

A

THESIS

entitled

*GEOCHEMICAL STUDIES ON COLLOIDAL AND
MACROMOLECULAR CONSTITUENTS IN SURFACE WATERS*

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*'The search for truth is in one way hard
and in another easy. For it is evident
that no one can master it fully or miss
it wholly. But each adds a little to
our knowledge about Nature, and from
all the facts assembled there arises
a certain grandeur.'* (Aristotle).

ABSTRACT

A qualitative and quantitative study has been made of colloidal and macromolecular constituents of surface waters in order to define their role in trace element geochemistry. Using ultrafiltration, the molecular size distribution of trace heavy metals in four rivers in the South-West of England has been defined, and with various analytical techniques it has been demonstrated that much of this colloidal material is uniform in composition. With the exception of iron, most trace metals appear in the filtrates of low molecular weight fractions and associated with small inorganic and organic species. Preliminary elemental and structural analysis has shown the dialyzates concentrated by ultrafiltration to be principally of an organic nature similar to that of the humic substances. Also associated with these non-dialyzable fractions are the common lighter elements. Many of the filterable forms of iron may occur as either a peptized colloidal species or as a macromolecular organic complex. Mössbauer spectroscopy results indicate that a broad range of similar functional groups of the non-dialyzable humic substances is occupied by iron in the Fe(III) oxidation state.

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C H A P T E R 1

1. INTRODUCTION TO COLLOIDAL AND MACROMOLECULAR CONSTITUENTS IN SURFACE WATERS

1.1 INTRODUCTION

Within any ecosystem, the organisms and their abiotic environment interact and so are interrelated. Under an aquatic regime these include surface-chemical phenomena which may determine the temporal and spatial distribution of many constituents. The effect of these interactions on the transition of elements is known to be a significant process in the accumulation and diagenesis of metals in sediments. Comparable reactions occurring within the colloiddally dispersed fractions of surface waters may play a considerable biogeochemical role, as yet poorly understood (Breger, 1968). This thesis presents a preliminary, qualitative and quantitative examination of the colloidal and polydisperse macromolecular composition of selected rivers and estuaries in the South-West of England. The work here is intended to outline and highlight the biogeochemical importance of the colloidal phase as a vehicle in the transport and distribution of major and minor elements.

Surface waters are frequently turbid and often discoloured. Those substances normally producing turbidity consist largely of clay minerals or microorganisms and span a broad size range. Substances producing discoloration, as distinct from turbidity, are considered to be either metal oxide precipitates or organic compounds with much smaller particle sizes. As there is no clear demarcation between colloidal and particulate suspensions, an intermediate pseudo or supracolloidal region of particle size may be distinguished arbitrarily. A distinction

between colloidal material and that in true solution also presents similar practical problems of definition, despite the frequent assumption that the dissolved state is well enough understood to be exempt from definition. Perhaps a true solution may be described as a completely uniform mixture in which each constituent, either charged or electrically neutral, wholly or partially solvated exhibits random motion and characteristic colligative properties. Between these extremes of dissolved and suspended states lies a wide range of particle sizes - the ill-defined colloidal and macromolecular fractions, which under favourable conditions may be retained in suspension indefinitely.

In 1951 Goetz and Tsuneishi described the use of membrane filtration in bacteriological analysis of water. The technique quickly found acceptance in the field of marine microbiology (Oppenheimer 1952) and as a microfiltration procedure in the general analysis of oceanographic samples (Goldberg, Baker and Fox, 1952; Fox, Oppenheimer and Kitteridge, 1953). The modern definition of the *dissolved* phase has arisen out of an acceptance of this traditional use of membrane filtration; the filtrate of a $0.45\mu\text{m}$ membrane is assumed to comprise all soluble species. This arbitrary but operational distinction approximates only to the classical 0.2 to $0.5\mu\text{m}$ upper size limit for dispersed colloids. The term soluble, as used by practising water chemists, frequently embraces a colloidal fraction since filterable and soluble are commonly regarded as synonymous. A conceptual redefinition of the soluble phase in natural waters proves difficult to apply in practice. Lammers (1971) has suggested a definition of the insoluble material in which the size range, from 0.01 to $200\mu\text{m}$, consists of combined colloidal and suspended particulate.

fractions. Particles smaller than $0.01\mu\text{m}$ are classified more realistically as dissolved since they exhibit colligative effects and dialyse through a semi-permeable membrane. Similar concepts have found acceptance elsewhere, particularly in the treatment of wastes and the maintenance of water quality (Cohen and Hannah, 1971; Rickert and Hunter, 1972). Nevertheless, such a distinction is of limited validity since macromolecular, organic compounds of colloidal dimensions do occur which are nondialysable but have colligative properties in solution. Perhaps the most practical size categories are those defined by Rickert and Hunter (1972):

<u>FRACTION</u>	<u>IDEAL SIZE LIMITS (μm)</u>
Particulate	>100
Supra-colloidal	$1 - 100$
Colloidal	$0.001 - 1$
Soluble	<0.001

The ratio of surface area to mass and to volume for particles of colloidal dimensions is of particular significance. For colloidal dispersions the ratio of *surface area to mass* is very high ranging from 24 to greater than 1000 and the cumulative effect of for example surface charge and ionogenic groups is sufficient to maintain the metastable condition by the counteraction of gravitational forces. Within this fraction the surface adsorption capacity is of considerable importance since such material will exhibit many properties consistent with surface chemical phenomena and an equilibrium with a heterogeneous continuous phase (Packham, 1964).

1.2 THE NATURE AND ORIGIN OF COLLOIDAL DISPERSIONS IN NATURAL WATERS

Several relatively homogeneous and related size groups may be found within the colloidal range such as bacteria and bacteriophages. Sols of pollutant and natural origin may be distinguished according to composition as either inorganic minerals or organic, carbonaceous material. Also, bacteria of less than $0.45\mu\text{m}$ have been isolated from marine samples (Anderson and Heffernan, 1965) constituting a viable, colloidal fraction of biogenic origin, which clearly includes viruses and phages as well as flagella and general cellular debris. Such material can be a significant colloidal constituent in a river subjected to sewage and trade effluent discharges. Of more general importance is the appreciable quantity of, as yet, un-resolved colloids and polymers found in rural drainage. These complex organic compounds arise from the decay of vegetation.

Colloidal and macromolecular dispersions are conveniently classified on the basis of composition and origin:

- I. Inorganic dispersions - metal oxides, hydroxides, silica and clays
- II. Autochthonous organic dispersions - biogenesis in situ
- III. Allochthonous organic dispersions - extraneous biogenic origin

This differentiation is artificial since it is unlikely that any or all of these groups behave as an independent entity within a natural aquatic regime.

1.2.1 Inorganic Colloidal Dispersions

These include metal oxide and hydroxide precipitates as well as some of the more friable clays and minerals such as bentonite, which are the finely divided products of erosion. In most natural waters, clays and clay-mineral colloids are aggregated into the larger supra - colloidal sizes with the influence of electrolytes so that physically, the floccules formed behave as suspended particles in a non-colloidal dispersion. Nevertheless formation of emulsoid colloids with hydrophilic organic polymers results in the establishment of a protected dispersion. Hood (1967) and Fotiyev (1968) have suggested that silica may be maintained as a discreet disperse phase by such a process. Similar mechanisms have also been postulated to account for the apparent supersaturation of iron in natural waters (Shapiro, 1964).

1.2.2 Autochthonous Organic, Colloidal Dispersions

Organic colloids of autochthonous origin in surface waters are generated by microorganisms within the water body. Certain species of marine bacteria of the genus Spirillum have been found to occur in a conventional membrane filtrate (Anderson and Heffernan, 1965). However, bacteria are in general regarded as having supra-colloidal dimensions i.e. $>0.1\mu\text{m}$ (Cohen and Hannah, 1971). Other viable biogenic material includes viruses (Thayer and Sproul, 1966), which exhibit many of the physical properties of colloids, and may assume a net negative charge within the pH range 3.5 to 5.9 with concomitant electrophoretic mobility. Virus-like particles have

recently been isolated from a number of bivalve species, a matter of considerable importance in Public Health which suggests a mechanism of trace element accumulation (Lammers, 1975). Carbohydrate-like material has also been reported to influence the physiology of oysters (Collier, Ray and Magnitzky, 1950; Collier et al, 1953).

A major source of autochthonous colloids arises from the vast yield of organic compounds secreted by living algae and generated upon bacterial degradation (Hellebust, 1965; Watt, 1966; Akiyama, 1972; Sorokin and Wyshkwarzev, 1973). The heterogeneous mixtures liberated by such processes include profoundly transformed carbohydrate and protein-like refractory polymers as well as more labile nutrient compounds. The influence of biological factors upon the fundamental physico-chemical process of adsorption is well recognized in effluent treatment. The role of bacterial exudates in coagulation and flocculation processes has received extensive investigation in their application to the maintenance of water supply quality. However, these processes in natural waters remain poorly understood. The cellular debris of secondary effluents which constitute the predominant colloidal fraction may be analogous to those in rivers under conditions of extreme dilution. A typical floccule-forming species is the bacterium genus Zooglea which has proved a convenient model for the study of extra-cellular polymer synthesis. The fibrils produced are responsible for the aggregated cell colonies so characteristic of these microorganisms. These exudates may play a significant role in the general accretion of particulate material. The isolated zooglear matrix of such bacterial

colonies has a marked affinity for transition metals (Friedman and Dougan, 1968; Dougan, Pfister and Frea, 1970). Microbial synthesis of metal-organic complexes has also been reported in the marine environment (Jones, 1970). The physical and chemical properties of the different, biological polymers generated by a heterogeneous microflora may govern the distribution of certain elements within the conventionally defined 'soluble' phase. A large proportion of the bacteria isolated from natural waters are variants of floccule-forming genera and grow in an aggregated manner to produce massive adherent particles that do not remain in suspension. Nevertheless, certain bacterial forms are reasonably assumed to act as colloids in rivers or lakes and, as a viable entity, they may profoundly influence surface-chemical phenomena within the colloidal-macromolecular size classes (Boyle et al, 1968).

Under eutrophic conditions, the copious generation of polymeric compounds such as complex polysaccharides and polypeptides is likely, especially during the declining growth of endogenous respiration phases. *Frequently* surface drainage is oligotrophic and as such autochthonous synthesis of colloids and molecules is likely to prove insignificant under such conditions. Allochthonous organic matter is relatively abundant in rivers and hence of primary significance in organic geochemistry.

1.2.3 Allochthonous Organic Colloidal Dispersions

The bulk of the organic matter supplied to the oceans is predominately a loosely defined group of refractory compounds described

variously as yellow organic acids, aquatic humus or simply as organic colour. Annually an estimated 3.6 to 7×10^8 tons of organic carbon is discharged into the world's oceans in this form, representing less than 1 per cent of the total budget for organic carbon in the marine environment (Skopintsev, 1967). The allochthonous yield of organic species in rivers and oceans also includes complex carbohydrates, reducing sugars, organic acids, complex ethers, proteins, amines and amino acids; few of these exhibit colloidal behaviour although aquatic humic substances are an important exception (Vallentyne, 1957; Semenov et al, 1966). Terms such as tannins, lignins, humin, humus, humic acid(s), humoliminic acid(s), fulvic acid(s) and yellow organic acid(s) have all been used in reference to the organic colouring matter found in lakes and rivers. Similar compounds in the marine environment are commonly referred to as 'Gelbstoff'. This plethora of jargon has undoubtedly contributed to the uncertainty surrounding the true nature of this group of compounds. The generally accepted classification of humic substances was outlined by Packham (1964) and is derived from soil science, (Table 1.1). Achard (1786) provides an early record of these humic substances but Berzelius in 1806 was perhaps the first to describe their occurrence in rivers. Many subsequent studies have demonstrated that these compounds are complex, macromolecular and largely refractory in nature; as a group they are variously labelled as heteropolycondensates, polydisperse polyelectrolytes or polyphenolic, polycarboxylic quinoid compounds.

Table 1.1

Classification of Humic Substances

Group	Alternative Names	Practical Definition
Humus	Humin, Ulmin	Insoluble in water and sodium hydroxide
Fulvic Acid(s)	Crenic or Apocrenic Acid(s)	Soluble in sodium hydroxide and mineral acids
Hymatomelanic Acid(s)	Haematomelanic or Ulmic Acid(s)	Soluble in sodium hydroxide and alcohol. Insoluble in mineral acids.
Humic Acid(s)	-	Soluble in sodium hydroxide. insoluble in mineral acids and alcohol.

The structure of these molecules has yet to be resolved but their ecological and geochemical significance is well established (Gruner, 1922; Rashid, 1971, 1972, 1974; Rashid and King, 1971; Rashid and Leonard, 1973; Rashid, Buckley and Robertson, 1972; Shapiro, 1957, 1958, 1964, 1966; Black and Christman, 1963; Christman, 1967; Christman and Ghassemi, 1966; Ghassemi and Christman, 1968).

Humic substances are polydisperse in natural waters (pH 5 to 8) with a broad range of molecular weight from less than 800 to as high as 200000. Clearly, this group of heterogeneous polymers spans the size classes defined earlier, permitting their description as either colloidal dispersion and nondialyzable or a true solution with colligative properties (Hansen and Schnitzer, 1969; Shapiro, 1964). It is however, convenient to differentiate the humic substances on the basis of size and apparent molecular weight. Black and Christman (1963)

demonstrated that most of the organic colouration in lakes is removed by ultrafiltration, of which 90% was found to lie in the 3.5 to 10nm size range. In contrast, Shapiro (1964) found humic - iron complexes to fall into a larger size class from 0.10 to 0.22 μm . The discrepancy in these observations may be explained by the variable and pH dependent association and dissociation of humic molecules (Wershaw and Pinkney, 1971; Gjessing, 1971).

1.3 THE ORIGIN OF HUMIC SUBSTANCES

It is commonly assumed that the humic substances in surface drainage are leached from soil humus where they are generated by the bacterial synthesis of organic acids from lignin residues (Haider and Martin, 1967; Matschke, 1971). This process may even continue within a stream (Peterson and Cummins, 1974). Organic colouration is also thought to originate from algal metabolism (Cristofalo, Kornreich and Ronkin, 1962; Yentsch and Reichert, 1962) and through the transformation of metabolites and exudates (Sieburth and Jensen, 1968, 1969). Although algae do contribute in this way, their role is probably limited since high concentrations of humic substances are known to occur even under the oligotrophic conditions of swamps and bogs. Humic acid formation is said to involve the polymerization of polyphenolic moieties liberated during the decay of vegetation (Oglesby, Christman and Driver, 1967). The oxidation of phenolic groups to quinones may facilitate condensation reactions with carbohydrates and proteinaceous material to produce the complex humic substances. A similar hypothesis has been suggested for the generation of marine

'Gelbstoff' which is thought to comprise similar compounds (Sieburth and Jensen, 1969). The exact chemical identity of the reactions in these processes will undoubtedly be highly variable with different environmental conditions. Nevertheless, common group characteristics and distinctive properties of these polymeric, organic acids have been established. Comparative studies have shown that soil and lacustrine organic matter bear a close similarity (Povoledo, 1964).

The organic component of river water and the proportions of various organic species must vary greatly in relation to climate as well as anthropogenic factors. In tropical and sub-tropical conditions the rate of organic production and decomposition is high, accordingly rivers in these regions are commonly discoloured. The total decompositional activity in temperate regions may have a comparable yield but will proceed at a slower rate. As a result allochthonous humic substances may accumulate within the soils of moorland and forested areas. A seasonal liberation of colouring matter into rivers may therefore be anticipated. The erosion of soils and sedimentary rocks also provides a source of metamorphosed and partially mineralized organic matter which is found in surface drainage (Bordovsky, 1965). Only the most stable organic residues are expected to persist within rivers under such a typical regime of microbial and oxidative degradation. A process of negative selectivity may therefore be postulated to account for the abundance of humic substances in rivers. There is, perhaps, some oblique evidence for the similarity between humic substances derived from differing environments. Remarkable agreement is found in the observations of independent workers,

particularly with regard to the complexation of iron by humic acids and low nitrogen content of humic substances.

