CHALMERS
tekniska högskola · Göteborg

Institutionen för
vattenförsörjnings- och avloppsteknik

Middlesex Polytechnic RESEARCH & CONSULTANCY

URBAN STORMWATER POLLUTION

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

Gregory M P Morrison

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LIST OF ILLUSTRATIONS

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	Research Assistants/Students	
Mr J B Ellis	Mr I Davies	)
Dr R Hamilton	Mrs A Gavens	) Part-time
	Mrs M A House	)
Mr D Jarrett	Ms H Roberts	SERC
Dr D M Revitt	Mr G Morrison	NERC
Mr B S Shutes	Mr P Beckwith	SERC
	Mr R Warren	SERC
	Mr O Harrop	NERC

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:

Hydrocarbons in Urban Sediments. D M Revitt, A Gavens, J B Ellis, Science Research Council. 1978-82.

Water Quality Indices in Urban Catchment Management. M A House, J B Ellis. Natural Environment Research Council, 1979-82.

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

Urban stormwater sediments and their response to hydraulic flow conditions in separate sewer systems. H Roberts, J B Ellis, R Hamilton. Science Research Council, 1980-83.

Rainfall-Runoff and water quality of highway surfaces.
J B Ellis, O Harrop. Natural Environment Research Council
and the Institute of Hydrology, Wallingford, 1980-84.

Heavy metal speciation in urban sediments. D M Revitt,,
J B Ellis, G Morrison. Natural Environment Research
Council and Swedish National Environmental Protection
Board. In collaboration with Urban Geohydrology Institute,
Chalmers University of Technology, Gothenburg, Sweden.
1982-1985.

Continuous, self-calibrating water quality monitoring.
J B Ellis, G S Morris. Water Research Centre, 1977-80.

Sampling strategies for stormwater quality modelling.
J B Ellis, Nuffield Foundation, 1976-80.

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.

Heavy Metals in street surface dusts. R Hamilton,
D M Revitt, R Warren. Greater London Council. 1981-84.

4. Research Reports in the current series include :

  No.1   Review, Objectives and Preliminary Considerations.
          J B Ellis. December 1976.

  No.2   Urban Stormwater: Macroinvertebrate Biology and
          Bacteriology. B S Shutes, J B Ellis, November 1977.

  No.3   Urban Stormwater: Water Quality Baseline Data.
          J B Ellis. December 1978.

  No.4   Water Quality Indices: A Management Tool.
          M A House. June 1980.

  No.5   The Selection of Determinands for Water Quality
          Classification. M A House. April 1981.

  No.6   Stormwater Pollution from Highway Surfaces:
          A Review. O Harrop. February 1983.

  No.7   Instrumentation and Method in Stormwater
          Monitoring. O Harrop (In Press).

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

FÖRORD

Samarbete mellan Middlesex Polytechnic, Centre for Urban Storm-
water 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",

Avsikten med projektet är att bestämma i vilken form tungmetal-
ler förekommer i dagvatten för att därigenom få bättre underlag
för att bedöma dagvattnets recipientpåverkan. I projektet kommer
dagvatten från avrinningsområden både i London och Göteborg att
undersökas, varför ungefär halva projekttiden kommer att för-
läggas till Göteborg och resten i London. Projektet skall vara
slutfört under 1985. Projeketet drivs som ett doktorandprojekt.
Den doktorand som valts för projektet är Gregory M P Morrison
författare till denna skrift.

Peter Balmér   Gilbert Svensson
professor   projektledare

ACKNOWLEDGEMENTS

SUMMARY

The aim of this report is to discuss physico-chemical
speciation techniques which allow the separation of bio-
available 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, exchange-
able,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 metallkoncentrationen 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 komplex-
bindningar 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, oxida-
tion med koncentrerade syror, UV-strålning eller med ozon.
Fysikalisk separering, baserad på storlek eller molekylvikt kan
utföras genom centrifugering, dialys, filtrering eller ultra-
filtrering.

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 asso-
cierade 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 titreringskurvan.

Baserat på tidigare arbeten föreslås ett specieringsschema för rutinanalys av dagvatten och liknande förorenade vatten. Den lösta fasen separeras i tre fraktioner: elektrokemiskt tillgänglig, Chelex-utbytbar och kolloidal. Koncentrationen av metall i varje fraktion beror på komplexbindningar. Den suspenderade fasen separeras också i tre fraktioner: utbytbar, karbonat och metallhydroxid samt organisk. Metallnivån i dessa fraktioner avgör hur lätt metaller frigörs till den lösta fasen. Fördelar och problem med denna metod diskuteras närmare i rapporten.

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. In-creased 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 antecedant 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 accumulat-ions. 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 concen-
tration for the catchment which he considers explains the lack
of first flush noted in some storm events. From these obser-
vations 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 heterogenous 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

Phase	Species Present	Diameter range (and MWt.)
Dissolved	Inorganically complexed metals.	
	Metals complexed with small organic molecules.	0-0.8 nm. (0-150)
	Free metal ions.	
Colloidal	Metals associated with Organic/Iron oxide colloids	0.8-400nm. $(150-10^7)$
	Metals complexed with organic fibrils.	
Suspended Solids and Particulates	Carbonate bound metals.	
	Metals adsorbed onto or bound to iron and manganese hydrous oxides.	> 400 nm. $(>10^7)$
	Organically complexed metal species.	
	Metals adsorbed onto, or contained within the lattice of, clays, sands and silts.	

Figure 1.1:   The Size Spectrum and Associations of
Metal Species in Natural Waters.
(From Stumm and Brauner 1975, Steinnes
1983).

