds to the results in Figure 6.2.

(ii) The surfaces of hydrous manganese oxides are chemically dynamic i.e. they are constantly being renewed, added to and changed in oxidation state (Hem 1980). This may mean that the metals associated with hydrous manganese oxide are co-precipitated rather than adsorbed. This explanation can explain the results of Figure 6.2 as metals will remain co-precipitated with the hydrous manganese oxides until the oxides are dissolved and the metals stripped off at low pH values.

6.2.3 Interactions of Heavy Metals with Organic Solids and Coatings.

Organic compounds may form coatings around suspended particles (Davis and Leckie, 1978, Lion et al. 1982) or remain as discrete particles. Leppard and Burnison (1983) have described colloidal organic fibrils, largely polysaccharides
\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.2.png}
\caption{Uptake Experiments of Hydrous Manganese Oxides (Gadde and Laitinen 1974).}
\end{figure}
secreted from algal cells, which also form coatings on particulates.

Certain organic ligands are capable of binding or complexing heavy metals and certainly soil and water derived humic and fulvic acids show this ability (Shuman and Cromer 1979). The complexing ability of organic fibrils is under investigation (Leppard and Burnison 1983). A higher stability constant is found for the complexation of heavy metals with humic substances (i.e. extracted by alkali) at higher pH values (Mantoura et al 1978).

A comparison of conditional stability constants at pH 8.0 for Fe(OH)$_3$ and organics has been made by Luoma and Davis (1983), as shown in Table 6.3. Copper is bound more strongly in organic matter than Fe(OH)$_3$ while cadmium, zinc and probably lead are preferentially associated with Fe(OH)$_3$.

6.3 Laboratory Simulation Studies of Real Systems

The laboratory simulation approach is necessarily a simplification of the real system, although it can give some indication of the behaviour of individual components.

Guy et al (1975) observed the change in heavy metal distribution between various particulate and dissolved components such as hydrous oxides, humic acids and clays, over a wide range of pH values. The model developed as a result of these studies appeared to be in qualitative agreement with reported distributions for natural waters. Typical values for Cu, Cd and Zn are given in Table 6.4. The results show that Cu has much greater tendency to attach to the particulate phase (represented by MnO$_2$ and clay) than either Cd or Zn. Cu also shows a greater attraction to dissolved humic acids.

The observed tendency of Cd and Zn to be readily solubilised in leachate studies on road sediments (Ellis and Revitt 1982) is in agreement with the results of Guy and Chakrabarti (1976) as both metals seem to prefer the dissolved phase.
<table>
<thead>
<tr>
<th></th>
<th>log $K_{pH 8.0}$ Fe(OH)$_3$</th>
<th>log $K_{pH 8.0}$ Organics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (II)</td>
<td>7.9</td>
<td>8.9 - 11.4</td>
</tr>
<tr>
<td>Pb (II)</td>
<td>9.6</td>
<td>-</td>
</tr>
<tr>
<td>Zn (II)</td>
<td>6.7</td>
<td>5.3 - 5.9</td>
</tr>
<tr>
<td>Cd (II)</td>
<td>5.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

**TABLE 6.3:** Conditional Stability Constants for Metal Complexation of Amorphous Fe(OH)$_3$ and Organic Material at pH 8.0. From Luoma and Davis (1983).
<table>
<thead>
<tr>
<th>METAL</th>
<th>$X_M$(mmol.metal/g.MnO$_2$)</th>
<th>$X_M$(mmol.metal/g.humic acid)</th>
<th>Distribution coefficient for clay.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>1.95</td>
<td>0.42</td>
<td>9.0</td>
</tr>
<tr>
<td>Zn</td>
<td>0.96</td>
<td>0.21</td>
<td>4.3</td>
</tr>
<tr>
<td>Cd</td>
<td>1.10</td>
<td>0.22</td>
<td>4.8</td>
</tr>
</tbody>
</table>

$$X_M = \text{Maximum sorption values}$$  
$$pH = 5.0$$  
$$\text{Ionic strength} = 1.0 \times 10^{-3} M \text{ potassium acetate}$$

6.4 The Product Approach (Mixing Experiments)

Mixing experiments were introduced by Sholkovitz (1976) to determine the composition of removal products (flocculants) due to the mixing of filtered river and sea waters at varying salinities. The results of these experiments showed that rapid flocculation of Fe, Mn, Al, P, organic carbon and humic acids occurs in the estuarine environment. Most of the precipitate is formed within one half hour of mixing, and is removed for analysis. A further 24 hours is required before enough precipitate forms for a second determination. The procedure is illustrated in Figure 6.3.