1.4 GEOCHEMICAL SIGNIFICANCE OF COLLOIDAL AND MACROMOLECULAR CONSTITUENTS OF SURFACE WATERS

Macromolecular organic acids are known to exert an influence upon the microflora and fauna of an aquatic habitat which may prove particularly significant when the ecosystem is under stress. A general biological interest in natural colloidal dispersions stems from studies of toxicity and nutrition in which the physical nature and the chemical speciation of minor elements and nutrients are thought to be critical factors (Elderfield, Thornton and Webb, 1971). Particles and molecules of colloidal dimensions may be utilized as merely an energy source or alternatively it is suggested that colloids could also act as a vehicle for certain essential growth factors. Many transition elements are co-factors of enzymes or vitamins, for example zinc is a constituent of the enzyme carbonic anhydrase while cobalt is present in vitamin B₁₂. Many metallo-organic compounds are of general physiological importance, the copper chelate haemocyanin is a common respiratory pigment and copper and manganese are essential for the synthesis of chlorophyll. Trace element toxicity and bio-availability are primary considerations in the conservation of biota subject to heavy metal pollution and chelation or complexation of these minor elements is a role commonly attributed to humic acids. Clearly competitive complexation with refractory organic ligands of this nature might prove either detrimental

or beneficial depending upon the context and the environmental conditions under consideration.

A potential to mobilize, transport and precipitate elements and minerals in natural waters has stimulated a geochemical interest in the nature of colloidal suspensions. As early as 1922 Gruner suggested that humic acids were responsible for the mobilization of silicates and the solubilization of iron. Similar views are maintained in regard to the formation of iron-rich deposits, and colloidal precipitation of metal oxides is postulated as a mechanism in the formation of manganese concretions (Breger, Zubovic and Chandler, 1972). Colloidal interactions commonly result in flocculation, whilst further reactions during precipitation such as complexation, hydrolysis or simple occlusion will result in a net removal of dissolved constituents. The potential to 'scavenge' elements in this manner emphasizes the significance of the colloidal state as a transitional phase between the true solution and the particulate suspension. The maintenance of iron and manganese in 'solution' and the general discolouration of raw source waters by organic polymers complicate water treatment processes in which coagulation is of primary importance (Hall and Packham, 1965).

The study of natural colloidal suspensions is a subject of academic interest with immediate practical application (Breger, 1968). Paradoxically, enigmatic observations in such diverse fields as biology, geochemistry and municipal water supply may find certain common answers from the study of natural colloidal phenomena. It

is not however, difficult to see why this type of research has received little attention until recently, The organic and inorganic constituents of the colloidal phase are difficult to recover and characterize and as a metastable condition, a dispersion is subject to continuing chemical reactions which may or may not establish an equilibrium. Such a system presents severe problems in sampling and analysis that are challenging to research. Nevertheless, a study of the colloidal and macromolecular fractions occurring in surface waters would seem essential to establish the validity of conventional, gross chemical analysis in current practice. It is pertinent to note that the possibility of surface chemical interactions is largely overlooked in discussions of chemical speciation which are frequently argued in terms of pure solution chemistry. It is to be hoped that a more rational interpretation of these problems taking proper account of the accumulation and degradation of organic polymers and colloids will arise from the growing awareness of their significance.

1.5 STUDY AREA AND SAMPLING PROGRAMME IN THE SOUTH-WEST OF ENGLAND

Preliminary monthly sampling was carried out at Launceston (site number 2 in fig. 1) on the Tamar River and later-on at four other selected localities which were sampled during July 1974 and in the succeeding five months. During the winter and early summer of 1975 this strategy was extended to include three other Cornish rivers: the Fowey, the Helford and the Carnon. The Helford River and the Carnon, which flows into Restronguet Creek, were included in a general geochemical survey of the area carried out during 1969-1970 (Thornton,

Watling and Darracott, 1975). Originally nineteen stations were sampled as a reconnaissance after which these were reduced to fifteen sampling sites which form the basis of this study.

The moderate to high regional rainfall together with the granite fissures of the moorland areas ensure a liberal supply of soft acid water to the region as a whole although on localized Devonian limestones the drainage may be of hard water. The soils of the South-West are rich along narrow alluvial deposits, particularly in the lower reaches of certain valleys and in the eastern region where the alluvium is extensive. The predominant Carboniferous and Devonian slates and shales yield Brown Earths of silty or clayey loam. Those soils derived from the Devonian beds are relatively free-draining but the wet clayey soils of the Carboniferous shales support only a poor pasture (Edmonds, Mckeown and Williams, 1969) and much of this land is given over to woodland. Sandy textured soils on the lower granite are cultivated locally but the high moors of this region with their characteristic peaty bogs and soils provide only rough grazing land but do also support coniferous plantations.

The upper reaches of the Tamar drain much of the Culm Measures along the Cornwall-Devon county boundary; to the south the flow skirts the Dartmoor Granite and traverses the Upper Devonian series. The Crossgate site (number 1, fig. 1) lies within the Culm Measures and receives substantial drainage from agricultural land. Three to four kilometres south of this point is the town of Launceston (2), a small community of some 5,000 inhabitants. Effluent

from an abattoir and a conventional sewage treatment plant enter the river at this point. Its course continues south through Wareham Wood to Gunnislake (3) on the Upper Devonian slates and to the east of a small granite mass. This area and much of the lower Tamar basin was a scene of extensive mining activity, principally for copper, arsenic and tungsten but also for silver (Dines, 1956). Little evidence of this past era remains today. The river flows through Blanchdown and Hatch Wood to Calstock (4) a small village situated at the upper limit of the tidal intrusion. From here the river again courses south to its confluence with the River Tavy above Saltash (5).

An organically enriched tributary of the River Fowey lies to the west of the main flow and rises on the Bodmin Granite at Dozmary Pool (6), a low depression in the peat alluvium and granite gravel. East Colliford (7) is a point on the periphery of the granite mass in an area of marginal land which supports merely subsistence farming. Further south on the middle Devonian slates is the village of St. Neot established during the past for the mining of copper, tin and lead; none of these metals were produced in large amounts (Dines, 1956). The third sampling site on this drainage at Quarry Wood (8) is situated downstream of this community. Here, the Fowey flows westwards through Hart Wood to Cutmadoc (9) and then turns south to Lostwithiel (10) on the Lower Devonian slates. This town of 2,000 people marks the upper limit of tidal influence and is an area in which successful mining trials for lead and copper were made.

The Carnon Valley drains the highly mineralized area of Gwennap-St. Day on the Mylor slates and to the east of the Carmanellis Granite. The river flows through Twelve Heads (12) and Hicks Mill to receive the outflow of the Great County Adit from surrounding mines upstream of the Bissoe site (13). This heavily polluted water is joined by a stream flowing through Perranwell (15) and is discharged into Restronguet Creek at Devoran (14).

Further south the Helford River flows eastwards to Falmouth Bay and a number of tributary streams. Constantine (16) lies to the north on the Carmanellis margin, while Trelowarren Mills (19) is to the south; the stream here drains the Devonian Gramscatho Beds and the Meneage Crush Zone. Gweek (18) is tidal and lies on the boundary of the Gramscatho and Mylor slates at the discharge of the stream from Gweek Mill (17).

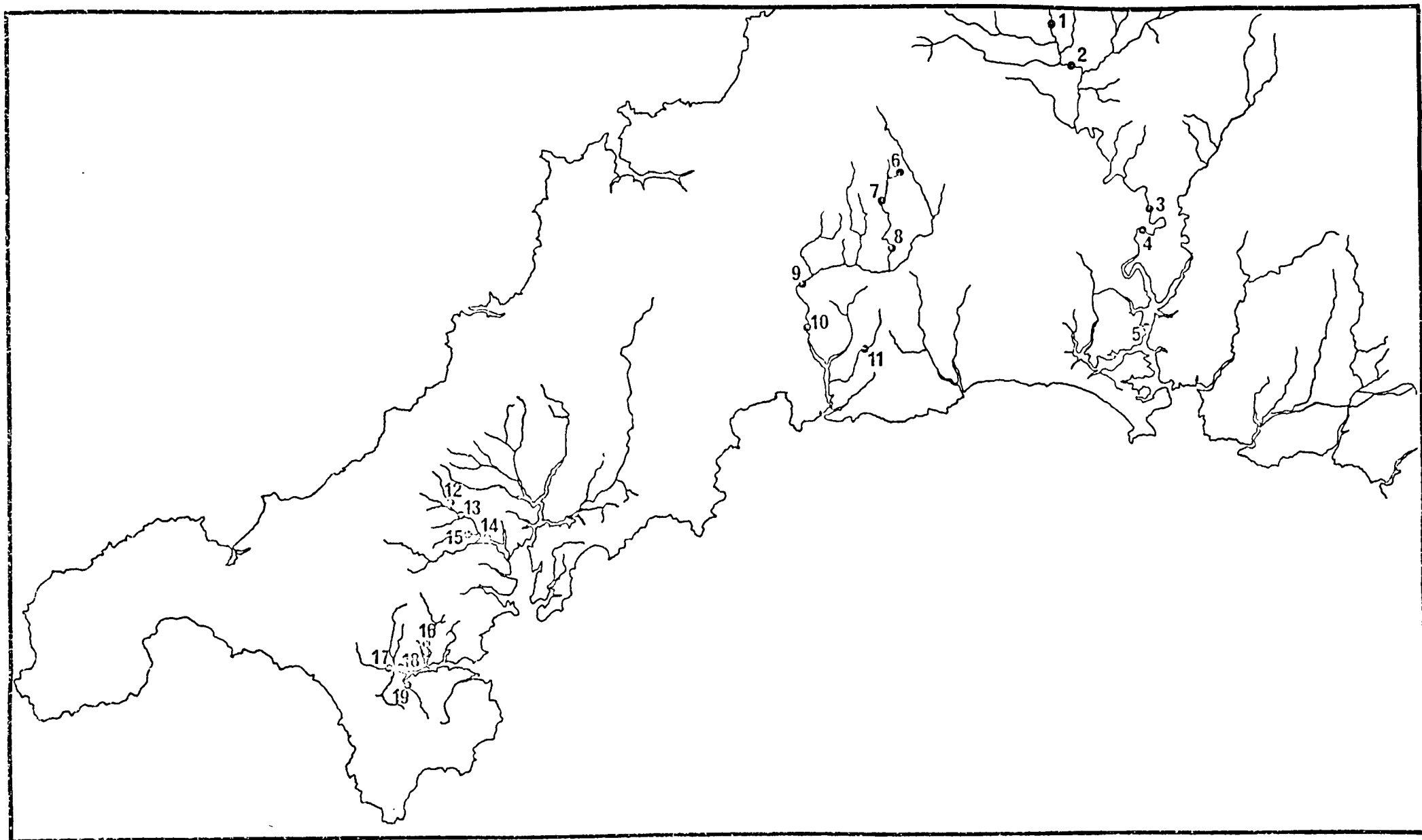


Fig. 1.1 Sampling sites in the South-West of England

C H A P T E R 2

2. CONCENTRATION AND FRACTIONATION OF 'DISSOLVED' ORGANIC COMPOUNDS IN NATURAL WATERS

2.1 INTRODUCTION

In trace organic geochemistry of saline and fresh water, the sampling and analysis of unknown and complex mixtures of compounds in dilute solution present considerable problems. A concentration procedure is a prerequisite for the analysis of the wide variety of pollutant and natural organic compounds which occur in surface waters. The methods advocated for the recovery of organic solutes generally involve chemical or physical techniques and often include a combination of several concentration and purification steps. Adsorption on activated carbon, anion exchange and macroreticular polystyrene resins, co-precipitation, solvent extraction, low temperature vacuum distillation, freeze dessication, centrifugation, dialysis and ultrafiltration are widely used methods. The choice of extraction procedure is determined largely by the nature of the analytical requirements and is crucial in any study of trace organic chemistry. Therefore, a brief review of procedures currently available is of value (Johnston, 1955; Jeffrey and Hood, 1958; Bader, Hood and Smith, 1960; Andelman and Caruso, 1971).

2.1.1 Solvent Extraction

Liquid-liquid extraction of organic material from water uses either a batch process or a continuous extraction technique with apparatus such as a Dean and Stark assembly (Goldberg, Delong and Sinclair, 1973). The efficiency of the removal is clearly dependent upon the chosen

extractant and the organic species in solution. Acetone, benzene, n-butanol, chloroform, ethyl acetate, ethyl ether, petroleum ether and phenol have been used as solvents and as general extractants in the study of such compounds as hydrocarbons, sterols and humic substances (Wilson and Armstrong, 1952; Khaylov, 1962; Shapiro, 1966; Williams, 1969; Foster and Morris, 1971; Saliot and Barbier, 1973; Barbier et al, 1973). A partial and selective recovery of dissolved organic compounds by such means is sufficient in qualitative analysis, for example humic and fulvic acids may be conveniently concentrated for this purpose with n-butanol (Shapiro, 1964, 1966a, 1966b). A variety of compounds may be isolated using several solvents either sequentially or mixed; humic acid fractions have also been obtained by Shapiro (1966a) with n-butanol extraction over the pH range 3.5 to 7.5. It has also been claimed that an 80% recovery of 'marine humus' can be achieved by the generation of chloroform emulsions (Khaylov, 1968).

2.1.2 Co-precipitation and Concentration by Adsorption

Co-precipitation of trace organic species, such as amino acids, has been used in the analysis of these compounds in sea water (Tatsumoto et al, 1961; Chau and Riley, 1966; Akiyama and Hanya, 1968). Recoveries with hydroxides and oxides of manganese, aluminium, copper, indium, gallium and iron as well as with calcium carbonate have been investigated, often with poor results. Also precipitation of the metal salts, for example 'lead humate', to concentrate humic acids is a method advocated by Klöcking and Mucke (1969) and by Weber and Wilson (1975). The most effective adsorbants are reported to be

iron oxides and alumina, but the reported efficiency of dissolved organic carbon (DOC) recovery varies between 21% and 80% (Williams and Zirino, 1964; Sridharan and Lee, 1972). Moed (1971) has reported good extraction of humic acids using alumina but again only 66% to 80% may be recovered; similar results have been recorded by De Haan (1972) who also used phosphate buffers as an eluant.

Polar and non-polar, organic molecules suffer a negative entropy change on hydration and dissolution from an organic phase. The magnitude of the entropy loss, which is a function of both the molecular weight and the degree of hydration, is 'conserved' by the regular orientation of polar water molecules about the solubilized compound. Adsorption from aqueous solution upon an inert surface results in a displacement of polar water from the surface and the decay of the hydration envelope about the solute. The concomitant entropy gain contributes to the Gibbs free energy of adsorption and hence, in part, promotes the dissolution.

Activated carbon is frequently used in the extraction of organic compounds from water and is a standard method of the American Public Health Association for the monitoring of municipal water supplies (Braus, Middleton and Walton, 1951; Middleton, Greenberg and Lee, 1962; Myrick and Ryckman, 1963; Robinson, O'Connor and Engelbrecht, 1967; Grigoropoulos and Smith, 1968; Hem, 1970; Buelow, Car^S_Awell and Symons, 1973; Boehm and Quinn, 1973; Kerr and Quinn, 1975). Qualitative and reproducible recovery necessitates a controlled partial dessication of the adsorbant to a constant water content. Activated carbon columns may then be purged with chloroform to yield the 'CCE fraction' followed by alcohol

and the 'CAE fraction'. Desorption from activated carbon is difficult to quantify; a large number of variables affect reproducibility but include principally volatile losses and irregularities in the initial drying stage.

Chemi-sorption and ionic binding upon adsorbants such as anion-exchange resins and alumina or activated carbon are frequently accompanied by a relatively high enthalpy of adsorption. This may account for the limited desorption and diminished recovery often experienced with such adsorbants (Mantoura and Riley, 1975). Synthetic polymers have been used often with improved recovery. Sieburth and Jensen (1968) reported 70% recoveries of 'yellow organic acids' using nylon, a polyamide, but in contrast only a 54% efficiency has been claimed by Mantoura and Riley (1975) for this method. Again the retention of certain compounds may partially involve irreversible interactions with free functional groups of the adsorbant surface. Polystyrene co-polymers in the form of macroreticular resins have, however, found a wide application for the extraction of vitamins, insecticides, surfactants, dyes and humic acids in dilute solution (Riley and Taylor, 1969; Gustafson and Paleos, 1971). Adsorption upon such a polymer matrix involves principally van der Waal-London interactions or hydrophobic bonding as evident from the low enthalpy of adsorption values which have been observed (Mantoura and Riley, 1975). The efficiency of such resins in organic extraction is attributed to this relatively low enthalpy of adsorption with many compounds; in such cases a high percentage of the total Gibbs free energy of adsorption is derived from the favourable entropy gain during dissolution. Such factors also govern the effectiveness of

other physico-chemical separation procedures. As defined by the qualitative rules of adsorption, a non-polar phase exhibits an increased affinity for the more non-polar constituents of a polar or aqueous system. In accordance with Traube's Rule, the hierarchy of adsorption favours high molecular weights, and for a homologous series of solutes, adsorption increases with molecular weight - the principle of affinity chromatography. A recovery of aqueous solutes with an organic solvent or an absorbant will exhibit a preferential extraction of macromolecular, non-polar species.