SOLUTION

SUSPENDED
SOLID

LIVING
CELL

K^{ML}

ML ⇌ ML

K'_{ML}

K^M K^θ

L^{Z-} ⇌ M^{Z+} ← L^{Z-} → ML

K^L

M^{Z-} ⇌ L^{Z-}
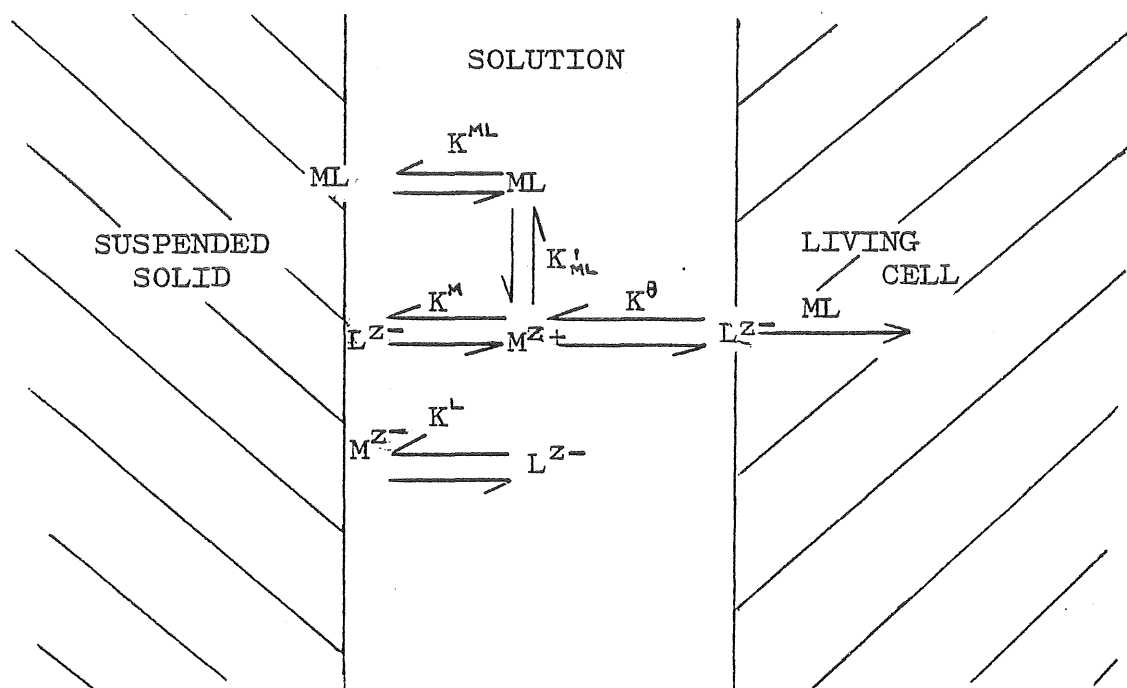
M	=	Metal
L	=	Ligand
K	=	Dissociation Constant
K^{ML}	=	Adsorption of ML on solid
K^L	=	Adsorption of L on solid
K^M	=	Adsorption of M on solid
K^θ	=	Adsorption of M on cell Membrane
K'_{ML}	=	Stability constant of M and L in solution.

FIGURE 1.2: The most relevant reactions of a
Toxic metal in a Water Sample
(After Buffle 1981). Diffusion
Rates are not included.

determination of toxic or bioavailable metals. An exact sep-
aration 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 screeening of waste-
waters 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, popul-
ation 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 experiment-
ally 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, Scendesmus
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 partic- ularly 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 Hyallela 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 :

$$[MA] = \frac{K[Maq^{2+}][A_{tot}]}{1 + K[Maq^{2+}]}$$

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 c opper are
particularly toxic because their respective acid dissociation
constants as bound water molecules are similar to the physiol-
ogical 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 deter-
mination 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 electro-deposition 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 :

Deposition (plating) step

$$ML \underset{k_b}{\overset{k_f}{\rightleftharpoons}} M^{2+} + L^{2-}$$

$$M^{2+} + Hg + 2e^- \rightleftharpoons M\,(Hg)$$
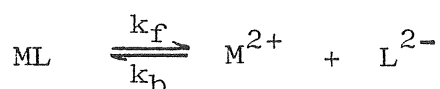
(1) ML = Species of interest.

(2) M^{2+} = Aquo metal ion
     L^{2-} = Free ligand

Stripping step

$$M\ (Hg) \underset{k_b}{\overset{k_f}{\rightleftharpoons}} M^{2+} + Hg + 2e^-$$

(3) k_f, k_b = Rate constants
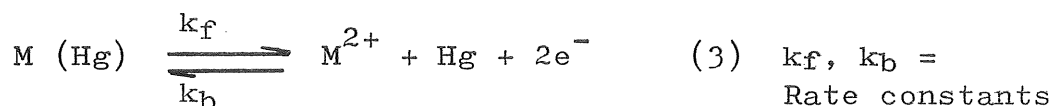
The contribution of a metal complex species (ML) to the ASV peak height or current relative to that of the free metal ion, depends on the rate constant for the dissociation of the complex (k_f in equation (1)) and the thickness of the diffusion layer. Diffusion layer thickness depends on the stirring 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 measurments 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 N_2/CO_2 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 KNO_3 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 electro-deposition 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.

DIFFUSION LAYER

∂

(a)

ION SELECTIVE ELECTRODE

BULK SOLUTION

ION SELECTIVE ELECTRODE

ZERO METAL FLUX

ZERO METAL FLUX

Surface

Free metal activity)

DIFFUSION LAYER

∂

(b) MODEL CELL

BULK SOLUTION

ASV ELECTRODE

METAL FLUX DEPENDING ON ANALYSIS CONDITIONS

METAL FLUX

Surface

Free metal concentration depending on ASV conditions.)

DIFFUSION LAYER

∂

(c) MODEL CELL

BULK SOLUTION

MODEL CELL

METAL FLUX

BIOLOGICAL ASSIMILATION

Surface

Surface sites occupied by metal)

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 ($Cd = 0.1 \ \mu g l^{-1}$, $Pb = 1.6 \ \mu g \ l^{-1}$) 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.

CHAPTER 3

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

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 bio-
available 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 1982.b). 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

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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 ($1\frac{1}{2}$ to 3 nm). The stability of imino-
acetate groups - log K $\cong$ 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 trans-
port.

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 in-
complete 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 assoc-
iated 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 ($-CH_2OCO-CH_2SH$) 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 introduced into quartz tubes and after the addition of a few drops of 30% H_2O_2, 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 HNO_3 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 adsorption, 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

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 HNO_3
(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):

$$r^2 = \frac{9\eta \ln (x_2/x_1)}{2(\rho - \rho_o)\omega^2 t}$$

t = centrifugation time

x_1, x_2 = initial and final distances of the particles from the axis of rotation.

ρ, ρ_o = densities of respectively the particle and the dispersing medium.

η = viscosity of the dispersing medium.

ω = 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 ultra-filters. 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

formulated.