Further studies (Boyle et al, 1977, Sholkovitz et al, 1978) demonstrated the important role of dissolved organic matter. The high molecular weight component of dissolved humic acids (0.1 μm - 0.45 μm filtered) which constitutes a small fraction of river dissolved organic material, is preferentially and rapidly flocculated during estuarine mixing.

The application of mixing experiments to the modelling of the impact of stormwater on river water would be interesting. During winter storms high concentrations of chlorides are frequently observed due to the use of de-icing salt. The resulting large change in ionic strength between incoming stormwater and ambient river water may give rise to changes in the speciation of the toxic metals. Other parameters which are often significantly different between stormwater and river water, and which may result in flocculation, include pH, organic material (both dissolved and particulate), suspended solids loadings and iron and manganese concentrations.

6.5 The Relevance of Theoretical Studies of Heavy Metals in Natural Waters.

A comparison of theoretical studies with the data from speciation schemes shows that some similarities exist between the two approaches. Copper is found mostly in the organic fraction of particulates because of the high stability of the
Water sample (1) e.g. river water

Filtration

Water sample (2) e.g. sea water and stormwater

Filtration

Fixed ratios of samples (1) and (2)

Mix and allow to stand \( \frac{1}{2} \) HR.

Collection of flocculants by filtration

Stand solutions for 24 HRS.

Collection of flocculants by filtration

Flocculants (24 HRS)

Flocculants (\( \frac{1}{2} \) HR.)

**Figure 6.3**: Procedure for Mixing Experiments (from Sholkovitz 1976).
resulting complexes; for the same reason lead is mostly associated with hydrous metal oxides. Zinc and cadmium tend to be more readily solubilised in the pH range of most aquatic samples.

Mathematical models, theoretical studies, laboratory simulation and mixing experiments are useful methods for interpreting heavy metal fractionation data from speciation schemes.
Organic ligands of both simple and complex structure occur in abundance in natural waters, typically at concentrations of 10 mg/l in river water. Approximately half of these organics are fulvic and humic acids (Saar and Weber 1982), both of which closely resemble organic extracts from soil (Reuter and Perdue 1977).

A large proportion of heavy metals in natural waters are believed to exist in complexed or chelated forms with organic ligands (natural or anthropogenic) which therefore act as regulators of metal availability (Chau 1973). Complexation may therefore control the geochemical transport and bioavailability of metals in the aquatic environment (van den Berg and Kramer 1979a).

In addition to increasing the possibility of heavy metal transport in natural waters, as well as through waste water treatment processes, complexing agents may hinder the removal of metals from water, increase the corrosion of metal surfaces and affect the oxidation state of metals in water (Kunkel and Manahan 1973).

Analysis of the complexation properties of these organic compounds is complicated by the irregular structure and wide range of components present. Several experimental methods have been developed to investigate the complexing behaviour of organic fractions in natural waters. Most methods involve a titrimetric procedure in which the ligands are reacted with a suitable metal ion until the end point, equivalent to the complexing capacity, is reached. A technique to detect remaining free metal is therefore required. Voltammetry is the most tested method (Shuman and Woodward, 1973, 1977, Shuman and Cromer, 1979) although it may measure weakly complexed metals.
in addition to free metal ion. Potentiometry, ion selective electrodes, solubilisation, bioassay and ion exchange are other available techniques.