2.1.3 Physical Separation

Non-selective extraction is more readily achieved by means of dessication and distillation, although soluble compounds recovered in this manner may be denatured. The risk of organic degradation is minimised with the use of vacuum evaporation, freeze-dessication or lyophilization (Ghassemi and Christman, 1968; Shapiro, 1961, 1966a, 1967, 1969; Black and Christman, 1963; Slonim and Crawley, 1966; Slonim, 1967; Midwood and Felbeck, 1968; Leenheer and Malcolm, 1973; Wershaw and Pinckney, 1973; Hall and Lee, 1974). A critical appraisal of freeze concentration of dilute organic compounds has been made by Baker (1967a, 1967b, 1969). Qualitative recovery is dependent upon mixing rates, ionic strength and sample pH. Dissolved salts induce morphological alterations within the forming ice, resulting in a non-specific entrapment of solute rich liquor and poor recovery in the concentrate, (Baker, 1970; Kammerer and Lee, 1969). Nevertheless, the technique is convenient in the dessication and concentration of

natural organic compounds or tissues (Fotiyev, 1968, 1970; Robertson, 1972; Rubenstein et al, 1973).

2.2 FRACTIONATION OF ORGANIC MIXTURES

The identification of the principal organic compounds in natural waters requires that the heterogeneous mixtures extracted are resolved into constituent groups. The methods of selective extraction, partial elution or precipitation outlined above are subjective but commonly employed (Ishiwatari, 1968; Midwood and Felbeck, 1968; Riley and Taylor, 1969; Cooper and Harriss, 1974; Shiroya and Kumada, 1974; Stuermer and Harvey, 1974; Weber and Wilson, 1975). Preparative electrophoresis techniques, of which there are many, may provide more satisfactory fractionation methods. However, the choice of a buffered ampholyte, which is both functional and compatible with the unresolved organic extract, presents further complications in the definition of optimum operating parameters (Fotiyev, 1968; Gjessing and Gjerdahl, 1972; Leenheer and Malcolm, 1973).

Gel permeation chromatography, a means of fractionation by molecular size, has proved most effective, and is widely used in the study of polydisperse systems which arise in biochemistry and organic geochemistry (Gjessing and Lee, 1967; Gjessing, 1967, 1971, 1973; Shapiro, 1966; Christman and Ghassemi, 1966; Christman and Minear, 1971; Hall and Lee, 1974; Wershaw and Pinkney, 1973).

The limitations of gel chromatography, however, must be fully appreciated in the interpretation of difficult separations. A knowledge of the possible interactions between unidentified solutes and the gel phase

is required. The molecular separation of polyaromatic, heterocyclic and phenolic compounds achieved by occlusion within or exclusion from the gel matrix is complicated by coulombic interactions and adsorption phenomena (Posner, 1963; Lindquist, 1967). Anomalous separations of this nature have been frequently overlooked, even in recent work (Christman and Minear, 1971; Brodsky, Prochazka and Vydrova, 1970; Rashid and Prakash, 1972; Hall and Lee, 1974). These interactions can be minimized by the use of electrolytes compatible with the heterogeneous mixture under separation. Amine buffers such as tris, an amino cation buffer - 2-amino-2(hydroxymethyl)-propane-1,3-diol - have proved most effective in the fractionation of humic acids and largely nullify gel-solute interactions (Swift and Posner, 1971; Mantoura and Riley, 1975).

The polydispersity of humic and fulvic acids is a function of pH. With increasing pH, these polyelectrolytes exhibit an increasing molecular weight due to aggregation and condensation (Gjessing, 1971; Wershaw and Pinkney, 1971). Separations performed under buffered conditions upon gel beds will not necessarily reflect the molecular size classes present under ambient conditions of pH in the environment. The calibration of this type of fractionation further assumes that molecules of comparable molecular weight exhibit similar chemical structure. In the absence of a defined superstructure, inhomogeneous polymers, of which humic acids may be regarded as an example, usually occur in a randomly coiled form. Inappropriate selections of calibrants has often resulted in the assignment of high and inaccurate molecular weights to humic acid fractions obtained by gel filtration (Wershaw

and Pinkney, 1973; Determan, 1969).

In conclusion, sample preconcentration and elaborate manipulation, together with the complex interpretation of results, are the primary disadvantages of separate fractionation and concentration techniques.

2.2.1 METHODS OF COMBINED FRACTIONATION AND CONCENTRATION

Centrifugation, classical dialysis and ultrafiltration facilitate, to a limited degree, a combined extraction, purification and fractionation of macromolecular compounds in dilute solution. Dialysis membranes and centrifugation found early applications in studies of lacustrine organic matter (Krogh and Lange, 1932; Birge and Juday, 1934). Continuous ultracentrifugation and reverse osmosis now provide direct but more refined methods of organic concentration and fractionation. Lammers (1966, 1971) has outlined the theory and practice of size fractionation in natural waters, and the use of centrifugation in the study of 'soluble' organic compounds in rivers and effluents has proved successful (Lammers, 1967; Rickert and Hunter, 1971).

Membrane filtration is a versatile means of separation and purification whereby particulate and colloidal size classes can be closely defined (Anderson and Heffernan, 1965; Ogura, 1970; Reed and Reed, 1970; Sharp, 1973; Sobsey et al, 1973). Conventional cellulose acetate or cellulose nitrate membranes are widely used in water chemistry for the recovery or removal of particulate material.

Adsorption losses and contamination arising from membrane filtration are a common source of error, particularly in organic analysis (Long and Cook, 1971; Quinn and Meyer, 1971; McMahon, 1973; Wageman and Graham, 1973; Hirsbrunner and Wangersky, 1975).

Non-cellulosic ultrafiltration membranes are available which facilitate the separation of complex organic mixtures into molecular size classes. The non-denaturant concentration of dilute organic solutions, rapid dialysis, evaluation of ion-binding phenomena and the approximation of molecular weights are all readily achieved using ultrafiltration. The method has found wide acceptance in the field of biochemistry (Blatt et al, 1967) and an increasing application in water chemistry. The limited fractionation, rather than total resolution achieved with ultrafiltration is convenient in the investigation of the colloidal and macromolecular fractions of natural waters. The concentration of viruses found in municipal and surface water is a further application of the technique (Sweet et al, 1971; Hobbie and Pomeroy, 1972; Sorber, Malina and Sagik, 1973; Rubenstein et al, 1973; Belfort, Rotem and Katzenelson, 1975). Fractionation of organic constituents in surface waters by this means was originally advocated by Gjessing (1970) and further evaluated by Wilander (1972). Assessments of ultrafiltration in pesticide removal (Chian, Bruce and Fang, 1975), water purification (Ashworth and Tristram, 1973; Bansal and Wiley, 1974), and the general study of trace organic contaminants (Klein et al, 1975; Deinzer, Melton and Mitchell, 1975) demonstrate the value of the technique. The method has also been used for the partial fractionation of marine organic matter in 'solution', (Ogura, 1974;

Sharp, 1973; Brown, 1975) and in the investigation of plankton growth parameters (Doig and Martin, 1974). As both a means of organic extraction (Milanovich, Ireland and Wilson, 1975) and fractionation (Gjessing, 1971, 1973) reverse osmosis by ultrafiltration compares favourably with conventional procedures. Nevertheless, the comparative studies of Gjessing (1971, 1973) must be viewed in the light of earlier comments made upon the interpretation of gel permeation chromatograms of un-buffered mixtures. Few of the techniques discussed are compatible with the combined requirements of organic and inorganic analysis. However, ultrafiltration may afford a simple means of achieving both objectives in the case of trace metal-organic analysis.

2.3 EVALUATION OF ION-BINDING PHENOMENA USING DIALYSIS AND ULTRAFILTRATION

An apparent complexation of trace elements by macromolecular organic compounds in natural waters has been demonstrated by means of radiotracers and conventional dialysis (Barsdate and Matson, 1967; Barsdate, 1968). Benes and Steinnes (1974, 1975) adopted a similar mode of experimentation but incorporating the principle of in situ dialysis outlined by Parker (1967). The use of radioisotopes in the study of the physico-chemical state of trace elements may have severe limitations arising from an assumption of isotopic equilibrium. Particularly slow exchange is observed with colloidal and organically complexed transition elements, as might be expected with a metastable state. The simplicity of this approach is still maintained, however, with the use of rapid ultrafiltration in conjunction with conventional analysis (Gjessing, 1970, 1973; Schindler and Alberts, 1974; Lindberg

and Harriss, 1974; Guy and Chakrabarti, 1975; Ramamoorthy and Kushner, 1975a, 1975b, 1975c; Smith, 1976). A critical appraisal of the use of ultrafiltration to separate organic complexes is not currently available, but Andren and Harriss (1975) experienced minimal adsorption losses for mercury. The retention of free major ions such as Ca^{2+} and Mg^{2+} is also indicated by Wilander (1972) in addition to apparent adsorption losses of phosphate, nitrate and ammonium; Schindler and Alberts (1974) failed to confirm these findings.

2.4 CONTAMINATION PROBLEMS IN TRACE ELEMENT ANALYSIS ARISING FROM PHYSICAL MANIPULATION AND PREPARATIVE FILTRATION OF WATER SAMPLES

The problems of contamination which arise from the general preparative procedures of trace metal analysis have recently been reviewed (Robertson, 1968, 1972). Cleaning of laboratory apparatus and sampling containers used during this study, as well as the investigation presented here, were conducted with reference to the recommendations made by Robertson (1968, 1972). A multi-element solvent extraction technique using sodium diethyl dithiocarbamate and chloroform was employed in the trace element analysis presented here (Applied Geochemistry Research Group, Technical Communication No. 62, 1975). The sensitivity of this method is 0.1 ppb for Zn, Cu and Cd, 1.0 ppb for Co, Mn and Ni, 1.0 to 2.0 ppb for Fe and 5.0 ppb for Pb (see Appendix A).

2.4.1 Sampling Procedures and Sample Storage

Ten litre high-density polyethylene bottles were used during sample collection and storage. The batch of new containers used

throughout this investigation was initially cleaned with a warm, 5% (v/v) solution of Decon 90, a laboratory surfactant recommended for the removal of radiotracers and general superficial contaminants. After thorough washing, the walls of each bottle were leached with 10% (v/v) nitric acid for a period of 14 days. During this time the acid was changed on several occasions. Finally the bottles were rinsed, filled with deionised water and allowed to age. As a matter of routine containers used for sampling were washed and filled with 10% (v/v) nitric acid and allowed to stand 48 hours before use. These vessels were washed out with deionised water, and in the field each container was rinsed with water from the sample site. No preservatives were used but all samples were refrigerated or deep-frozen within 36 hours.

2.4.2 Sample Contamination Arising from the Use of Glass-Fibre Filters

Adsorption losses of dissolved organic compounds preclude the use of conventional 0.45 μ m cellulose acetate membranes in organic analysis. Comparable particle retention characteristics are, however, exhibited by glass-fibre filters (Sheldon, 1972) but these can prove a contamination hazard (Wageman and Graham, 1974). Whatman (GF/C) glass-fibre filters were analysed as follows: one sample after washing with 1M Ultragrade hydrochloric acid, was ignited at 500°C and digested in Analar hydrofluoric acid; another sample was analysed, as supplied by the manufacturers, for the purposes of a control. The results in Table 2.1 indicate that this type of filter is a potential source of iron and zinc contamination.

TABLE 2.1

Trace-Metal Analysis of Whatman (GF/C) Glass-Fibre Filter

	Concentration $\mu\text{g g}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
Acid-Washed Filter	650	29750	4.5	11.9	3.2	11.9	53.0	7.9
Control	660	30260	4.3	11.4	3.1	15.0	55.0	7.6

Soaking in 1M hydrochloric acid does not appear to significantly mobilize these impurities. Nonetheless filtration of double distilled deionised water through a rinsed, acid-washed GF/C filter confirmed that zinc and iron leached from this type of filter will not contribute markedly to the overall blank determination of this method (Table 2.2).

TABLE 2.2

Analysis of Distilled Deionised Water Filtered through a
Whatman (GF/C) Glass-Fibre Filter

	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
	2.0	1.5	0.5	<1.0	0.2	<1.0	<1.0	<5.0
Whatman GF/C Filtrates	2.5	2.0	1.0	<1.0	0.1	<1.0	<1.0	<5.0
	2.0	1.0	0.5	<1.0	0.1	<1.0	<1.0	<5.0
Mean	2.2	1.5	0.8	<1.0	0.1	<1.0	<1.0	<5.0
Blank	1.5	2.0	0.1	<1.0	0.1	<1.0	<1.0	<5.0

Distilled deionised water and a 0.5M solution of Spec Pure ammonium EDTA (ethylene diamine tetra-acetic acid) remained uncontaminated with the major ions Ca^{2+} and K^{+} after filtration through an acid-washed GF/C filter. The detection limits for Ca and K using direct flame-atomic

absorption spectrophotometry are 0.1 and 0.05 ppm respectively. Acid-washed Whatman (GF/C) filters were used throughout this study, for the removal of suspended particulate material in water samples. The 90 mm diameter filters can be used conveniently with available ultrafiltration apparatus. Both filtration cells and filters were washed with one litre of deionised water followed by a further rinsing with a 500 ml aliquot of the sample in preparation for filtration of the sample.

2.4.3 Ultrafiltration Apparatus and its Use in Trace Metal Analysis

A self-contained, ultrafiltration cell (Amicon Model TCF-10) with an integral peristaltic pump was used in this study. The system facilitates the recirculation of the dialyzate through a thin, spiral channel. The laminar flow created by this circulation is designed to minimise the accumulation of any concentrate at the membrane surface. The membrane, retained by an 'O'-ring upon a polythene support, lies within the shallow well of the Delrin base-block. This lower block forms an end-cap to a cylindrical, polycarbonate sleeve which is retained by another end-cap fitted with a pressure release valve. Silicone rubber 'O'-rings are used to seal the joints of the apparatus which is assembled on a rigid frame. A fibre-glass reservoir is also an accessory available with Amicon equipment, and is used to maintain a closed system during the filtration of large samples and to effectively supplement the 500 ml volume of the cell. Conventional cleaning mixtures used in the care of laboratory glassware, may not be used with Delrin which is an acetal resin; the ultrafiltration apparatus is susceptible to attack from acids as well as organic solvents. However,

a warm 5% (v/v) solution of Decon 90 has proved a suitable cleaning agent. All the tubing used with the cell was decontaminated by soaking in 10% (v/v) nitric acid.

2.4.4 Contamination Using Ultrafiltration Apparatus and Accessory Equipment

Trials were carried out in duplicate using distilled deionised water at pH 4 to 5; controls were maintained in acid-washed polythene bottles or covered polythene beakers during each test. The results in Table 2.3 show the levels of contamination found in distilled deionised water (500 ml) circulated continuously through the cell for a period of 30 minutes. Separate leaching trials with three acid-washed, silicone rubber 'O'-rings were also carried out. The 500 ml of distilled water used in this experiment was found not to be significantly contaminated by these components after five days.

TABLE 2.3

Trace Element Contamination Associated with the Cell Unit and Components

	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
Recycled 'Dialyzate'	2.0	1.9	1.4	<1.0	0.2	1.0	<1.0	<5.0
Blank Determination	3.0	3.6	0.3	<1.0	0.2	1.0	<1.0	<5.0
Leaching of 'O' ring	1.0	2.5	0.9	1.0	0.3	1.0	<1.0	<5.0
Blank Determination	2.0	2.9	0.4	<1.0	0.1	2.0	<1.0	<5.0

The 2.4 litre, glass-fibre accessory reservoir was filled with

10% (v/v) nitric acid and allowed to stand for 10 days. The acid was changed after 2 and 7 days during this period. The container was then washed and filled with double distilled deionised water which was analysed after 48 hours. This trial was repeated after soaking the reservoir with 50% (v/v) nitric acid for five days and again after similar treatment for a further period of five days. Levels of trace element contamination from this apparatus are given in Table 2.4. A sample of the fibre glass used in the manufacture of the reservoir was provided for analysis. Two one gramme samples of the deaminated material were obtained, one of these was ignited and the remaining residue was subjected to a hydrofluoric acid attack (Sample A); a control (Sample B) was digested without prior ignition. The results of this analysis are given in Table 2.5. A tube supports the gas-line in the reservoir cap and this polyvinyl chloride (P.V.C.) tube was removed during leaching tests and subjected to trials separately. Even after acid-washing this minor component, immersed in 500 ml of distilled deionised water, proved to be a significant source of zinc contamination (Table 2.6). Initially the polythene gas-pipe, which also comes into sample contact in the reservoir, produced iron and zinc contamination of a 500 ml blank but this diminished with the ageing of the polythene (Table 2.6).