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 colloidally 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 pre-dominantly 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

```
                    Water
                    Sample
                      |
                  0.45 μm
                  filtration
                      |
        ┌─────────────┘
        |
   Particulate
                      |
                   Soluble
                      |
        ┌─────────────┴──────────────────────────┐
        |                                         |
    ASV labile                                 Bound
        |                                   (ASV labile
                                             - total)
  ┌─────┼──────┬──────────┐                       |
  |     |      |          |            ┌─────┬────┴────┬──────┐
pH 4.8  Chelex  U.V.    U.V.           |     |         |      |
ASV     labile  irradiated  irradiated pH 4.8  Chelex  U.V.   U.V.
labile          + Chelex   Bound       labile  irrad-  irrad-
                labile                          iated   iated
                                                        + Chelex
                                                        labile
```
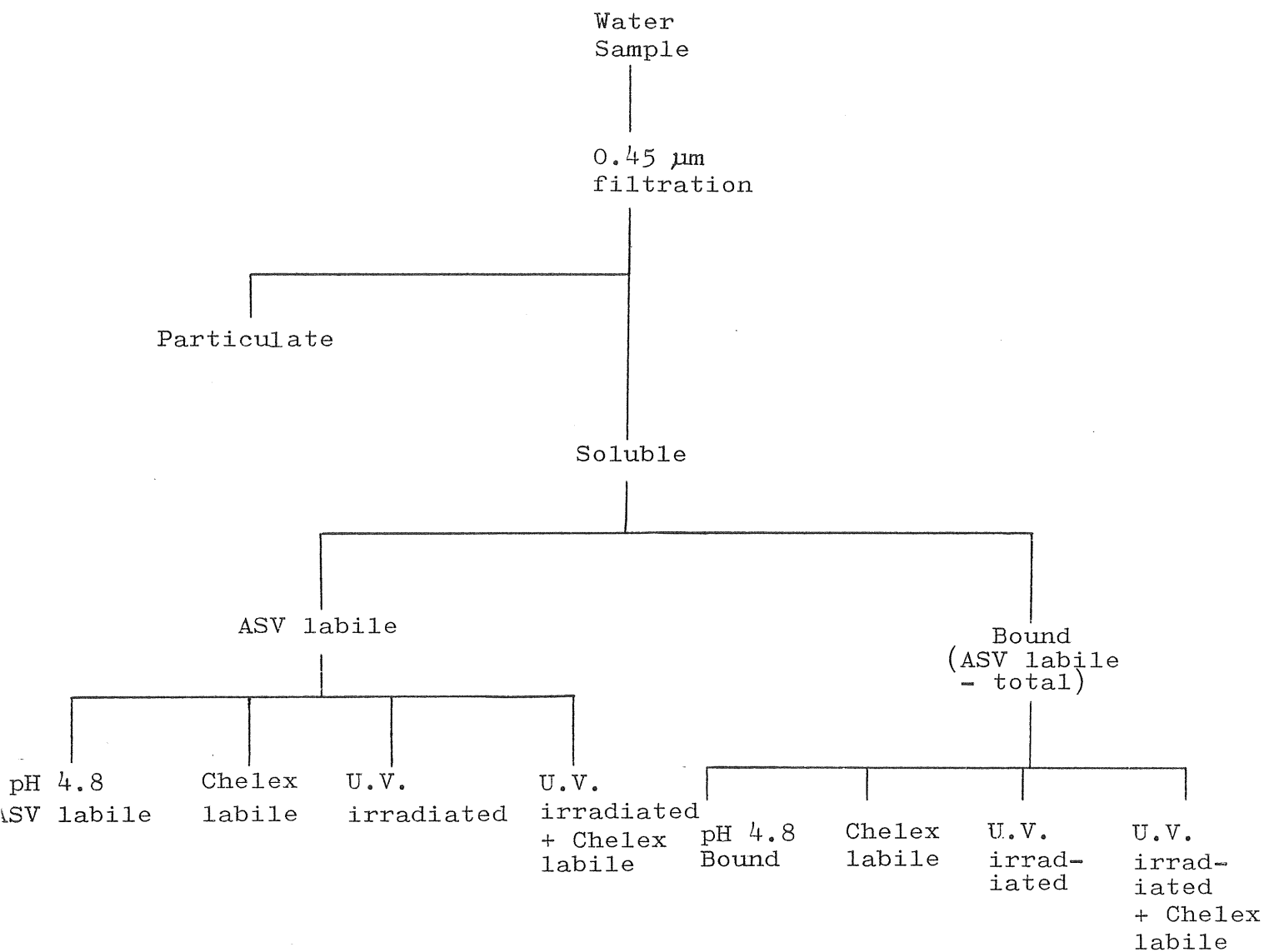
FIG. 4.1:   Speciation Scheme of Batley and Florence (1976b)

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 complexs 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. Assoc-
iations 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 to-
gether 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 ultra-
filtration 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.

- 32 -
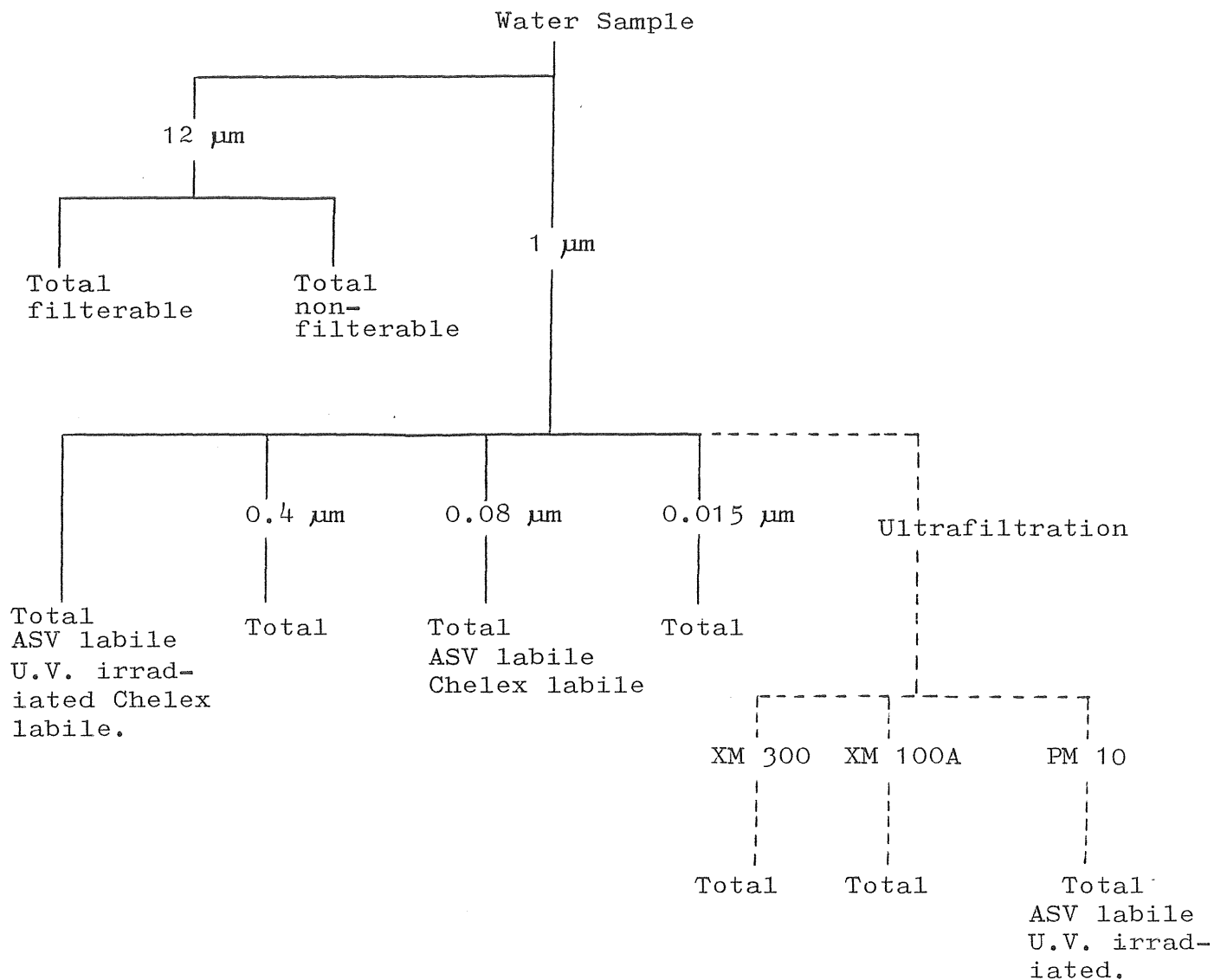
FIG. 4.2 : The Physical Separation Scheme of
Laxen and Harrison (1981a).