7.1 Polarography and Voltammetry

Preliminary polarographic studies of consecutively formed complex ions (Deford and Hume, 1953) were developed by Matson (1968) who used Anodic Stripping Voltammetry as a sensitive technique to detect free, as opposed to organically complexed copper. Copper is a satisfactory metal for the titration as it forms strong, specific complexes with many organic compounds.

Methods for determining Apparent Complexing Capacity (Chau 1973, Chau and Lum-Shue-Chan, 1974) and later Conditional Stability Constants (Shuman and Woodward, 1973, 1977) have since been developed. The method is rapid, sensitive, allows the determination of total ligand concentration and is applicable over a wide pH range.

7.1.1. Determination of Apparent Complexing Capacity.

The technique is essentially a complexometric titration of metal ion against ligand. Where ligands are titrated sequentially, those with the highest stability constants are complexed first of all (Crosser and Allen 1977).

A typical titration curve for copper with a water sample is illustrated in Figure 7.1. When many ligands are present, and their individual concentrations are small, a smooth curve as shown by the dashed line in the figure is typical. In this case the sharp changes in stability constants are offset by small changes in concentration. Changes in titration slope are therefore related to the product of the concentration of the ligand times its Conditional Stability Constant.

The Apparent Complexing Capacity is found by extrapolation of the free metal ion (final slope) to the x-axis. There appears to
FIGURE 7.1: Titration of a Water Sample with Copper
have been some ambiguity in the literature regarding this determination. Some authors have used the value of \( C_L \), which is the value at the point of inflection of the final and the directly preceding slopes (Duinker and Kramer 1977, Hart 1981, Shuman and Woodward 1977). This value is then used in the calculation of Conditional Stability Constants. Other authors (Eisenreich et al 1980, Laxen and Harrison 1981b, 1983) appear to have used the recommended procedure of Chau (1973) as illustrated in Figure 7.1, and this is the commonly preferred method.

7.1.2. Calculation of the Conditional Stability Constant.

The Conditional Stability Constant can be calculated on the basis of formation of the complex:

\[
\begin{align*}
\text{aM} + \text{bL} & \rightleftharpoons \text{NaL}_b \\
K'_{\text{ML}} &= \frac{[\text{NaL}_b]}{[\text{M}]^a [\text{L}]^b}
\end{align*}
\]

where \( a \) and \( b \) depend on the stoichiometry,
\( L = \text{ligand} \)
\( M = \text{metal} \)
\( K'_{\text{ML}} = \text{Stability Constant} \)

For a given Complexation Capacity, the initial slope becomes lower for increasing values of the Conditional Stability Constant. Using ASV titration data, Shuman and Woodward (1973, 1977) derived the following equation:

\[
i_{s} \approx \frac{k}{(K)^a} \cdot \frac{(C_M/a)^{\frac{b}{a}}}{(C_L - b/a \cdot C_M)^{b/a}}
\]

where \( i_{s}/k = \text{slope of graph after all complexing sites are filled} \).
\( C_M = \text{Concentration of metal added} \)
\( C_L = \text{Concentration of total ligand at the intercept} \).

For a 1:1 complex a plot of \( i_s \) vs \( [C_M/(C_L - C_M)] \) has a slope of \( k/K' \), while for a 1:2 complex a plot of \( i_s \) vs \( C_M/(C_L - 2C_M)^2 \) has a slope of \( k/K' \).
With low stability constants the calculation is accurate, within 20%, for titration values up to halfway to the endpoint \( C_L \). The error decreases for higher stability constants. Some kinetic dissociation has been observed for humic and fulvic acids (Shuman and Cromer 1979) and the extent is dependent on ligand concentration. After applying a suitable correction factor the stability constant was not significantly changed, while the Complexation Capacity was slightly increased.

In environmental samples displacement reactions can take place in addition to the direct reaction in equation (1). (Neubecker and Allen 1983).

\[
M + M'L \rightleftharpoons ML + M' \\
K'_{ML} = \frac{[ML][M']}{[M][M'L]} \tag{3}
\]

\( M' \) = another metal

Therefore, the conditions for measurement e.g. pH, ionic strength, competing complexation are different from those relevant to the determination of the Thermodynamic Stability Constant and the term Conditional Stability Constant is therefore used. The changes in the Conditional Stability Constant during titration may be offset by small changes in concentration and hence it may only be possible to quote an Average Conditional Stability Constant for a plethora of ligands.