2.4.5 Trace Element Contamination Using Ultrafiltration Membranes

Throughout these contamination trials membrane preparation was carried out according to the manufacturer's recommendations. The Diapor particulate filters in the DP series of Amicon Microporous

TABLE 2.4

Contamination Associated with Accessory Fibre-Glass Reservoir

	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
Contamination of Distilled Deionised water held in the Reservoir after acid-washing using 10% nitric acid	20.0	18.4	7.2	2.0	0.6	2.0	1.0	<5.0
	17.0	10.0	5.0	2.0	0.6	2.0	1.0	<5.0
Distilled water Blank determination	1.0	2.1	0.6	<1.0	0.2	1.0	<1.0	<5.0
Contamination of Distilled Deionised water after washing with 50% nitric acid	13.0	9.5	2.1	1.0	0.3	1.0	<1.0	<5.0
	10.0	8.5	2.0	1.0	0.1	<1.0	1.0	<5.0
Mean	11.5	9.0	2.05	1.0	0.2	<1.0	<1.0	<5.0
	8.0	7.2	1.4	1.0	0.3	2.0	1.0	<5.0
	6.0	6.8	1.6	1.0	0.2	<1.0	<1.0	<5.0
Mean	7.0	7.0	1.5	1.0	0.25	<1.0	<1.0	<5.0
Distilled water Blank determination	1.5	2.0	<0.1	<1.0	0.1	<1.0	<1.0	<5.0
Contamination of Distilled Deionised water after further leaching of the Reservoir with 50% nitric acid	6.5	9.7	2.0	<1.0	0.1	1.5	<1.0	<5.0
	6.0	8.8	2.0	<1.0	0.1	1.0	<1.0	<5.0
Mean	6.3	9.3	2.0	<1.0	0.1	1.3	<1.0	<5.0
Distilled water Blank determination	2.0	1.0	<0.1	<1.0	0.1	<1.0	<1.0	<5.0

TABLE 2.5

Analysis of Fibre-Glass Sample from Amicon Reservoir

	Concentration $\mu\text{g g}^{-1}$									Concentration mg g^{-1}			
	Fe	Zn	Cu	Co	Cd	Cr	Ni	Mn	Pb	Ca	Mg	Ti	Al
SAMPLE A	460.0	23.0	6.2	20.0	3.9	27.0	25.0	15.0	26.0	103.0	1.4	0.5	68.0
SAMPLE B	570.0	21.0	5.4	17.6	3.3	28.0	22.0	14.3	23.5	84.7	1.4	0.8	71.3

TABLE 2.6

Contamination Associated with Accessory Tubing

	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
Contamination of Distilled Deionised water	2.0	5.2	0.8	1.0	0.2	2.0	<1.0	<5.0
by P.V.C. Gas-line Support immersed for 5 days	2.0	4.1	0.6	<1.0	0.2	2.0	<1.0	<5.0
Mean	2.0	4.7	0.7	<1.0	0.2	2.0	<1.0	<5.0
Distilled water Blank determination	2.5	2.6	0.5	1.0	0.1	1.0	<1.0	<5.0
Contamination with Polyethylene Pressure	10.0	5.8	0.6	1.0	0.1	<1.0	<1.0	<5.0
Tubing immersed in Distilled Deionised water	5.0	1.5	1.0	1.0	0.1	1.0	<1.0	<5.0
Distilled water Blank determination	5.0	2.0	0.9	<1.0	<0.1	1.0	<1.0	<5.0

filters were soaked in 1M hydrochloric acid for 48 hours prior to use. Membranes of the XM series are supplied 'ready for use' and were merely rinsed by filtration of 500 ml of distilled deionised water. Diaflo Ultrafilters of the PM and UM ranges were soaked in distilled water with several changes over a 48 hour period.

A 500 ml volume of distilled deionised water was filtered through each membrane to wash out any possible impurities. During these trials one litre of distilled deionised water was circulated within the cell and filtered twice through the membrane in use. The cell and pump tubing were cleaned after each membrane trial to prevent cross-contamination of ultrafiltrates. Trace element contamination of successive filtrates from each of the membranes tested can be seen from the results in Table 2.7. The membranes used in a second trial were all prepared as before but were also soaked in 1M hydrochloric acid. Ultrafiltrate contamination arising from the six membrane types tested after acid-washing and rinsing is recorded in Table 2.8.

The results of these contamination studies have shown that the Whatman (GF/C), glass-fibre filter can be reasonably used in trace metal analysis and is perhaps the only pre-filter suitable for use in a study of organo-metallic species of trace elements in solution. All the Amicon microporous filters and ultrafiltration membranes used, with the exception of the UM-05 membrane, contaminated distilled deionised water. Zinc, copper and iron were the principal impurities leached from these filters, and zinc contamination was particularly marked with membranes of the XM series. Acid-washing

TABLE 2.7

Sequential Filtrate Contamination using Ultrafiltration Membranes

Membrane Type	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
DP045	3.0	9.7	5.3	<1.0	0.2	1.0	<1.0	<5.0
	3.4	9.7	4.9	<1.0	0.2	1.1	<1.0	<5.0
DP02	2.0	7.0	3.8	<1.0	0.3	1.0	<1.0	<5.0
	4.0	7.8	4.2	<1.0	0.3	2.2	<1.0	<5.0
XM300	4.1	31.0	0.9	<1.0	0.5	2.0	<1.0	<5.0
	3.0	5.8	1.3	<1.0	0.2	2.0	<1.0	<5.0
XM50	2.0	34.0	1.4	<1.0	0.2	2.0	<1.0	<5.0
	2.0	5.0	0.5	<1.0	0.2	2.0	<1.0	<5.0
PM-10	2.0	6.8	1.2	<1.0	0.2	1.0	<1.0	<5.0
	3.0	5.9	1.1	<1.0	0.2	1.0	<1.0	<5.0
UM-05	2.0	1.1	1.5	<1.0	0.2	1.0	<1.0	<5.0
Blank	3.0	3.6	0.3	<1.0	0.2	1.0	<1.0	<5.0
	2.0	4.0	0.4	<1.0	0.2	2.0	<1.0	<5.0

TABLE 2.8

Sequential Filtrate Contamination using Ethanol-Acid washed Membranes

Membrane Type	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
DP045	7.0	3.8	4.0	2.0	0.2	1.0	<1.0	<5.0
	5.0	3.8	4.2	<1.0	0.2	<1.0	<1.0	<5.0
DP02	6.0	7.2	2.2	1.0	0.2	2.0	<1.0	<5.0
	2.0	7.0	2.5	1.0	0.3	<1.0	<1.0	<5.0
XM300	6.6	41.0	2.2	1.1	0.6	<1.0	<1.0	<5.0
	6.0	40.0	2.5	1.0	0.9	<1.0	<1.0	<5.0
XM50	5.0	19.0	2.1	<1.0	0.4	<1.0	<1.0	<5.0
	4.4	18.6	2.2	<1.0	0.2	<1.0	<1.0	<5.0
PM-10	2.0	2.5	0.7	<1.0	0.2	<1.0	<1.0	<5.0
	1.5	2.0	<0.1	<1.0	0.2	<1.0	<1.0	<5.0
UM-05	2.0	2.0	<0.5	1.0	0.2	<1.0	<1.0	<5.0
	2.5	1.5	<0.1	1.0	0.1	<1.0	<1.0	<5.0
Blank	2.5	2.0	0.5	<1.0	0.1	<1.0	<1.0	<5.0
	2.0	1.0	<0.1	<1.0	0.2	<1.0	<1.0	<5.0

and rinsing failed to significantly improve blank determinations in many cases. However, filtration blanks with acid-washed PM-10 membranes were reduced to acceptable levels after rinsing with distilled water. The TCF 10 ultrafiltration cell and silicone rubber 'O'-ring seals are satisfactorily decontaminated with a warm, dilute solution of Decon 90 laboratory surfactant. The accessory fibre-glass reservoir together with component tubing has proved a persistent source of contamination.

2.5 ADSORPTION LOSSES OF TRACE ELEMENTS USING AN ULTRAFILTRATION CELL AND A PM-10 MEMBRANE

A mixed, aqueous standard containing zinc, copper, cobalt, cadmium, iron, manganese, nickel and lead, all at a concentration of $100 \mu\text{g l}^{-1}$, was filtered through an acid-washed PM-10 membrane. Successive aliquots of the filtrate were taken and analysed in duplicate, their mean concentrations are given in Table 2.9. After decontamination of the cell assembly a marked discolouration of the base-block well was observed. This suspect and apparently corroded surface stained green upon the application of a sodium diethyl dithiocarbamate solution, indicating that this was perhaps the cause of the observed adsorption losses. No staining of the reconditioned surfaces was apparent and the trial was repeated. The cell was filled with a fresh standard and allowed to stand for 12 hours; a further two litres of this standard solution was filtered through the membrane. The membrane used was washed firstly with a 10% (v/v) ethanol solution to remove preservatives (phenolphthalein and glycerin) and then allowed to soak

TABLE 2.9

Successive PM-10 Filtrate Aliquots of a Standard Solution

	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
Aqueous Standard	94.0	97.5	98.0	101	95.0	102	95.0	100
Filtrate 1.	89.0	99.0	89.0	89.0	85.0	90.0	88.0	100
Filtrate 2.	96.0	99.0	88.0	88.0	87.0	82.0	90.0	100
Filtrate 3.	83.0	95.0	92.0	92.0	85.0	79.0	83.0	100

TABLE 2.10

Successive Filtrate Aliquots of a Standard Solution
using the Reconditioned Ultrafiltration Cell.

	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
Aqueous Standard	100	100	94.0	90.0	100	100	95.0	100
Filtrate 1.	100	98.0	100	90.0	92.0	98.0	87.0	100
Filtrate 2.	98.0	98.0	100	92.0	95.0	100	93.0	100
Standard held in Cell for 12 hours	75.0	85.0	85.0	90.0	76.0	90.0	83.0	90.0
% Adsorption	25.0	15.0	9.6	0.0	23.0	10.0	7.9	-

TABLE 2.11

Contamination and Adsorption Trials using Polymethylmethacrylate (Plexiglass)Ultrafiltration Cell with Ethanol - Acid washed PM-10 Membrane

	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
Blank Determination of Distilled Deionised Water Held in Filtration Cell for 12 hours and filtered through PM-10 Membrane	1.0	1.7	0.9	1.0	0.7	1.0	1.0	5.0
Aqueous Standard Solution held in Cell for 24 hours and filtered through PM-10 Membrane	104.0	105.0	99.7	93.0	90.0	92.0	94.0	107.0
Standard Solution	105.0	104.9	99.5	93.0	90.0	92.0	94.0	107.0
Standard filtered through PM-10 Membrane	97.0	102.0	100.0	100.0	96.0	104.0	100.0	104.0
Standard Solution	97.0	98.0	96.0	100.0	96.0	108.0	103.0	106.0
Second Standard Solution Filtered through PM-10 Membrane	106.0	105.0	104.0	100.0	90.0	97.0	103.0	118.0
Standard Solution	102.0	98.7	103.0	100.0	89.0	100.0	100.0	118.0

TABLE 2.12

Influence of Membrane Preservatives upon the Solvent Extraction
of Diethyl Dithiocarbamate Complexes of Trace Metals

	Concentration $\mu\text{g l}^{-1}$							
	Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
Standard + 10 ml 0.1% Phenolphthalein	102.0	105.0	96.0	93.0	102.0	93.5	106.0	99.0
Standard Solution	104.0	103.0	95.0	94.0	100.0	94.0	102.0	101.0
Standard + 1 ml 0.1% Phenolphthalein	96.0	96.2	92.5	102.0	94.5	102.0	95.0	103.0
Standard Solution	97.0	97.0	94.0	98.0	96.0	102.0	95.0	105.0
Standard + 1 ml of Glycerol	91.0	103.0	96.0	93.0	93.0	96.0	105.0	104.0
Standard Solution	93.0	98.0	94.0	93.0	94.0	98.0	102.0	105.0

in 1M hydrochloric acid. This preparation is thought to be necessary to avoid any possible inhibition of the solvent extraction technique used in the analysis; the results of this trial are given in Table 2.10. A simplified prototype cell of polymethyl methacrylate was constructed in an attempt to minimise contamination and adsorption losses further (Robertson, 1968, 1972). The results obtained with this cell are tabulated in Table 2.11.

2.5.1 Investigation into the Possible Inhibition of Trace Element Solvent Extraction by the Interference of Membrane Preservatives

Phenolphthalein and glycerin are used by manufacturers to prevent membrane desiccation during shipment. An aqueous, multi-element standard of $100 \mu\text{g l}^{-1}$ was made up. To one litre aliquots of this standard were added:

- a) 10 ml of a 0.1% phenolphthalein solution
- b) 1 ml of a 0.1% phenolphthalein solution
- c) 1 ml of Analar glycerol

to give three test solutions. No apparent inhibition of the chloroform extraction was observed in this analysis (Table 2.12); nevertheless ethanol-acid washing is recommended for the complete decontamination of the membranes and for the removal of preservatives.

2.6 DISCUSSION AND CONCLUSIONS

A review of the concentration and separation methods currently available clearly indicates that few techniques are available which

are compatible with the combined requirements of trace metal and organic analysis. Many of the concentration methods developed for organic compounds fail to yield a complete recovery and are commonly selective; in addition, a further separation procedure is often essential. In trace element analysis it is desirable to minimise sample manipulation to avoid contamination. It is therefore preferable to use a simple and combined concentration and fractionation technique to separate organo-metallic complexes from water samples. Centrifugation of bulk water samples is impractical for the isolation of particles $<1\mu\text{m}$ (Lammers, 1971). Ultrafiltration is perhaps the only immediate, alternative method available for the concentration and separation of organic, trace element complexes in dilute solution.

Benes and Steinnes (1974) have argued against the use of conventional sampling and analysis in the study of trace metal speciation and point out the following limitations:

1. Adsorption may negatively affect ultrafiltration results because of the large inner surface of fine membranes and the small filterable volume of water.
2. Different forms of a trace element are adsorbed selectively and so disturb the solution equilibrium during sampling, transport and analysis.
3. The usual methods of preventing adsorption losses, i.e. acidification or freezing of the solution cannot be used in the study of trace element speciation in which the maintenance of sample equilibria is crucial.

A method of in situ dialysis has been suggested to overcome some of

these difficulties (Benes and Steinnes, 1974, 1975). A dialysis bag or cell filled with purified water is immersed in situ for a period of 10 days to establish the Donnan Membrane equilibria with metastable complexes of the macromolecular organic species. Analysis of the 'dialyzate' and an ambient water sample is expected to give a time integrated indication of the trace element concentration complexed by colloidal fractions of organic matter. However, the proliferation of microorganisms upon apparatus immersed in any natural body of water is commonly observed during measurements of productivity. Localized biological activity may create artifacts with this method associated with accelerated growth of bacteria and their secretions. In addition, bacterial degradation of dialysis membranes during protracted periods of use should also be anticipated under such circumstances. The recent observations by Sieburth (1975) on the microbial degradation of cellulose dialysis membranes would seem to preclude any experimentation of this nature in the marine environment. Nevertheless, since the difficulties encountered in conventional sampling, storage and analysis must be considered in the interpretation of results, the use of high performance, ultrafiltration membranes and simplified apparatus in the field cannot be dismissed. Such a methodology would be appropriate for the detailed examination of a few samples but would undoubtedly prove impractical in a more general survey.