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 $< 1 \mu m$ fraction and the total metal of the $< 0.015 \mu m$ 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 $\mu g/L$. $CuCO_3$ 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 different-
iation 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 satis-
factory 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 est-
uarine 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 collids, 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.

CHAPTER 5

THE SEPARATION OF HEAVY METAL
FRACTIONS IN SUSPENDED SOLIDS
AND SEDIMENTS

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 there-
fore 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 concen-
trations 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 treat-
ment 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 $MgCl_2$
(Tessier et al 1979, Eisenreich et al 1980), $BaCl_2$- triethanol-
amine (Forstner and Patchineelam 1980) and NH_4OAc (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) $\longrightarrow$ Fe (II),
Mn (IV) $\longrightarrow$ 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 $Na_4P_2O_7$
(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 HNO_3. 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)

EXTRACTANT	% ORGANIC CARBON EXTRACTED OR DIGESTED
1) 1M $MgCl_2$, pH 7.0	5.4
2) 0.04M $NH_4OH.HCl$ in Glacial Acetic Acid (25% v/v)	0.0
3) 30% H_2O_2 at pH 2.0 and 0.02M HNO_3	56.2
4) 0.1M NaOH	33.0
5) Concentrated HNO_3	55.9
6) Concentrated HNO_3 and $HClO_4$ (9:1)	92.3

TABLE 5.1: Analysis of Sequential Scheme Extractants for the Decomposition or Removal of Organic Matter in a Road Sediment.

FRACTIONS	EXTRACTION MEDIUM	INTERPRETATION
1. Soluble	H_2O	Easily soluble metals
2. Exchangeable	$0.2M$ $BaCl_2$ -- triethanolamine pH 8.1	Easily extractable fraction (e.g. exchangeable) cations.
3. Humic and fulvic acid.	$0.1N$ NaOH	Metals attached to humic and fulvic acids.
4. Carbonate	Acidic cation exchange	Metals associated with carbonates.
5. Easily reducible	$0.1N$ NH_2 OH.HCl + $0.01N$ HNO_3	Metals bound to Mn-oxides, amorphous Fe-oxides.
6. Strongly bound organic	30% H_2O_2 + $1N$ NH_4OAc	Organic residues and sulphides.
7. Moderately reducible	$1N$ NH_2OH HCl + 25% acetic acid	Hydrous Fe-oxide associated metals.
8. Residual	$HF/HClO_4$	Inorganic residues

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-lithogeneous) 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 straight forward. 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.

FRACTIONS	EXTRACTION MEDIUM	INTERPRETATION
1. Exchangeable	$MgCl_2$ (1M) pH 7.0 1 hour	Weakly adsorbed on clays, hydrous oxides, humic acids.
2. Bound to carbonates	NaOAc (1M) pH 5.0 with acetic acid. 5 hours.	Associated with sedimentary carbonates.
3. Bound to iron and manganese oxides	0.04M $NH_2OH.HCl$ in 25% (v/v) HOAc. 5 hours. 96°C. pH 2.0.	Associated with hydrous oxides as nodules, concretions, cement between particles or coatings.
4. Bound to organic matter	30% H_2O_2/0.02M HNO_3 pH 2.0. 5 hours. 85°C. Subsequently NH_4OAc	Complexed or peptized with natural organic matter. Associated with sulphides.
5. Residual	$HF/HClO_4$ (5:1)	Within the crystalline structure of primary and secondary minerals.

TABLE 5.3: Sequential Extraction Scheme for Sediments and Suspended Solids (Tessier et al 1979).

Elbe   Rhine   Seine   Magdelene   G Ruaha

ZINC

COPPER

LEAD

CADMIUM

increase in river pollution
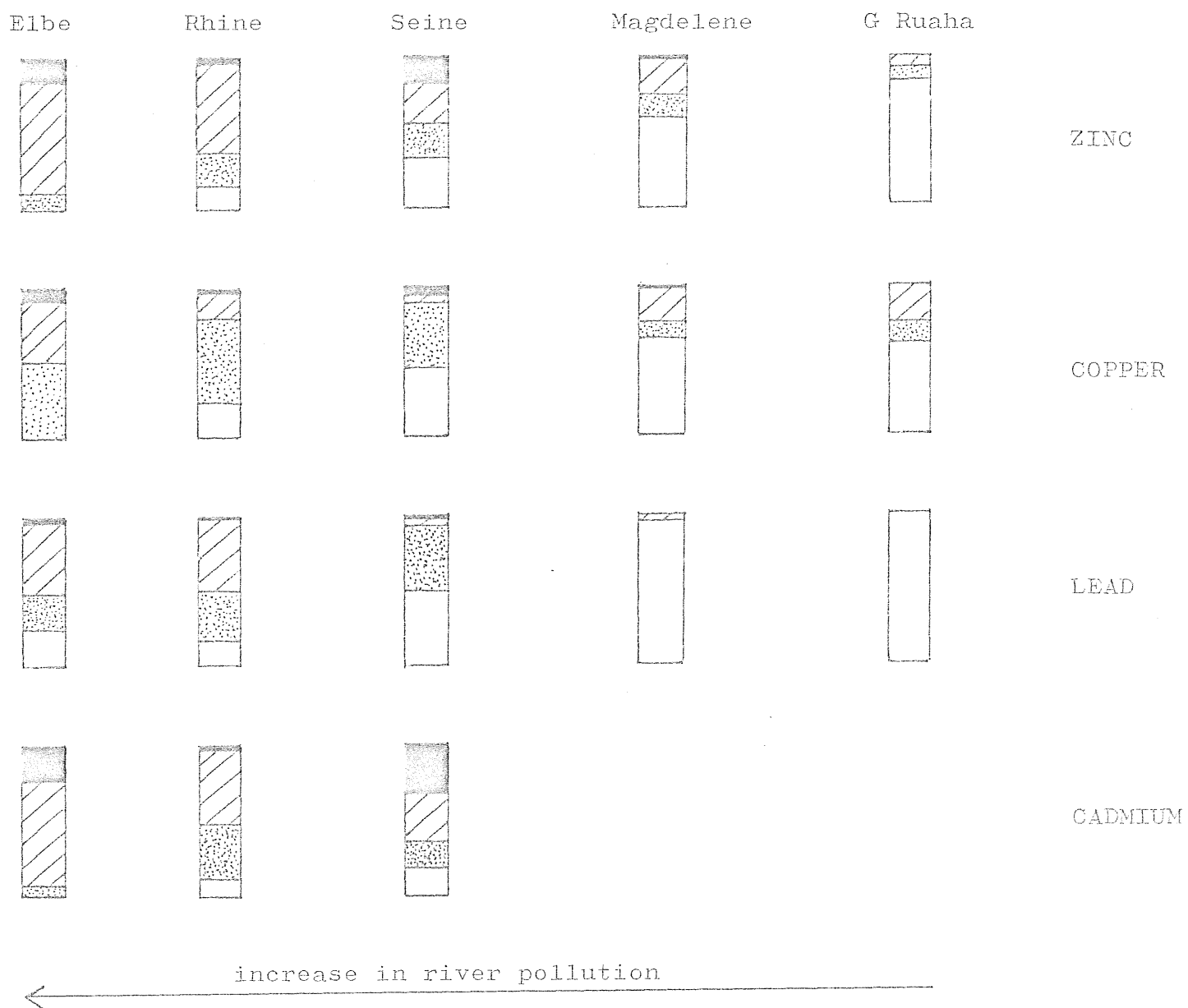
FIGURE 5.1:   Sequential Extraction Scheme applied to
              Polluted and Unpolluted River Sediments
              (from Salomons and Forstner 1980)