Although the results depend on the experimental conditions the technique is simple and straightforward to carry out. Some workers have found problems with the use of ASV as the measurement technique in that metal complexes may be reduced by the electro-chemical procedure. This problem can be overcome by operating at potentials only high enough to reduce the free metal ion. Another area of concern is that surface active organic material may be adsorbed to the electrode surface causing erroneous free metal values.

The use of this method has been criticised by Tuschall and Brezonik (1982) and shown to give low stability constant values compared to other methods. The subject remains controversial.
as is indicated by a recent exchange of comments (Bhat and Weber 1982).

However, Brisbin (1980) found a close relationship between the ASV titration technique and the amount of copper required to inhibit the growth rate of *Selenastrum capricornutum*. The technique is probably therefore valid to predict the toxic relationships of trace metals and the biota of natural waters.

7.2 Other Methods

Potentiometric titration has been widely used by soil scientists (Schnitzer and Kahan 1972) for determining the complexing capacity of isolated materials. As a direct technique an insufficient change of pH is found in natural waters due to the low concentrations of complexing ligands and the high concentration of bicarbonate. Shuman and Cromer (1979) carried out a potentiometric titration on humic and fulvic acids isolated from lake water. The copper complexation capacity from ASV titration data only represented 15% of the theoretical capacity from total acidity.

Ion selective electrodes in theory only measure free ion and should therefore be very suitable. However, it is generally difficult to achieve Nernstian response below a filterable copper concentration of 1 µM. Buffle et al (1977) have applied the technique to fresh waters, but difficulties arise in obtaining a stable response, probably due to the presence of organic ligands.

Solubilisation finds most application when studying strong chelating agents such as EDTA and NTA. The procedure, introduced by Kunkel and Manahan (1973), is based on the solubilisation of metal by the addition of ligands to a metal hydroxide suspension at pH 10.0. The precipitate is filtered and soluble copper determined by Atomic Absorption Spectrophotometry. A concentration of copper in the filtrate greater than 15 µg/l must be due to complexation by water sample constituents (under the conditions of the experiment). Despite the simplicity of the
technique there are many problems including:

i) the requirement of highly alkaline pH conditions (Campbell et al, 1977)

ii) incorrect results are obtained with weak ligands (Frimmel et al, 1980).

iii) the necessity of boiling the solution for 1 hour (Neubecker and Allen 1983).

iv) the slow attainment of equilibrium between the solution and the precipitate, precipitate ageing and the operational definition of solubility in terms of filtration through a 0.45 µm filter (Crosser and Allen, 1977).

Biological response or bioassay is related to the response of micro-organisms (Davey et al, 1973) to the toxic forms of heavy metals. By measuring a metabolic parameter as a function of added copper ion, the fraction of bioavailable (free) copper in solution can be determined. A titration-like curve may be formed from which an endpoint is estimated (Davey et al 1973, Gatcher et al 1978, Gillespie and Vacarro 1978).

Ion exchange, introduced by Schubert (1948), has been well tested by soil scientists (Zunino et al 1972, Schnitzer and Kahn 1972) although the ion exchanger tends to strip the metal from its complexes. The ion exchanger behaves as a second ligand onto which the metal can become bound.

A more acceptable technique, utilising a weak ion exchanger, MnO₂, in place of synthetic resins has been introduced by van den Berg and Kramer (1979). Most free copper ion is bound and the hydrous oxide does not remove metals from complexes. The method works well for complexes with Conditional Stability Constants lower than log K = 10 and also for low ligand concentration (0.2 mg/l organic C). The formation of ternary complexes of organic ligands at the oxide surface (Davis and Leckie, 1978) is the main criticism of this technique.
7.3 Application and Interpretation of Heavy Metal Complexing Measurements.