A scrutiny of trace element contamination and adsorption losses arising from the use of ultrafiltration membranes is warranted. The limited investigation presented here has confirmed that certain modern materials used in the construction of apparatus are a contamination

hazard. Membrane filters are also a potential source of contamination, particularly of zinc. With the elimination of contaminating components from filtration apparatus and the thorough washing-out of acid-washed membranes, blanks can be reduced to acceptable levels. The problems of adsorption losses are more difficult to assess in the light of recent observations (Benes, 1969; Benes and Steinnes, 1974). Using electrochemical methods Stumm and Bilinski (1972) have also demonstrated the selective adsorption of simple lead carbonate complexes. However, the adsorption losses of free-ionic species from aqueous solution observed during this study have been minimal. Ultrafiltration may therefore be used, with certain reservations, to distinguish between concentrations of macromolecular complexes and more simple trace element species in natural waters. It is suggested that the following conditions should be observed to minimise contamination and adsorption losses:

1. Filtration to remove particulate matter is carried out rapidly to minimise adsorption losses and to maintain a constant size of particle retention.
2. All apparatus, filters and membranes used should be decontaminated and washed out with distilled water; with 90 mm membranes a volume of not less than 1 litre should be used, followed by a sample aliquot of not less than 500 ml.
3. High performance membranes are used.
4. A sample of not less than 5 litres is used for preparation and analysis.

These conditions can only be met with the use of glass-fibre filters and cross-linked polyolefin membranes such as those of the Amicon PM range. Both the Whatman (GF/C), glass-fibre filter and the Amicon PM-10 ultrafiltration membrane facilitate rapid filtration and minimal contamination of a sample. Using the Whatman (GF/C) filter a 10 litre water sample can be filtered within an hour. A maintenance of particle size retention is important particularly during this preparative pre-filtration. This can most readily be achieved by allowing the sample to siphon onto the surface of a large filter. In this way as the pores of the filter are occluded the area of the filter used gradually increases to maintain an even flow rate and consistent particle size retention. Using a PM-10 membrane it is then possible to filter a one litre aliquot within 90 minutes removing all the organic species with molecular weights in excess of 10,000. The colloidal and macromolecular fractions of an eight litre sample can be conveniently concentrated within 6 hours using a single 90 mm PM-10 membrane. The Amicon TC5D system is a bulk concentrator which has been used to concentrate dissolved humic acids from a 40 litre sample in 3 hours using PM-10 membranes. This stainless steel apparatus is clearly unsuitable for use in trace element analysis. Also sample volumes of this size are impractical except for the concentration of dilute polymers for extensive analysis and experimentation. Nevertheless, the use of a non-metallic thin channel, modular system of ultrafiltration with an array of PM-10 membranes would perhaps facilitate the rapid isolation and concentration of macromolecular fractions for trace element analysis.

The choice of a modern synthetic polymer to be used in the fabrication of apparatus is crucial since trace element catalysts and stabilizers are commonly used in the manufacture of these products (Robertson, 1972). The reactions and surface-chemical behaviour of these plastics and resins can create systematic errors in analysis. The results of the simple adsorption study presented here are sufficient to emphasise the need for care in the cleaning and use of this type of apparatus. Acid attack clearly denatures the surface of the acetal resin Delrin, a highly crystalline form of polymerised formaldehyde. The adsorption losses, first observed with the Amicon filtration cell are attributed to a sorption of trace elements upon surfaces which were worn and etched. Dilute acid draining from a membrane is sufficient to have caused the corrosion of one of the acetal resin blocks in the cell assembly. The use of polymethyl methacrylate apparatus in trace element analysis is to be favoured since this material is not a source of contamination (Robertson, 1968, 1972) and minimal adsorption losses have been experienced with this type of apparatus.

Ultrafiltration using decontaminated membranes provides perhaps the only direct means of discriminating between free ionic trace element species and their more complex colloidal forms. The method is also simple, facilitating the concentration of dilute material for detailed analysis and identification. The non-denaturant separation and concentration of molecular sizes which can be achieved with ultrafiltration makes this the most appropriate method currently available for the study of little known organo-metallic forms in natural waters.

CHAPTER 3

3. COLLOIDAL AND ORGANO-METALLIC COMPLEXES OF IRON IN NATURAL WATERS

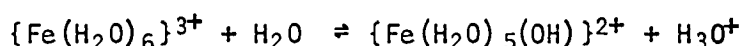
3.1 INTRODUCTION

As a practical procedure it is difficult to distinguish between material occurring in true solution and that occurring as a sol. The current, arbitrary definition of the particulate and soluble phases in water has already been discussed in Chapter 1. However, an operational distinction using membrane filtration may prove particularly inappropriate to the readily hydrolysed elements such as Al, Fe, Mn and Ti. Hydrolysis products of these elements may be expected to occur in discrete particle size fractions of less than $0.45\mu\text{m}$ (Shigematsu et al, 1970; Kennedy, Zellweger and Jones, 1974). With respect to iron the definition's value is limited still further since much of the so-called soluble iron of lakes and rivers is colloidal but intimately associated with organic matter. A rigorous adherence to conventional membrane filtration may lead to adsorption losses of organically complexed iron manifest as a systematic error in analysis.

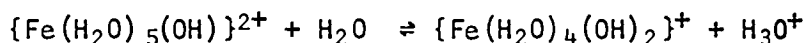
Above pH 4.8 the solubilities of most Fe(III) species are calculated to be less than $10\ \mu\text{g l}^{-1}$ and on theoretical consideration Fe(III) may be regarded as insoluble at the pH and Eh of most natural waters. Nevertheless, the world average concentration of iron reported in river water lies at $670\ \mu\text{g l}^{-1}$ (Riley and Chester, 1971); levels 10^3 times the value expected from the solubilities of known Fe(III) species are not uncommonly reported. Cooper (1948) postulated as many as ten iron species to occur in sea water and similar conclusions have been derived from a consideration of fresh water conditions (Stumm and Lee, 1960). In contrast Sillén (1961) has suggested that under marine conditions 99% of all 'soluble' iron must

be present as organic complexes and that the remaining dissolved forms, principally hydrated Fe(II) and Fe(III) species, exist only in relatively small amounts. A number of recent observations clearly indicate that, at least in fresh water lakes and rivers, iron in apparent solution does occur in organic species. It is convenient at this point, however, to consider initially the behaviour of iron under pH and Eh conditions typical of surface waters.

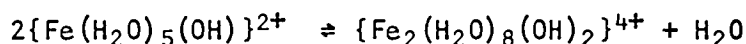
Within the pH range 6 to 8, a number of poly-valent metals including iron are slowly hydrolyzed forming hydroxo-complexes (Stumm and Lee, 1960):



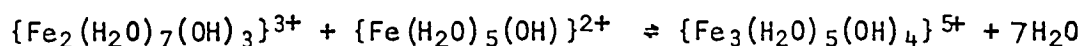
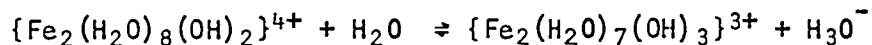
Upon the protonation of a solvent water molecule, a polar molecule of solvation is coordinated as a hydroxyl group; this process may continue as protonation of the hydration envelope progresses:



Such hydroxo-complexes have a pronounced tendency to polymerize via hydroxo-bridges:



a process which marks a preliminary stage in the phenomenon of iron 'ageing'. Hydrolysis of dimeric ions may yield more complex hydroxides with a consequent extension of polymerization through further hydroxo-bridges:



Colloidal hydroxo-polymers and, ultimately, insoluble hydrous ferric oxides are formed. The polymeric structure of the oxides formed initially adopts a more regular configuration during ageing, the

mineral α -goethite (α -FeOOH) being the final product. Lengweiler et al, (1961) have demonstrated that colloidal iron hydroxide dispersions are formed even from dilute solution at pH 6.0 to 7.3. Marchand (1974) using radio-tracer techniques has also found iron to remain principally in a colloidal state in sea water. Similarly the equilibrium product, $\text{Fe}(\text{OH})_{2.7}\text{Cl}_{0.3}(\text{H}_2\text{O})_3$ generated during the hydrolysis of iron in artificial sea water, has been shown by Chow (1965) to have the structure of goethite.

The maintenance of polymeric iron species as discrete sols requires explanation. The relative stability of such a hydrophobic dispersion is dependent upon numerous physico-chemical factors and is a function of the intricate particulate, colloidal and ionic interactions of the heterogeneous system in surface waters. Many naturally occurring colloidal and dissolved organic constituents of lakes and rivers in particular the humic substances, comprise carboxyl, hydroxyl, phenolic and quinoid functional groups within a loosely condensed aromatic structure. Many of these compounds may be present in 'solution' as reversible aggregates or as single molecules (Wershaw and Pinckney, 1971). An organic, hydrophilic dispersion such as this does not exhibit the phenomena associated with a phase boundary and a diffuse, double-layer charge. The adsorption of such material upon a hydrophobic dispersion would produce a stabilized or protected colloid with a concomitant negative charge. A complexation with poly-valent cations and specific interactions with colloidal and dissolved humic or fulvic acids may readily account for the stabilization of iron hydroxo-polymer

dispersions. Peptization of iron oxides and silicates was a mechanism postulated by Gruner (1922) to account for the mobilization of iron and the occurrence of sedimentary iron formations. More recently Shapiro (1964) further emphasised the possible significance of humic acids in the peptization and solubilization of iron in lakes. Hydrophobic organic compounds such as alkanes, fatty acids, and dialkyl phthalates are solubilized by fulvic acids (Matsuda and Schnitzer, 1971) and a similar agency may be involved in the stabilization of colloidal iron (Schnitzer and Khan, 1972). The sorption of dissolved organic matter upon inert surfaces is a surface chemical phenomenon known to occur in sea water and which may be directly illustrated using micro-electrophoresis (Neihof and Loeb, 1972, 1974). Apparently any positively charged (hydrophobic) material immersed in sea water suffers a reversal of charge due to the adsorption of 'dissolved' organic matter. Both high and low molecular weight compounds appear responsible for this charge reversal. The contribution of minor, inorganic constituents to this process is thought to be insignificant. Further investigation is required to establish that similar processes also occur in the waters of lakes and rivers.

Multivalent anions may effect a destabilization of colloidal iron sufficient to establish equilibria antagonistic to the process of peptization. Sulphate ions readily coagulate hydrophobic ferric oxide sols by the partial neutralization of charge and reduction in the diffuse double-layer. Colloidal precipitates of ferric hydroxides commonly contain substantial occlusions of mineral anions as impurities (eg. $\text{Fe}(\text{OH})_{2.7}\text{Cl}_{0.3}(\text{H}_2\text{O})_3$). Co-precipitation in this case arises

from complex formation since the adsorption of anions is negligible. The presence of Fe(III) is, however, known to enhance the adsorption of phosphate and sulphate ions upon mica (Langdon, Perrott and Wilson, 1973). The strong tendency for complex formation between HPO_4^{2-} and Fe^{3+} ions is of special significance as a control of eutrophication, being a convenient means of effecting inorganic phosphate removal by precipitation of ferric-hydroxo complexes. In the presence of humic acid-iron complexes the co-precipitate formed with phosphates is regarded as a precursor of ^{the} mineral vivianite (Kovalev and Generalova, 1974).

3.1.1 The Bio-geochemical Significance of Organo-metallic Species of Iron

The formation and occurrence of polymeric iron species may account for some observations made upon the bio-geochemistry of iron in natural waters (Hem, 1972). Marine microorganisms are thought to produce iron-binding compounds or siderochromes which are effectively iron-specific, transfer compounds. Synthesis of such chelating agents also occurs extensively in soils and may continue in lakes and rivers. Iron deficiency stimulates a vast production of these hydroxamic acids (Goyne and Carpenter, 1975). The speciation and availability of iron are of biological significance in so far as they may dictate the biological productivity of a water body (Schelske, 1962). In the estimation of iron bio-availability in natural waters, considerable analytical problems arise (Kawaguchi and Matsuo, 1958; Shapiro, 1967, 1969; Johnston, 1964; Vuille, 1968). The procedures which have been adopted to date are generally somewhat unsatisfactory

since many employ chemical manipulations to assay a fundamentally biological phenomenon. Differential reduction of ferric iron in conjunction with colorimetric analysis using orth-bathophenanthroline has been suggested as one method. Analysis of free ferric ions using potassium thiocyanate has been found to correlate closely with iron-availability as determined by bio-assay (Shapiro, 1967, 1969). A definition of 'metabolically bound' iron is given by Davies (1970) and the concentration of this accumulated fraction, as determined using EDTA, is related to growth rate in phytoplankton. The synthesis of essential enzymes and pigments such as nitrogenase and chlorophylls is held to account for the iron requirement. Also, it has been frequently suggested that ageing and organic complexation limit availability (Johnston, 1964). The use of an 'iron index' as a predictive tool for the occurrence of red-tides has also been advocated in this context (Martin and Martin, 1974). The intimate relationship between both biological and geochemical cycles of iron is clearly illustrated and reviewed by Kuznetsov (1968).

3.1.2 Organo-metallic Complexes of Iron in Natural Waters

The apparent supersaturation of natural waters with respect to Fe(III) species can only partially be accounted for by the stabilization of colloidal polymeric forms. The presence of dissolved organic matter may also affect the geochemistry of iron by complexation and hence regulate, in part, solubility and redox potential. Anaerobically fermenting vegetation found in bogs and swamps may mobilize iron, and the retention of iron in 'true' solution in organically enriched, surface waters is a consistent feature of moorland drainage (Hissel, 1955;

Van Beneden, 1956; Shapiro, 1964, 1966).

Schnitzer and Skinner (1966) and Malcolm et al. (1968) have shown that Fe(II) and Fe(III) humic acid complexes effectively solubilize iron at pH values greater than 3.1. Shapiro (1964, 1966a, 1966b) demonstrated that solvent extracted humic acids exhibit a maximum affinity for iron at pH 10 and has suggested a mechanism of peptization to account for these observations. This interpretation has been brought into question (Christman, 1967) since further consideration of Shapiro's work in the light of more realistic molecular weight values indicates that chelation and a ligand exchange reaction may equally account for these observations. The apparent large increase in molecular weight, observed by Ghassemi and Christman (1968), during gel permeation chromatography of lacustrine organic acids was attributed to organometallic complexation manifest as a progressive dissociation of iron complexes with increasing pH. Organically bound iron has been found to readily permeate many grades of Sephadex gels unlike free or inorganic iron species which are bound irreversibly upon the cross-linked, dextran matrix. Plumb and Lee (1973) have shown that complexed and unbound iron fractions can be determined using affinity chromatography and Sephadex gels.

A relationship between 'soluble' iron, particle size, pH and organic acid concentration is indicated from the body of evidence within the literature. However, there is no consistent relationship between the concentration of humic substances and iron in natural waters (Lamar, 1968; Hem, 1970). Sources of iron

unrelated to the concentration and origin of the organic complexes may obscure the fundamental principle (Perdue, Beck and Reuter, 1976). In addition, competitive chelation of aluminium is a further complicating factor. Colloidal processes involving peptization as well as organic complexation by humic acids may give rise to complicated correlations not readily interpreted in terms of either process independently.

Iron in both Fe(II) and Fe(III) oxidation states may be stabilized in aqueous aerobic conditions by organic matter. Fe(II) organic complexes analogous to humic acids are resistant to oxidation for considerable periods of time (Theis and Singer, 1974; Akiyama, 1973; Hem, 1960). Theis and Singer (1974) demonstrated that both tannic and humic acids are capable of reducing free Fe^{3+} ions but that this process is limited by the progressive hydrolysis of the iron. Similar observations have been reported during the analysis of ferrous species in lakes and rivers (Gjessing, 1964; Shapiro, 1966). However, Hem (1960) considered the chelating ability of tannic acid to be weak and insignificant in natural waters. A dynamic equilibrium between organic complexes and the processes of hydrolysis has been advocated (Christman, 1967; Theis and Singer, 1974). Clearly the accurate sampling and analysis of such a system presents severe practical problems. In the absence of specific analytical techniques for the characterization and determination of this loosely defined group of organic compounds and their complexes, it is unlikely that the true nature of iron-organic associations can yet be fully understood.

3.1.3 The Dispersion and Precipitation of Colloidal and Organo-metallic Complexes of Iron in Rivers and Estuaries

The characteristic behaviour of iron in the dissolved and undissolved phases of surface waters is closely related as a consequence of reactions involving both phases (Hahn and Stumm, 1970). The processes of adsorption and complexation as well as the phenomena of dissolution, coagulation and precipitation dictate the geochemistry of all species of iron transported by rivers and discharged to estuaries. Clearly coagulation, which leads to aggregation and sedimentation of colloids, determines to a large degree the composition of the suspended particulate material of natural waters. In sea water, hydrous iron oxides are thought to scavenge the ionic species of the trace metals (Goldberg, 1954; Krauskopf, 1956; Aston and Chester, 1973). This mechanism of co-precipitation is postulated upon the unjustified assumption that dissolved iron forms exclusively unstabilized and colloidal hydroxo-complexes. The theoretical behaviour of this simple model predicts that the stability of the colloidal dispersion is governed by the classical laws of adsorption and coagulation defined by the Hardy-Schulze rule. However, the stabilization of polymeric colloidal forms by humic and fulvic acids may inhibit or arrest this process. The precipitation of colloidal iron in estuaries requires a more detailed explanation which will be complicated by the existence of organic complexation and peptization (Williams and Chan, 1966; Eisma, 1975; Holliday and Liss, 1976).