NH_4OAc

$NH_2OH.HCl$

H_2O_2/HCl

RESISTANT
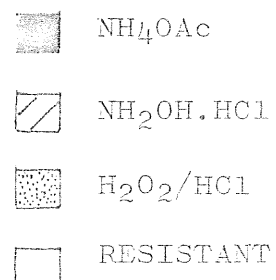
	FRACTIONS	EXTRACTION MEDIUM	INTERPRETATION
1.	Exchangeable	$0.5M$ $MgCl_2$, 7 hr.	Adsorbed, ion exchangeable
2.	Organic	$0.4N$ $Na_4P_2O_7$, 10 hr. pH 7.0	Particulate organic coatings.
3.	Bound to hydrous oxides	$0.3N$ HCl 30 min. $90^{\circ}C$	Hydrous metal oxide coatings.
4.	Residual	Conc. HNO_3/HCl/HF	Crystalline bound metals.

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 inter-
pretation. 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 exper-
iments. 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 know 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/1) 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 sea
water has compared favourable with analytical work (Millero 1974,
Whitfield, 1975).

In freshwaters two other important interactions must be taken
into account :

(i)   The adsoprtion 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

| CONTROL MECHANISM	TYPE OF PARTICULATE	EFFECTS ON HEAVY METALS
1. Surface exchange	Fe_2O_3, silica, alumina, clays.	Regulator. A small change in pH may cause significant adsorption or desorption.
2. Co-precipitation, surface exchange.	MnO_2	Removal. An effective sink unless low pH is encountered.
3. Complexation	Organic solids and coatings.	Removal, depending on complexation capacity and stability constant.

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.

$$SOH + M^{z+} \rightleftharpoons SO^- M^{z+} + H^+$$

$$SOH + M^{z+} + H_2O \rightleftharpoons SO^- MOH^{(z-1)+} + 2H^+$$
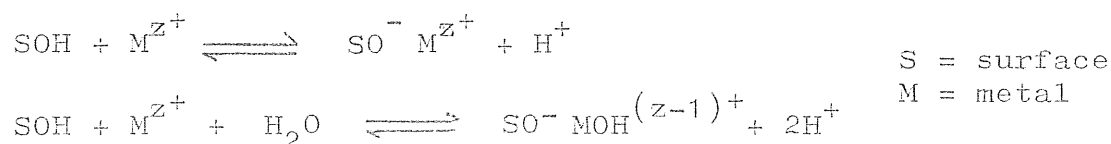
S = surface
M = metal

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 β_1^*. Log β_1^* is the hydrolysis constant
for addition of the first -OH to the metal ion,

$$M^{z+} + H_2O \rightleftharpoons M(OH)^+ + H^+$$

$$\beta_1^* = \frac{[M(OH)^+][H^+]}{[M^{z+}]}$$
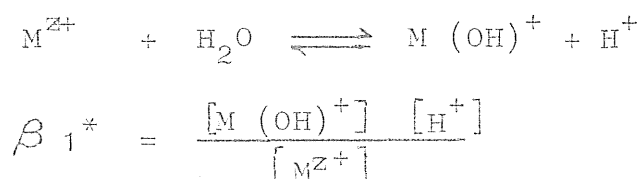
Consideration of the first hydrolysis constants (Table 6.2)
shows that the % adsorption should decrease in the order
$Cu^{2+} > Pb^{2+} > Zn^{2+} > Cd^{2+}$, at a given pH. In fact adsorption
of the metal ion begins to occur at a value of pβ_1^* - 1.5 (Gadde
and Laitinen 1974, Davis and Leckie 1978, Lion et al 1982). The
values of pβ^* - 1.5, illustrated in Table 6.2, can be compared
to the results for metal ion adsorption on hydrous iron oxides

	Cu^{2+}	Pb^{2+}	Zn^{2+}	Cd^{2+}
First Hydrolysis Constant, $p^*\beta_1$	7.0	7.5	8.5	9.5
$p^*\beta_1 - 1.5$	5.5	6.0	7.0	8.0

TABLE 6.2: Hydrolysis Constants for Heavy Metal Ions
(from Florence and Batley 1980)