There is still a certain amount of critical discussion regarding the measurement of heavy metal complexation capacity and the presently available techniques require further development. However, the importance of the ability of organic ligands to complex heavy metals is well recognised. For this reason the ASV titration method, if standardised with respect to analytical conditions, is a simple technique for monitoring the spatial and temporal variations of complexing organic ligands in polluted waters.
Consideration of the literature, which has been reviewed in this report, has enabled a speciation scheme to be devised for heavy metals in natural waters (Figure 8.1). The scheme is at present being tested for stormwater runoff as part of a project being carried out by the Middlesex Polytechnic Urban Pollution Research Centre, in collaboration with the Urban Geohydrology Unit, Chalmers University of Technology, Gothenburg, under the auspices of NERC and SNV awards.

8.1 Description of the Speciation Scheme

One requirement is for a well balanced scheme which is experimentally easy to perform and is not extensively time consuming. A further objective is the provision of a complete analysis (both dissolved and particulate associated metals) which will provide relevant information on bioavailable and total heavy metal levels.

A preliminary separation allows an operationally defined distinction between the dissolved and suspended solid phases. This is achieved by filtration through a 0.4 μm Nucleopore polycarbonate filter. The heavy metals in the soluble and insoluble phases are then chemically extracted into different fractions depending on their relative ease of release. The fractions have been chosen to enable comparisons with the relative bioavailability of the metals.

The dissolved phase is separated into fractions depending on the complexation strength of the heavy metal. The free ions and weakly complexed metals are determined directly using Differential Pulse Anodic Stripping Voltammetry (DPASV) and are termed the Electrochemically Available fraction. Those heavy metals which are more strongly complexed are removed.
Polluted Water sample

FILTRATION
0.4 μm

DISSOLVED PHASE

SUSPENDED SOLID PHASE

ELECTRO-CHEMICALLY AVAILABLE FRACTION

CHELEX-100 REMOVEABLE FRACTION

STRONGLY BOUND FRACTION

EXCHANGEABLE FRACTION

CARBONATE AND HYDROUS METAL OXIDE FRACTION

ORGANIC FRACTION

FIGURE 8.1: A Speciation Scheme for the Analysis of Heavy Metals in Polluted Waters.
using a Chelex Batch method. Both the Electrochemically Available and Chelex-100 Removeable fractions may be biologically available. The remaining Total Dissolved fraction, measured after acid digestion, is probably largely inert to organisms.

The suspended solid phase is divided into fractions according to the ease of metal release. Heavy metals which exchange with MgCl₂ may be released from the suspended solid phase under normal pH conditions in stormwater. Reducing conditions, such as those found in anoxic sediments e.g. in gullypots, are necessary to release the metals associated with carbonates or hydrous metal oxides. Total "organically bound" metal is probably largely unavailable to organisms.

8.2 Chemical Treatment of the Samples

All equipment is pre-soaked in 10% HNO₃ for 24 hours and washed with Milli Q Double Distilled Water (M.D.D.). The dissolved and suspended solid phases in stormwater are separated by filtration through a 0.4 µm Nucleopore polycarbonate filter, using a polyethylene Millipore filtration system. Only water pressure is applied to prevent algal or bacterial cell breakage. The suspended solid phase is air dried (50°C) to constant weight and stored in a dessicator.

Dissolved organic carbon levels and pH are routinely monitored. Particulate organic carbon, complexation capacity, chloride concentration and conductivity are also measured to provide useful background data.

The dissolved phase is separated into the three fractions outlined below:

(i) Electrochemically available. An aliquot (10 cm³) of the dissolved phase is determined directly for heavy metals by Differential Pulse Anodic Stripping Voltammetry. 2M sodium
acetate (0.1 ml) which acts as a buffer and support electrolyte, at the pH of the natural sample is added before analysis. The analytical conditions are as follows:

- Plating time = 180 seconds
- Equilibration time = 30 seconds
- Deoxygenation period = 240 seconds
- Initial Potential = -1.3V, -0.9V or -0.3V.
- Scan rate = 2mV/sec

ii) Chelex-100 Removeable. An aliquot (50 ml) of the dissolved phase is added to prepared Calcium Chelex-100 (500 mg) and equilibrated by shaking continuously for 24 hours. The heavy metals are then eluted off the Chelex resin with 1M HNO₃ (10 ml) and washed with M.D.D. water (10 ml). After making up to 25 ml the metal levels in the eluate (7 ml) are determined after the addition of 2M sodium acetate (3 ml) by Differential Pulse Anodic Stripping Voltammetry.

iii) Total metal. The strongly bound metals are released by acid oxidation. Concentrated nitric acid (0.18 ml) and concentrated perchloric acid (0.02 ml) are added directly to an aliquot (25 ml) of the dissolved phase. After evaporation to dryness in a fume cupboard the metals are taken up in 1M HNO₃ (10 ml) and determined in the same way as the previous fraction.

The suspended solid phase is separated into three different fractions according to the ease of release of the metals. The first two digestions are carried out in polyethylene tubes in a water bath at controlled temperatures. The final digestion is carried out in a small glass beaker (50 ml).

(i) Exchangeable heavy metals. 1M MgCl₂ at pH 7.0 (5 ml) is added to the dried suspended solid phase and agitated at room temperature for 1 hour. The supernatant liquid is separated by centrifugation at 5000 rpm for 45 minutes and again after washing with M.D.D. water (5 ml). The two sus-
ernatants are combined and an aliquot (9ml) determined by DPASV, after the addition of 2M sodium acetate (1ml).

ii) Carbonate and hydrous metal oxide associated heavy metals.

The suspended solids from (i) above are digested with 0.04M NH₂OH·HCl in 25% glacial acetic acid (10 ml) at 96°C with occasional agitation for 5 hours. The solids are washed with M.D.D. water (10 ml) as before. An aliquot of the combined supernatants (7 ml) is determined by Differential Pulse Anodic Stripping Voltammetry after the addition of 2M sodium acetate (3ml).

iii) Total heavy metal. These strongly bound metals are released by digestion with nitric and perchloric acids (9:1). Concentrated nitric acid (18 ml) and concentrated perchloric acid (2 ml) are added directly to the suspended solid phase from (ii). The mixture is evaporated to dryness in a fume cupboard, then taken up in 1M HNO₃ (10 ml) and the supernatants combined. Heavy metals are determined in the same way as the previous fractions.

8.3 Problems Encountered in the Determinations.

Obtaining low and consistent blank values is difficult at the trace level. Low blank values, about 0.1 µg/l, can be obtained in a fairly clean laboratory and with rapid analysis preventing air contamination. Materials coming into contact with the sample can be rendered metal free by acid washing (10% HNO₃) for at least 24 hours, followed by rinsing with M.D.D. water. All materials coming into contact with the sample should be polyethylene or polycarbonate, as other materials may contaminate the sample (e.g. rubber) or adsorb metals from the sample (e.g. glass). Amber glass ground stoppered bottles are used for storing samples due to be analysed for organic carbon. These vessels are prepared by soaking in chromic acid overnight, followed by thorough rinsing with M.D.D. water.
Intermetallic compounds interfere during sample analysis by Differential Pulse Anodic Stripping Voltammetry. The copper peak shows an increase in height in the presence of zinc. It is therefore preferable to analyse copper and zinc separately. In freshwater samples some interference is seen in the voltammogram for the Electrochemically available fraction, particularly between -0.5V and -0.2V. However, the lead and cadmium peaks are usually clearly visible and are confirmed by the standard addition method.

Some care needs to be taken when carrying out standard additions due to possible weak complexation of the added metal by organic ligands in the sample. This effect reduces the gradient of the analysis slope and so leads to elevated results. This can be prevented by utilising at least three standard additions in the analysis.


GETSY, J.P. LEVERSEE, G.J. and WILLIAMS, D.R. (1977). "Effects of Naturally Occurring Aquatic Organic Fractions on Cd Toxicity to Siomocephalus serrulatus (Daphinidae) and Gambusia affinis (Poeciliidae)." Water Res., 11,