A progressive dilution of humic acids and their iron complexes

downstream might be anticipated not only as a function of river discharge but also as a result of coagulation. Gjessing (1970) has demonstrated both the precipitation of humic acids and the removal of iron which occurs in organically enriched streams, during conditions of reduced flow. The decrease in humic acid concentration is also correlated with an apparent reduction in polydispersity which favours molecular weights of less than 5,000. These observations are in agreement with the distribution of molecular weights reported by Brogden (1971) for humic acids in Florida Gulf Coast rivers and estuaries. The precipitation of iron in terms of the Derjaguin-Landau-Verwey-Overbeek (DLVO) Theory which is implicit in the early work of Goldberg (1954) and Krauskopf (1956) is based on the diffuse double layer model as applied to hydrophobic dispersions. Coagulation and aggregation of peptized and complexed organo-metallic species of iron must be defined in terms of the surface chemistry of hydrophilic dispersions.

Carboxyl and hydroxyl functional groups of humic acids will be fully dissociated at the pH of river water. As a consequence, a mutual repulsion of the negative functional groups will occur within the poly-electrolyte forcing the molecule to assume a stretched configuration. A polymer of this type will undergo these characteristic changes in structure even when exposed to a dilute electrolyte. Complexation of cations by the carboxyl and hydroxyl groups would cause a progressive reduction in the intramolecular forces of repulsion within the polymer and so favour tighter molecular coiling (Ong and Bisque, 1968). The Fuoss Effect describes this type of

polymer behaviour and may govern the extent to which humic acids peptize colloidal dispersions and complex cations in general. The dispersion of colloidal species of iron may largely depend upon antagonistic equilibria between the processes of destabilization, such as the complexation of multivalent anions or the effects of strong electrolytes, and the stabilizing influences of organic ligands and polymers.

Refractory organic colloids and non-labile complexes may also undergo a gradual 'dissolution' and tend toward a more hydrophobic condition. This process would involve the expulsion of water of hydration from the polymer structure during coiling and hence a reduction in solvation. A mechanism of this nature may account for the progressive reduction in the polydispersity of humic and fulvic acids already described. The rapid precipitation, under estuarine conditions of organo-metallic species of iron, associated with the macro-molecular fractions, demands further explanation (Sholkovitz, 1976). Humic acids upon admixture with the saline water of an estuary will undoubtedly suffer a syneresis in the presence of the high chloride ion concentration. The molecular size measurements of Brogden (1971) and Mantoura (1976, personal communication) for humic acids in estuarine conditions record a marked reduction in apparent molecular weight which is possibly associated with molecular coiling and shrinkage. Precipitation of peptized and complexed cations with humic substances would then be expected to occur as a result of molecular bridging and flocculation. A precipitation of humic and fulvic acid complexes would also be expected from the known

chemistry of these compounds. The ionic strength of sea water is approximately 0.7, assuming a salinity of 35‰ at 25°C. Schnitzer and Hansen (1970) have investigated the effect of ionic strength (μ) on the stability of fulvic acid complexes. Over the range from 0.0 to 0.15 μ Fe(III) and Al(III) complexes exhibit the greatest stability but when the ionic strength approximates to 0.2 these and other complexes precipitate. From this argument it would seem reasonable to suggest that iron in either a peptized, colloidal form or a simple organic complex will be precipitated within an estuary.

3.1.4 Size Fractionation of Organo-metallic and Colloidal Iron Species

The distribution of size fractions of 'soluble' iron and the molecular size fraction of humic acid in natural waters suggests a correlation between the polydispersity of these organic acids and 'filterable' iron. Black and Christman (1963), using membrane filtration, observed that most of the coloured, colloidal suspension in rivers is found in the size fractions of $<0.01\mu\text{m}$. The percentage of the dispersion retained by a $0.1\mu\text{m}$ filter varied from 4 to 38% but the dialyzate of a $0.0048\mu\text{m}$ membrane filter frequently concentrated 90% of the organic colour. In contrast, Shapiro (1964) found that most of the 'soluble' iron in lake water could be removed by filtration through a $0.1\mu\text{m}$ membrane and concluded that the colloidal species of iron remain in the 0.1 to $0.22\mu\text{m}$ size range. These results are in good agreement with the work of Lamar (1968) who reported the levels of iron occurring in submicron size fractions

of two samples of highly coloured water from Florida and Alaska. The peptization of iron by humic acids is suggested by Shapiro (1964) to account for these observations and is also implicit in Lamar's later conclusions. More recent studies on the separation of macromolecular iron species by ultrafiltration indicate that the iron occurring in these fractions may, at least in part, be complexed by humic acids (Tryland and Gjessing, 1975). The addition of EDTA in dilute solution to filtered samples is sufficient to liberate approximately 2/3 of the iron from the dialyzate of an Amicon UM-10 ultrafiltration membrane - nominal molecular weight cut-off 10,000. This filterable fraction is also pH dependent and ^{this} ^{for} may account ^{for} the low retention of colloidal iron reported by Black and Christman (1963) for 0.1 μ m membranes.

Interpretation of these observations in terms of either colloidal peptization or organic complexation need not be mutually exclusive since both are compatible with the known behaviour and postulated structure of humic substances. These compounds are envisaged as a broken network of poorly condensed aromatic ring structures punctured by voids or holes of various dimensions which may entrap organic molecules and inorganic metals and their oxides. Interactions with peripheral functional groups may also result in the complexation of cations (Schnitzer and Khan, 1972). Size fractionation of these organo-metallic species is clearly an important prerequisite to the study and rational interpretation of the complicated processes involved in the geochemistry of iron and other important trace metals.

3.2 SEPARATION OF COLLOIDAL IRON FROM RIVER WATER BY ULTRAFILTRATION

3.2.1 Sampling and Analysis

A preliminary investigation of colloidal iron and associated trace metals in the Tamar River was carried out from July to October 1974. During this period the river was sampled at the five stations described in Chapter I. Four rivers, including the Tamar, were later sampled at strategic points to provide samples from a broader range of polluted and uncontaminated conditions. All water samples were refrigerated or stored deep-frozen until required for analysis.

Each sample was filtered through a rinsed, acid-washed Whatman (GF/C) glass-fibre filter to remove suspended particulate matter. A one litre aliquot of this primary filtrate was acidified to pH 1 and retained deep-frozen for later analysis. The bulk of the filtrate was passed through a rinsed, acid-washed PM-10 ultra-filtration membrane. One litre of this secondary ultrafiltrate was acidified and kept for analysis. The concentrated colloidal material isolated during filtration was dialyzed with 100ml of deionised water and recovered from the membrane surface. The acidified filtrate aliquots were analysed for trace metals using a chelation-solvent extraction technique (Applied Geochemistry Research Group, Technical Communication No. 62, 1975). In this procedure chloroform extracts of diethyl dithio-carbamate complexes recovered from a sample are acidified with 2ml of concentrated 'Ultragrade' nitric acid and evaporated. The residue from each extraction was taken up in either 5 or 10ml of 1M Ultragrade hydrochloric acid and analyzed using

a Perkin Elmer 403 atomic absorption spectrophotometer.

3.2.2 Results

The concentration in the Whatman (GF/C) filtrate of each sample may be regarded as a gross analysis of all the 'soluble' iron species. Filtration through an Amicon PM-10 ultrafiltration membrane effectively concentrates and removes the colloidal and macromolecular iron forms from solution. The concentration of iron in this secondary filtrate may therefore be regarded as the concentration of the non-colloidal, or dialyzable forms. The colloidal iron concentration is calculated from the difference in the concentration found in the total 'soluble' fraction of the primary filtrate and that passing into the secondary ultrafiltrate.

3.2.2.1 The Tamar River 1974/1975

The concentration of the total 'soluble' iron together with the concentration of the non-colloidal, dialyzable fractions found during the summer of 1974 and the winter of 1975 are shown in figures 3.1 and 3.2. Total 'soluble' levels in this drainage ranged from $7 \mu\text{g l}^{-1}$ at the tidal limit to as high as $500 \mu\text{g l}^{-1}$ in the upper reaches of the river at Crossgate. The dialyzable fraction occurring in the PM-10 ultrafiltrate, which perhaps includes a predominance of species that are genuinely in solution, occurred in a relatively narrow range of concentrations (5 to $35 \mu\text{g l}^{-1}$). The variation in the concentration of total 'soluble' iron during the period of this study was considerable although the highest levels were recorded

TABLE 3.1

Colloidal Iron as Percentage of Total 'Soluble' Concentration in Tamar River (1974/75)

Sampling Point	M O N T H								
	5/74	7/74	8/74	9/74	10/74	2/75	3/75	4/75	6/75
Crossgate	-	97.0	92.6	-	92.7	64.0	65.0	63.3	80.3
Launceston	98.6	80.0	91.8	94.0	-	30.8	14.3	53.8	50.0
Gunnislake	-	92.8	94.5	91.2	93.2	14.3	50.0	37.8	70.8
Calstock	-	77.5	87.3	90.0	91.0	50.0	76.5	62.1	81.5

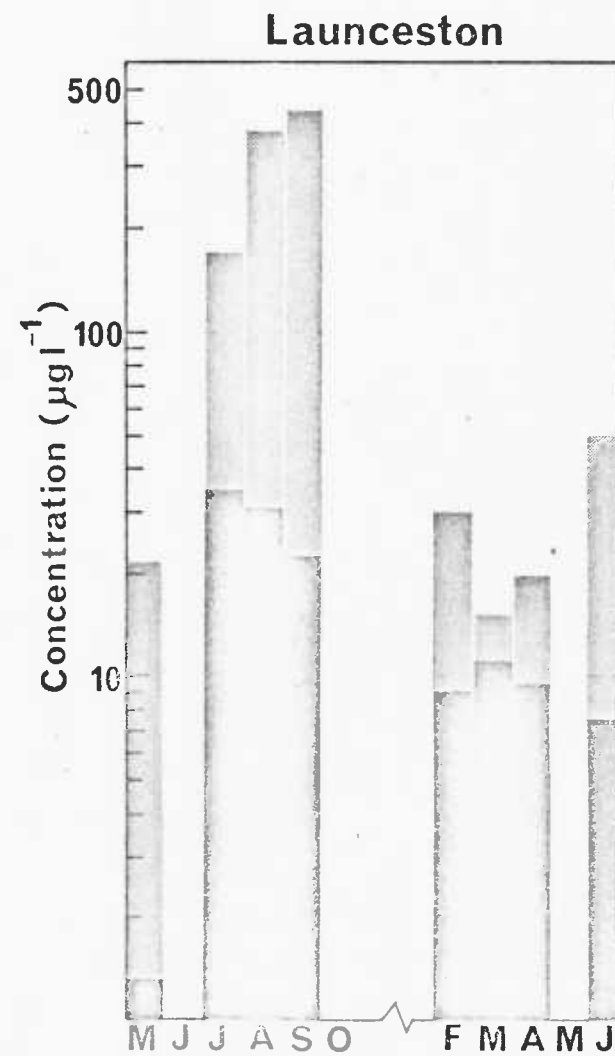
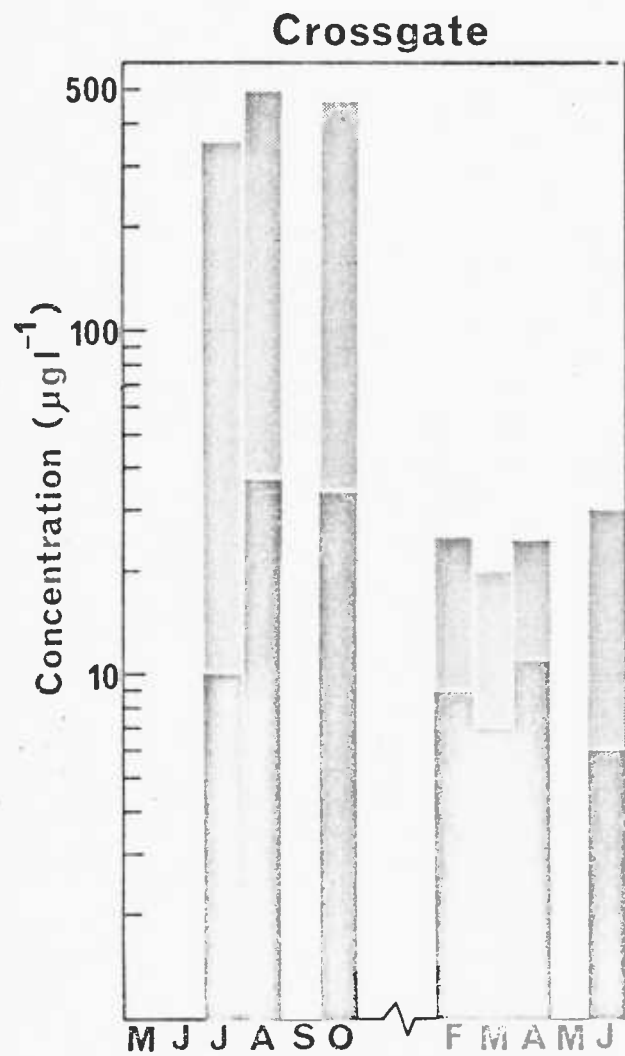


Fig. 3.1 Colloidal and 'soluble' iron concentrations in the Tamar River 1974/75
 (colloidal soluble)

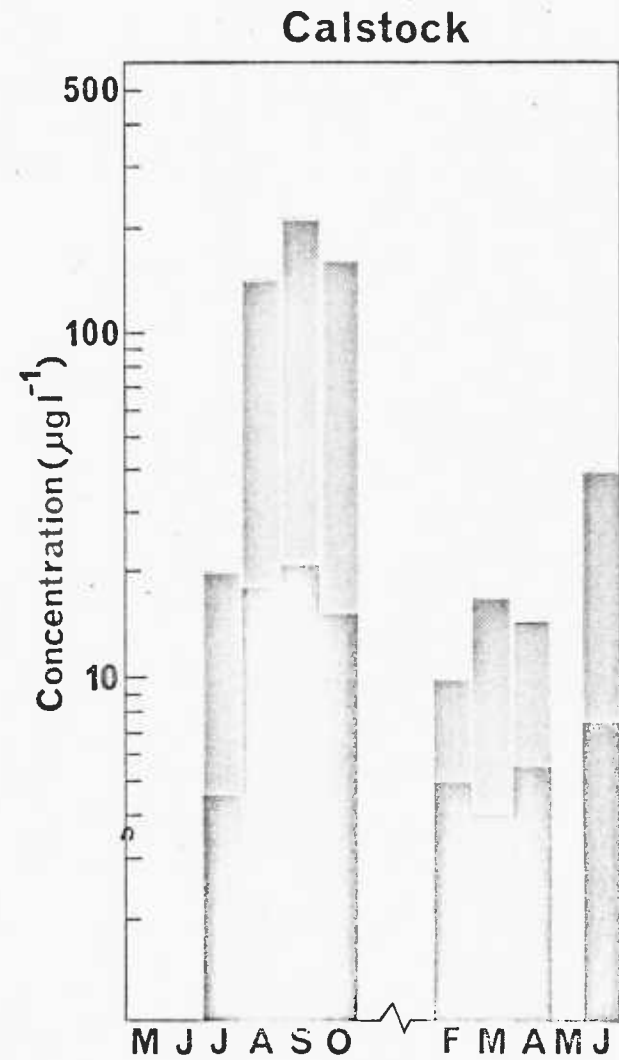
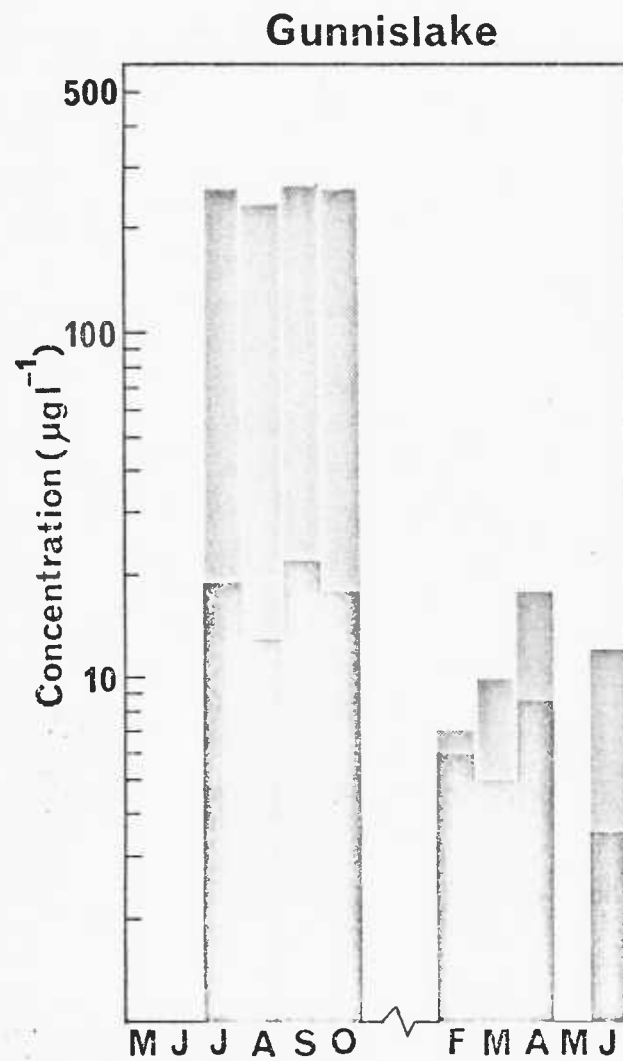
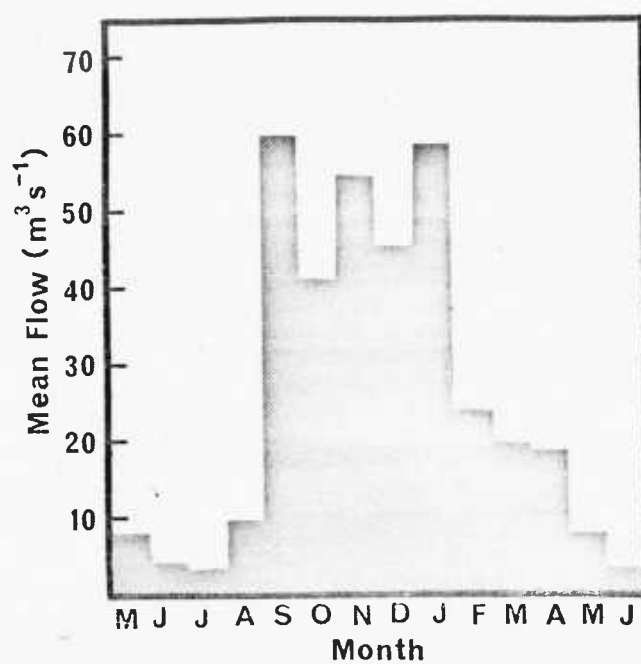