(Figure 6.1). 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 nagative 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
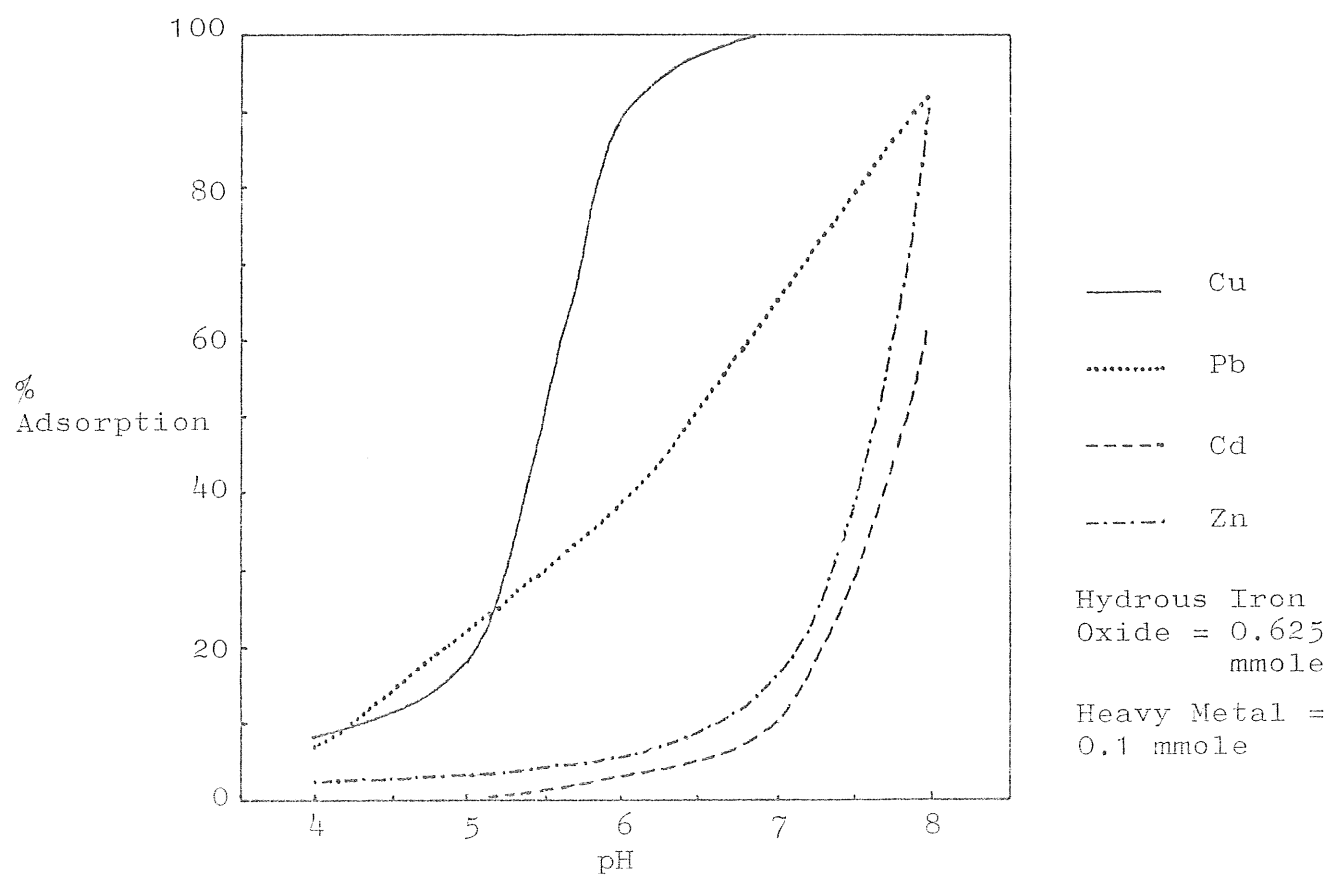
FIGURE 6.1: Uptake Experiments for Hydrous Iron
Oxides(Davis and Leckie 1978, Gadde
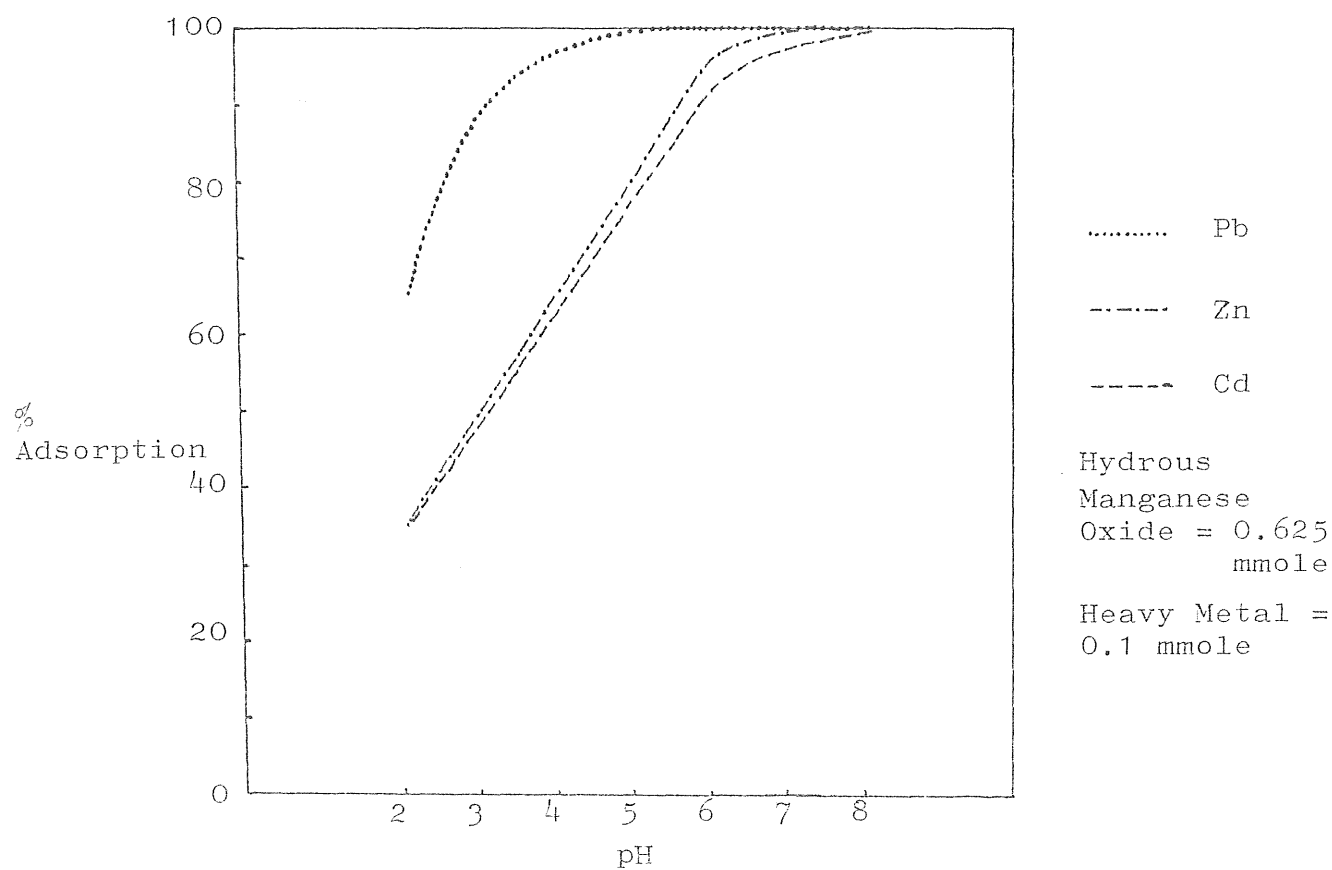and Laitinen 1974).

FIGURE 6.2:   Uptake Experiments of Hydrous
              Manganese Oxides (Gadde and Laitinen
              1974).

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 investig-
ation (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 simplific-
ation 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 distrib-
utions 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.

	log K, pH 8.0 Fe(OH)$_3$	log K, pH 8.0 Organics
Cu (II)	7.9	8.9 - 11.4
Pb (II)	9.6	-
Zn (II)	6.7	5.3 - 5.9
Cd (II)	5.7	4.7

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).

METAL	X_M (mmol.metal/ g.MnO$_2$)	X_M (mmol.metal/ g.humic acid)	Distribution coefficient for clay.
Cu	1.95	0.42	9.0
Zn	0.96	0.21	4.3
Cd	1.10	0.22	4.8

X_M = Maximum sorption values

pH = 5.0

Ionic strength = 1.0 x 10^{-3}M potassium acetate

TABLE 6.4: Sorption Characteristics of Humic Acid, MnO$_2$ and Bentonite. From Guy et al (1975).

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 ($0.1 \mu m$ - $0.45 \mu m$ 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

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

FILTRATION

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.

FLOCCULANTS
($\frac{1}{2}$ HR.)

COLLECTION OF
FLOCCULANTS BY
FILTRATION

FLOCCULANTS
(24 HRS)
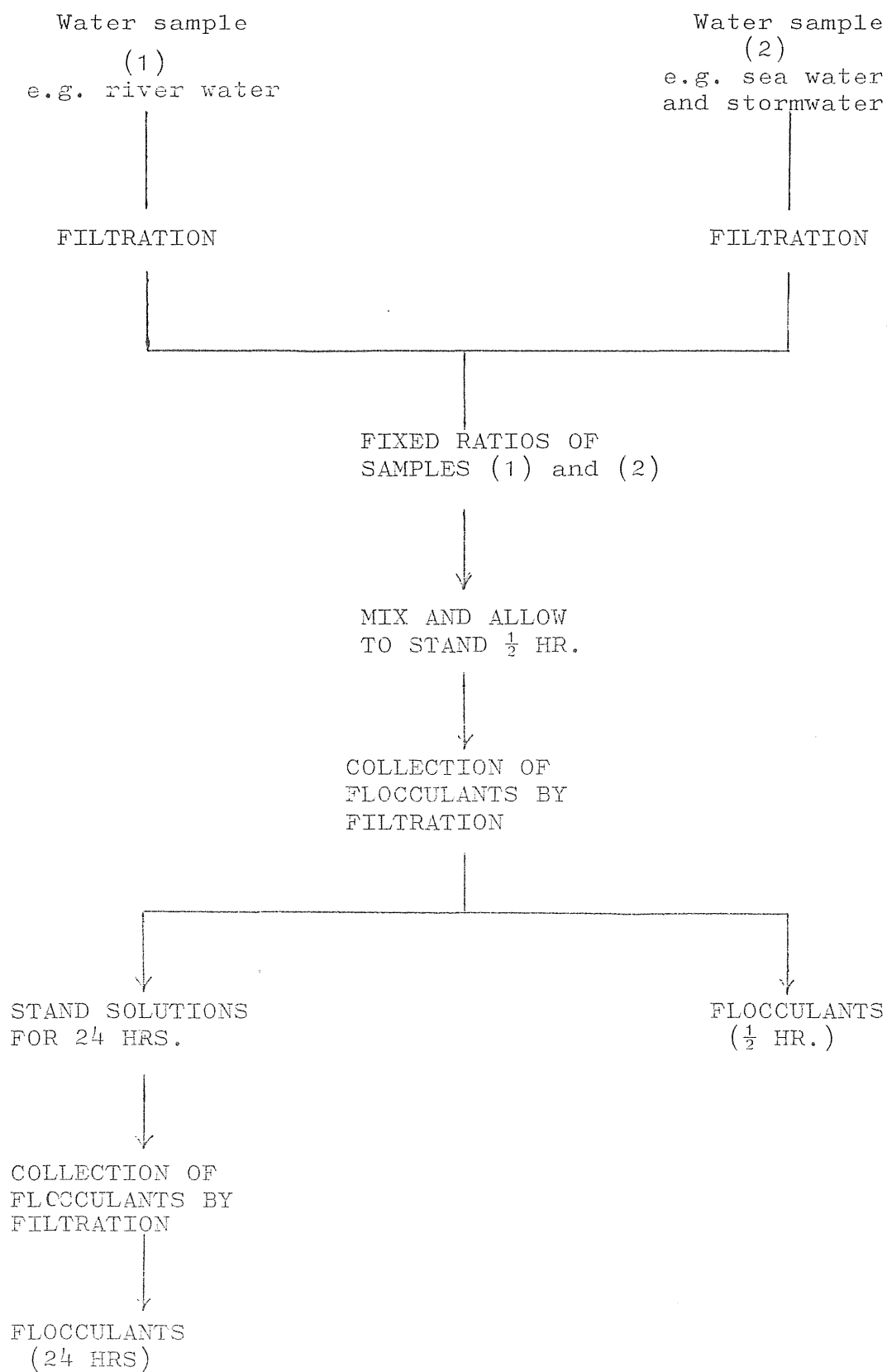
FIGURE 6.3:   Procedure for Mixing Experiments
              (from Sholkovitz 1976).

- 59 -

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.

CHAPTER 7

APPARENT HEAVY METAL COMPLEXING CAPACITIES AND CONDITIONAL STABILITY CONSTANTS IN THE AQUEOUS ENVIRONMENT.

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 determin-
ation 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 sequent-
ially, 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
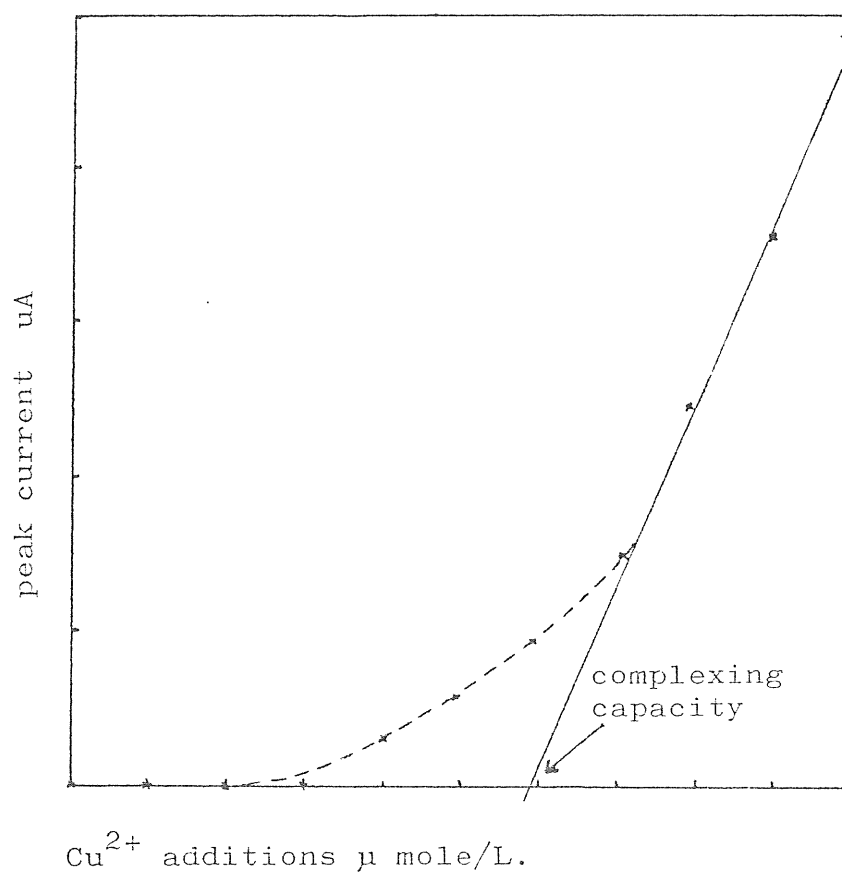the free metal ion (final slope) to the x-axis. There appears to