Fig. 3.2 Colloidal and 'soluble' iron concentrations in the Tamar River 1974/75
 (■ colloidal ■ soluble)

(a)



(b)

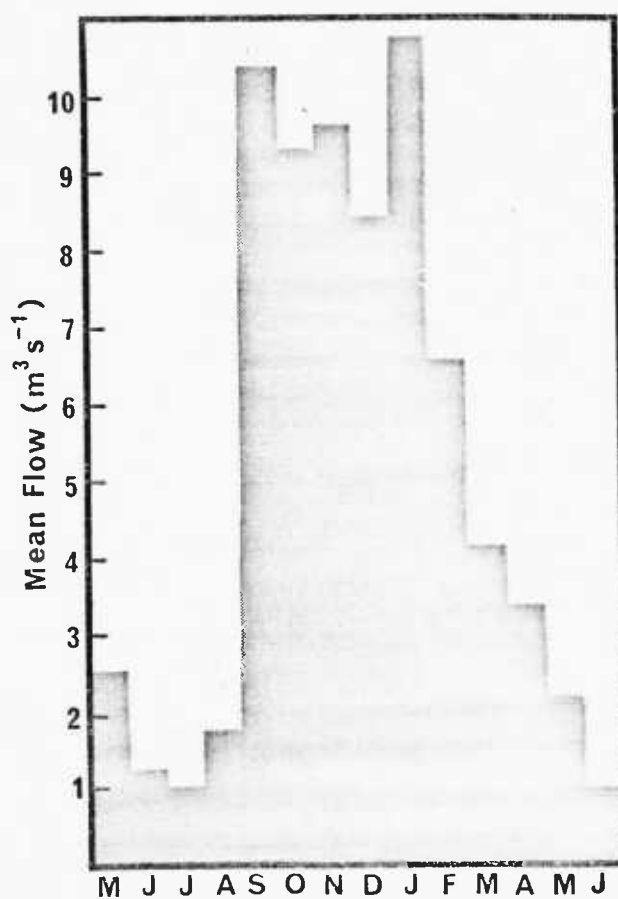


Fig. 3.3 (a) Mean Tamar River discharge (Gunnislake May 1974-June 1975)

(b) Mean Fowey River discharge (Restromel Two May 1974-June 1975)

during the summer months with the influx of a high percentage of colloidal material. A marked reduction in mean concentration and percentage colloidal iron is evident during the early months of 1975. An inverse relationship between total 'soluble' iron and river flow (fig. 3.3) is perhaps also indicated from this study. Both the total concentration and the percentage of colloidal iron diminished progressively downstream to the tidal limit at Gunnislake. Within the upper estuary, marked by the Calstock station, the proportion of colloidal iron in the total concentration again increases. However, no evidence for the passage or formation of a colloidal dispersion in the open estuary at Saltash could be found using this technique.

3.2.2.2 The River Fowey 1975

Trends similar to those outlined above can be seen in the Helford and the River Fowey, however, sampling of these catchments was not extensive and some stations were sampled on only four occasions. In the upper reaches of the Fowey the highest concentrations and the greatest percentage of colloidal iron were found at Dozmary Pool and East Colliford (fig. 3.4). A gradual reduction in the total concentration is observed downstream particularly on the lower moor and in low land regions which are typified by the examples of Quarry Wood and Lostwithiel. The general fall in the amount of 'soluble' iron found in this drainage in later months may be correlated with reduced run-off (fig. 3.3) and a consequent decline in the concentration of humic acid complexes discharged into the moorland catchment. The proportion of colloidal iron occurring at each station

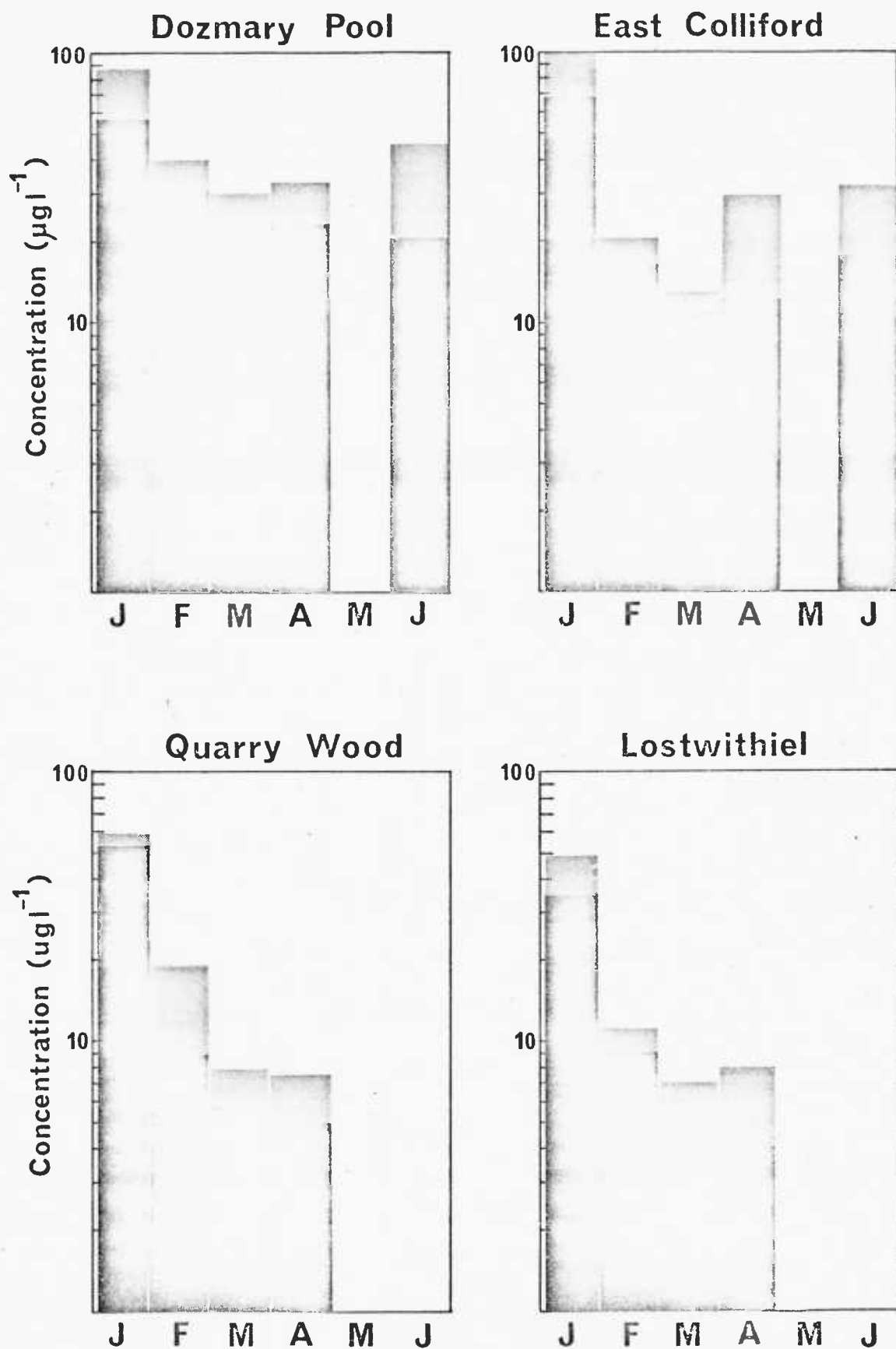


Fig. 3.4 Colloidal and 'soluble' iron concentration in the moorland catchment of the River Fowey 1975 (■ colloidal ■ soluble)

in this river does not show obvious trends but was commonly between 20 and 40% of the total concentration (Table 3.2).

3.2.2.3 The Helford and the River Carnon 1975

The tributaries of the unmineralized Helford valley are typified by the stations at Constantine and Trelowarren. The estuary was always sampled on a low tide at Gweek Quay. The range of the total concentration of iron found in this catchment was 6 to 38 $\mu\text{g l}^{-1}$ (fig. 3.5). Some colloidal iron was detectable in all samples and at Trelowarren, 27 to 46% of the total 'soluble' concentration was non-dialyzable. A greater variation in the percentage of this colloidal fraction was found at Constantine and in the estuary. Only low concentrations were found in the river discharge at Gweek Quay and on only two occasions was a significant percentage of colloidal material determined.

Remarkably, there was no colloidal iron in the River Carnon at the three points sampled: Twelve Heads and Bissoe, as well as Devoran which marks the limit of tidal influence in Restronguet Creek. Some of the highest total iron concentrations recorded during this investigation were found at Bissoe and Devoran (fig. 3.6). A negligible uv absorbance at 250 nm was a characteristic feature of waters from this drainage unlike the organically enriched conditions of many of the other localities sampled. The localized concentration of iron at Bissoe which lies below the Great County Adit suggests that these elevated levels are associated with acidic mine drainage from this area. The wide variation in concentration from month to

TABLE 3.2

Colloidal Iron as Percentage of Total 'Soluble' Concentration
in some Rivers and Estuaries in the South-west of England

River and Sampling Point	M O N T H				
	1/75	2/75	3/75	4/75	6/75
	%	%	%	%	%
<u>River Fowey:</u>					
Dozmary Pool	34.0	22.5	44.2	-	53.3
East Colliford	30.0	22.7	15.4	59.7	46.3
Quarry Wood	6.0	30.0	28.8	33.3	-
Cutmadoc	9.6	ND*	-	-	-
Lostwithiel	28.0	18.0	ND*	50.0	ND*
Wilake	35.7	-	ND*	23.5	-
Penpoll	25.0	-	-	-	-
<u>River Carnon:</u>					
Twelve Heads	ND*	ND*	ND*	ND*	ND*
Hicks Mill	ND*	-	-	-	-
Bissoe	ND*	ND*	ND*	ND*	ND*
Perranwell	ND*	-	-	-	-
Devoran	ND*	ND*	ND*	ND*	ND*
<u>River Helford:</u>					
Constantine	7.1	33.0	13.3	31.7	29.8
Gweek Mill	26.3	-	-	-	-
Gweek Quay	18.2	ND*	43.0	ND*	-
Trelowarren	31.6	46.2	27.3	38.2	28.6

* None Detectable - ND

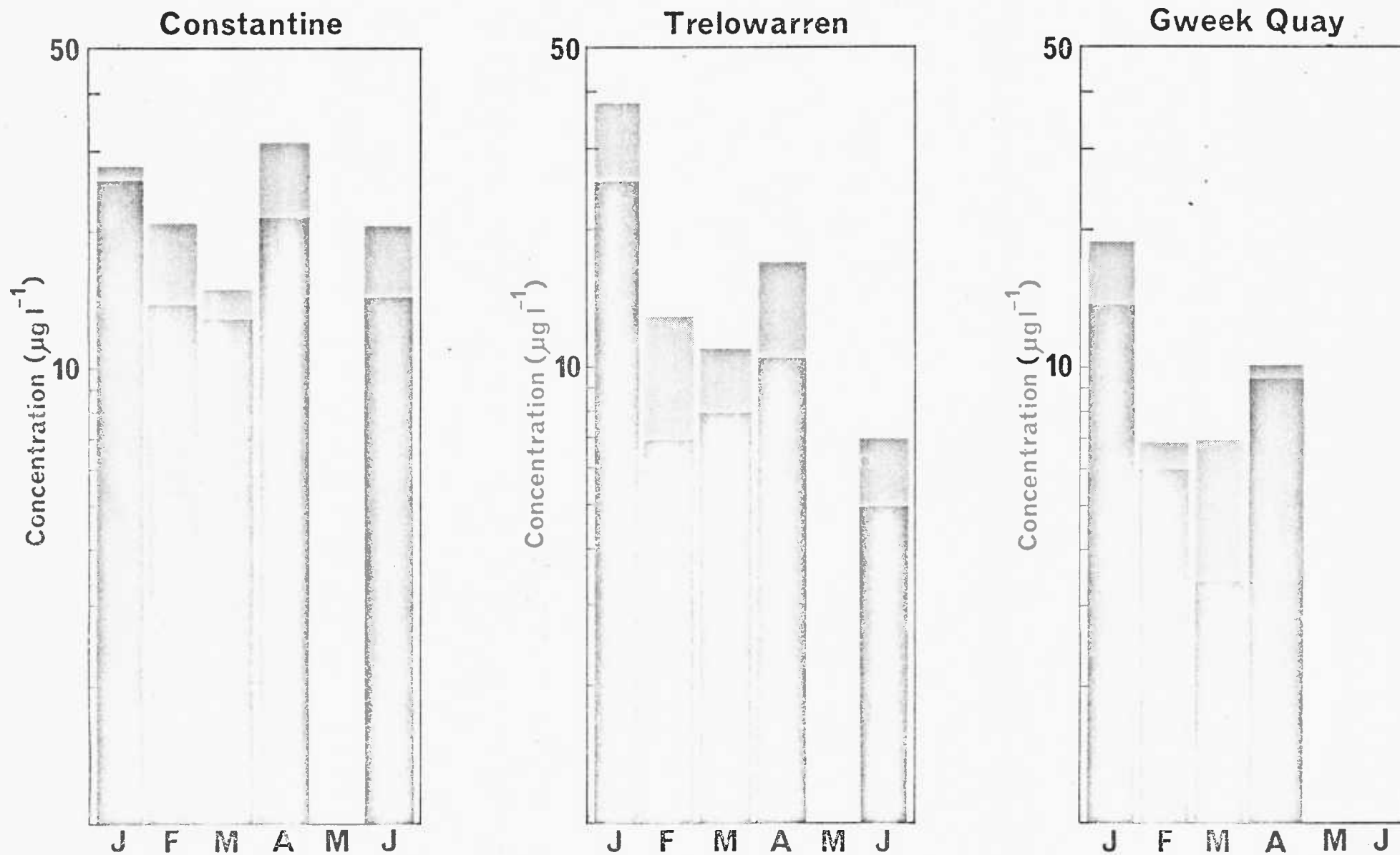


Fig. 3.5 Colloidal and 'soluble' iron concentrations in the Helford Valley drainage basin 1975
 (■ colloidal ■ soluble)