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 :

$$aM \; + \; bL \; \rightleftharpoons \; M_aL_b \qquad\qquad K'_{ML} = \frac{[M_aL_b]}{[M]^a \, [L]^b} \qquad\qquad (1)$$
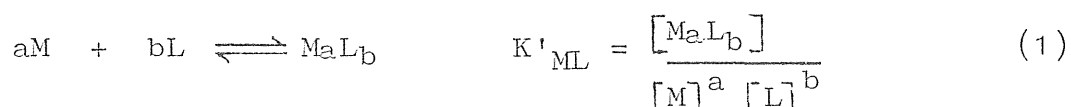
where a and b depend on the stoichiometry,

L = ligand

M = metal

K'_{ML} = 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 :

$$i_s \approx \frac{k}{(K')^{\frac{1}{a}}} \cdot \frac{(C_M/a)^{\frac{1}{a}}}{(C_L - b/a \, C_M)^{b/a}} \qquad\qquad (2)$$

where i_s/k = slope of graph after all complexing sites are filled.

C_M = Concentration of metal added

C_L = Concentration of total ligand at the intercept,

For a 1:1 complex a plot of i_s vs $\left[C_M/(C_L - C_M)\right]$ 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).

$$M + M'L \rightleftharpoons ML + M' \qquad K'_{ML} = \frac{[ML][M']}{[M][M'L]} \qquad (3)$$
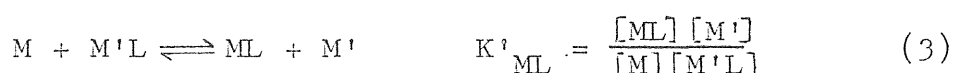
M' = another metal

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 capricomutum. 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 $\mu g/l$ 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,
MnO_2, 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.

CHAPTER 8

A SPECIATION SCHEME FOR THE ANALYSIS OF HEAVY METALS 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
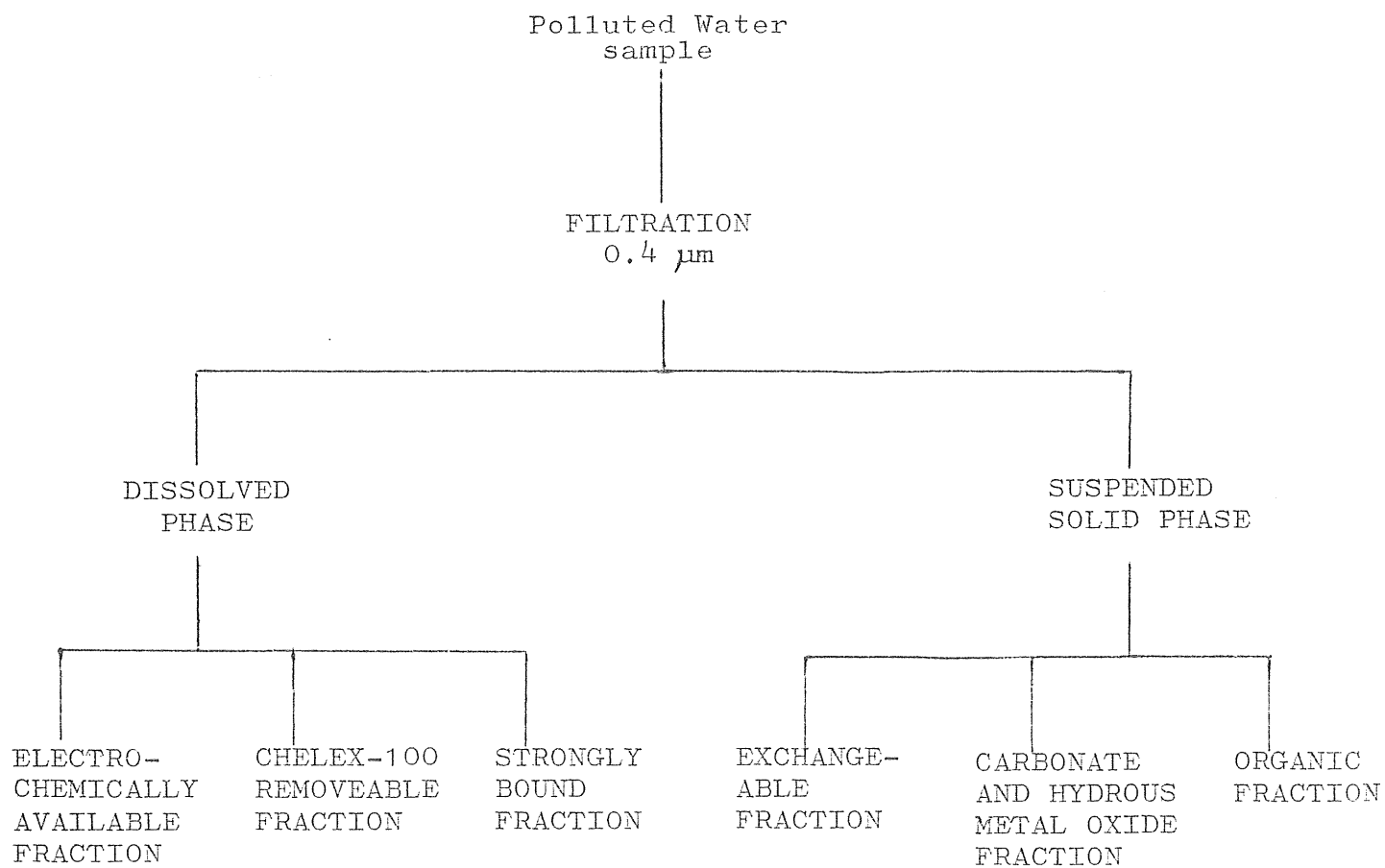
FIGURE 8.1 : A Speciation Scheme for the Analysis of
Heavy Metals in Polluted Waters.

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
$MgCl_2$ 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% HNO_3 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 $(50^{o}C)$ 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 cm^3) 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 HNO_3 (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 (3ml) 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 HNO_3 (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 $MgCl_2$ 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 su--

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 $NH_2OH.HCl$ in 25% glacial acetic acid (10 ml)
        at $96^{o}C$ 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 Volt-
        ammetry 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 HNO_3
        (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 $\mu g/l$, can be obtained in a
fairly clean laboratory and with rapid analysis preventing air con-
tamination. Materials coming into contact with the sample can
be rendered metal free by acid washing (10% HNO_3) 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 poly-
carbonate, 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.

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