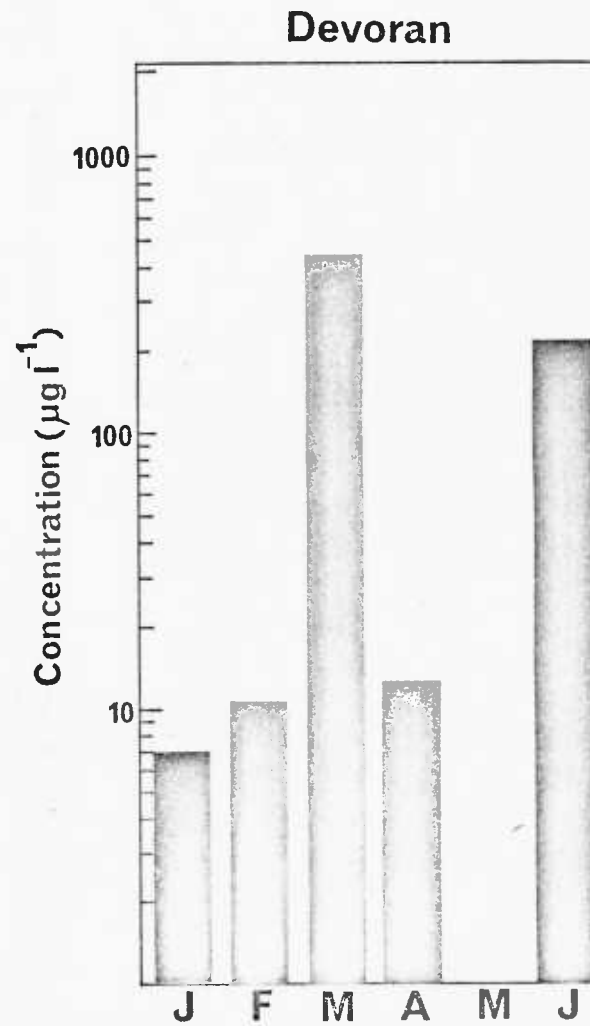
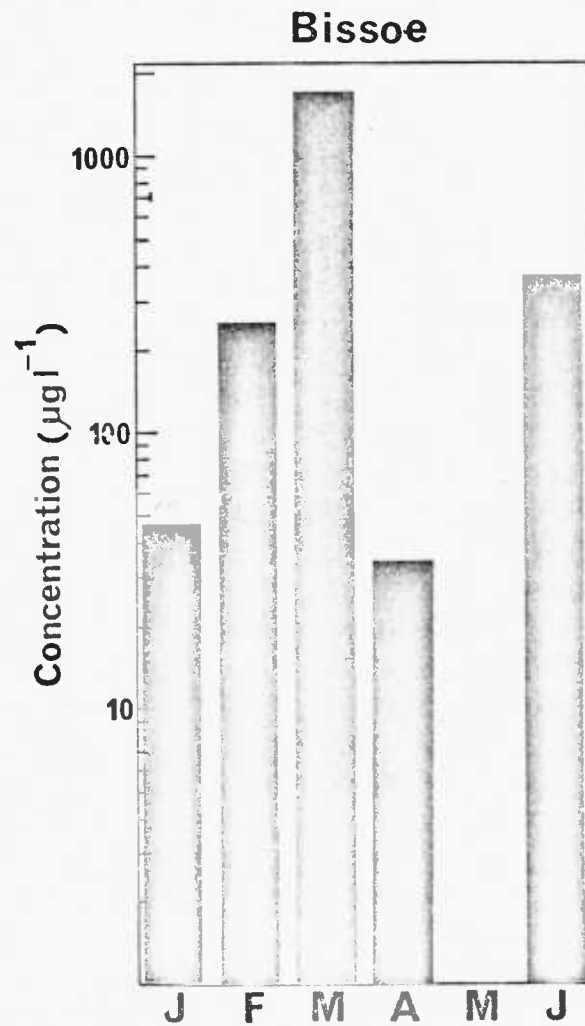
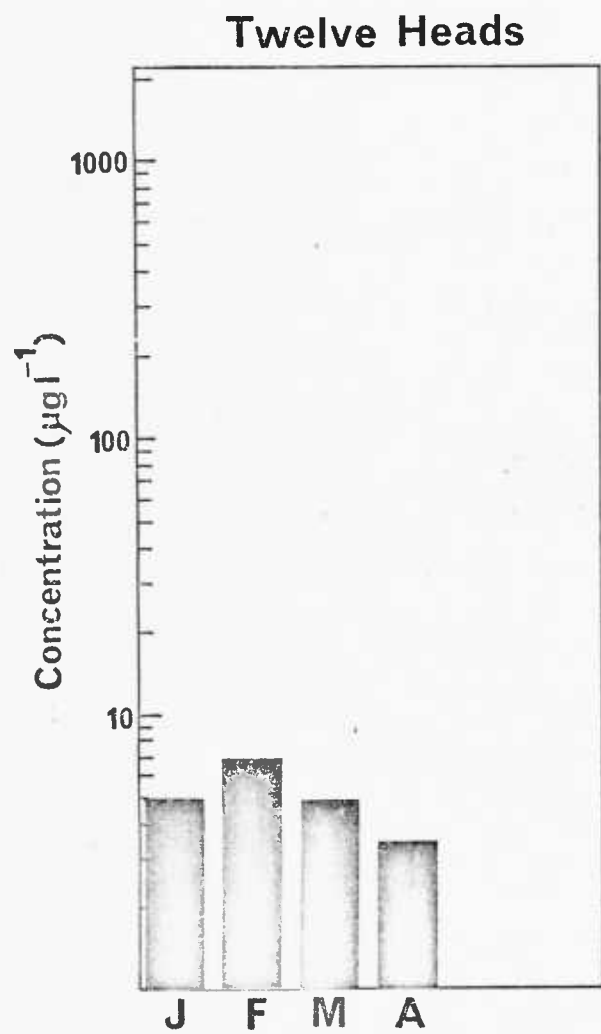


Fig. 3.6 Soluble iron concentrations in the River Carnon 1975

month is probably a reflection of discontinuous discharge and variations in river flow.

3.3 DISCUSSION AND CONCLUSIONS

The levels of total 'soluble' iron found in the Tamar during this study are mostly in agreement with previous findings and the reports of organically bound forms occurring in these waters (Butler and Tibbitts, 1972; Bryan and Hummerstone, 1973). Seasonal variations in the concentration of 'soluble' iron at a fixed station in the Tamar estuary have been described previously (Butler and Tibbitts, 1972). Monitoring a point at the confluence of the River Tavy with the Tamar it has been possible to show that an unreactive, organically bound iron fraction is liberated from filtered samples upon uv irradiation. Butler and Tibbitts (1972) have also demonstrated a good correlation between uv absorbance at 260 nm and the concentration of iron.

'Soluble' iron concentration in the Tamar estuary have been found to vary from 3 to 50 $\mu\text{g l}^{-1}$ (Butler and Tibbitts, 1972) and from 5 to 75 $\mu\text{g l}^{-1}$ (Bryan and Hummerstone, 1973); these results are largely substantiated by the observations in this study. Nevertheless, in contrast to the findings of Butler and Tibbitts (1972) the highest concentration determined over the period 1974/75 was found during the period from July to October 1974. Relatively low concentrations were observed during the winter months from February to May 1975

^{these}
 and [^]do not significantly differ from the data of Butler and Tibbitts. The differences observed in the present study may be the result of the variable influences of such factors as river flow and run-off which will differ from year to year. In addition the moorland drainage discharged by the River Tavy may have influenced the conditions at the point sampled in this previous study.

A bound, macromolecular iron species was also found in the Helford and the River Fowey. The highest concentration of iron was found in the moorland streams at Dozmary Pool and East Colliford on the northern catchment of the Fowey. The percentage of colloidal iron present in these waters ranged from 15 to 60% and was a characteristic feature of these organically enriched streams. Colloidal iron with a 'molecular weight' $>10,000$, has also been found in similar conditions by Gjessing (1970), but is reported here to occur as more than 90% of the total concentration. A marked reduction in the total and colloidal iron concentrations at low land stations can clearly be seen (fig. 3.4; Table 3.1) and is in accordance with observations made on similar peat drainage (Tryland and Gjessing, 1975).

Among the tributaries of the Helford River the highest concentrations of iron were found at Constantine which receives the organically enriched drainage from the Carmanellis Granite. Colloidal iron levels ranged from 2 to $10 \mu\text{g l}^{-1}$ and from 7 to 33% of the total concentration at this point. A greater percentage of non-dialyzable iron was found at Trelowarren (fig. 3.5), but not unexpectedly total and colloidal iron concentrations were most variable in the estuary at

Gweek Quay. On two occasions no colloidal iron was detectable in the waters from Gweek Mill, however, in February 43% of the total concentration was found to be colloidal. These findings are somewhat at variance with the results of a previous survey of the area in which typical concentrations of total 'soluble' iron were found to be in excess of $29 \mu\text{g l}^{-1}$ with a mean of $396 \mu\text{g l}^{-1}$ (Thornton, Watling and Darracott, 1975). A mean concentration of $217 \mu\text{g l}^{-1}$ Fe for the Helford estuary was also reported in this previous work.

During the present investigation a broad range of concentrations (5 to $1750 \mu\text{g l}^{-1}$) was found in the Carnon River in agreement with previous findings (Bryan and Hummerstone, 1973; Thornton, Darracott and Watling, 1975). However, no colloidal or organically bound iron was *detected* in this drainage (fig. 3.6).

Iron found in the dialyzable fraction of less than 10,000 'molecular weight' for most samples rarely exceeded $25 \mu\text{g l}^{-1}$ and commonly approached $10 \mu\text{g l}^{-1}$. Inorganic iron species may account for the occurrence of these trace levels apparently in true solution. Organically bound iron remaining in the ultrafiltrate probably accounts for the higher concentrations of 'dialyzable' iron found in the waters of the Fowey and Tamar (Chapter 5). However, the concentrations of soluble, non-colloidal iron in excess of 1.0 ppm found in the Carnon River require explanation. These waters contain little organic material in solution and it is therefore unlikely that low molecular weight species of complexed iron are present. Nevertheless, the elevated levels are clearly localized and vary

over a wide range of concentrations (Bryan and Hummerstone, 1973; Thornton, Watling and Darracott, 1975). Rich iron coatings are a feature of the stream sediments in this drainage which is dominated by the continuing mining activity in the area. The discontinuous discharge of iron enriched mine drainage into this stream together with fluctuating river flow probably accounts for the high but variable concentrations of unbound iron (Lamar, 1968; Hem, 1972).

The findings of this study are in broad agreement with previous work and have shown that a significant fraction of the iron occurring in natural waters is colloidal and organically bound (Shapiro, 1964; Lamar, 1968; Gjessing, 1970; Tryland and Gjessing, 1975). These complexes are clearly sufficiently refractory to resist reduction by hydroxylamine and hydrochloric acid (Butler and Tibbitts, 1972). However, both EDTA (Tryland and Gjessing, 1975) and diethyl dithiocarbamate seem effective in chelating a significant proportion of the iron apparently complexed by humic substances in moorland streams. The amount of colloidal iron reported in swamps and peaty drainage is very variable but in extreme conditions 80 to 95% of the iron occurring in 'solution' may be colloidal (Lamar, 1968; Gjessing, 1970). A similar proportion of the iron in the upper reaches of the Tamar river is apparently transported as a discrete, colloidal dispersion. However, the macromolecular fractions of moorland drainage, typified by the River Fowey, also include a significant but smaller percentage of the total concentration of iron.

The precipitation of iron within the estuaries investigated

in this study has already been suggested (Bryan and Hummerstone, 1973). No organic complexes could be detected in the Tamar estuary either during this work or by Butler and Tibbitts (1972); this absence perhaps provides some evidence for the precipitation of these organo-metallic species under saline conditions.

4. MÖSSBAUER SPECTROSCOPY OF FULVIC ACID-IRON COMPLEXES

4.1 INTRODUCTION

The association between iron and fulvic or humic acids and the oxidation state of the iron which is maintained in these refractory compounds remains a matter for speculation. A resolution of this uncertainty does present considerable analytical problems which may be largely overcome with the use of Mössbauer Spectroscopy - Nuclear Gamma Resonance Spectroscopy (NGR) (Wertheim, 1964; May, 1971). The Mössbauer Effect discovered in 1957 refers to the increased scattering of gamma rays by an absorber or target when the free-atom recoil energy is less than the characteristic quantized energy of lattice vibration. The gamma ray absorption spectrum of an absorber is found to be sensitive to a change in radiation source or absorber. Mössbauer or NGR spectroscopy is essentially the study of absorption spectra of recoilless gamma ray emission.

Classical Mössbauer measurements are recorded for many of the model organic and inorganic compounds of tin and iron, but among the transition elements the effect has also been observed with nickel and zinc (Danon, 1966, 1971). In conjunction with other spectroscopic methods, NGR spectroscopy provides a powerful tool in stereochemical studies of iron and a number of other elements. The technique facilitates a detailed examination of structural variations around a nucleus and its electronic configuration and is a method sensitive to the symmetry and nature of the ligands which form the immediate environment of the atom (Duncan and Golding, 1965). Structural

CHAPTER 4

variations of the Fe(II) state may be correlated with electronic spectral measurements and interpreted in terms of Fe(II) to Fe(III) charge transfer for compounds of mixed oxidation states. Mössbauer measurements may distinguish various simultaneously occurring phases and within these phases various molecular environments (Georgescu and Nistor, 1973). NGR methods for estimating Fe(III) to Fe(II) ratios and Fe(II) population sites from peak areas may provide useful information on the stages of an oxidation-reduction process and its products.

The diagnostic parameters of NGR spectroscopy are complex but the purpose of any spectrometer is to produce a known and precise energy shift to a point of resonance - the so called isomer shift. Using the same gamma-ray source resonance is achieved between a reference absorber and the absorber of interest. The standard procedure, using constant velocity or constant acceleration spectrometers, exploits a Doppler shift induced by a relative motion between the source and the absorbing sample. In this way a narrow energy band of gamma ray emission is generated which includes the resonant frequency or velocity of the absorber. The change in resonance observed with different compounds of the same element reflect differences in the charge density about the atom, which influence its nuclear resonance. The resonance velocity measurement is particularly sensitive to the configuration of the valency electrons, which in the case of iron involve the 3s, 3p, 3d and 4s electron shells. The electronic configuration of these orbitals determines the charge density of these and inner shells at the nucleus which responds to variable

shielding effects of the valency electrons. Energy levels within the nucleus are influenced by the changes in the electrostatic interactions between the nuclear and electronic charges.

A simple isomer shift is only observed when the charge density about the nucleus is uniform. A deviation from this condition will arise with an asymmetric charge distribution at the nucleus. These multipolar electrostatic interactions result in a redistribution of charge and changes in the configuration of nuclear energy levels. Under these conditions gamma-ray resonance absorption is observed as a quadrupole splitting or division of the resonance velocities. The extent of this splitting is correlated with the number of substates of the nuclear energy levels. In the case of ^{57}Fe there are as many as six peaks to a symmetrical spectrum. Such a system becomes more complex with the development of a magnetic hyperfine structure commonly illustrative of magnetic splitting of the nuclear energy levels. This type of spectrum is found with natural iron and its alloys as well as with compounds of Fe(II) such as FeF_2 . The magnetic hyperfine structure reflects the transition of a gamma-ray from one particular magnetic sublevel of an excited nuclear state to a sublevel of the ground state.

NGR spectroscopy is clearly a method which is highly sensitive to the chemical condition of iron in many compounds and is therefore eminently suitable for the study of the complex Fe speciation in humic substances.

4.2 INSTRUMENTATION OF NUCLEAR GAMMA RESONANCE SPECTROSCOPY

The instrumental methods of NGR spectroscopy have been outlined by Spijkerman (1971) and the more advanced developments in this field have been reviewed by Kalvius and Kankeleit (1972). Most commercially available instruments are electro-mechanical spectrometers coupled with a multichannel analyzer; in general a spectrometer consists of two main parts: a gamma-ray detection and recording system and a Doppler velocity source drive.

Mössbauer sources are not monochromatic and emit radiation energies which are both higher and lower than that of the recoilless or Mössbauer gamma-ray. The detection system employed must only record the Mössbauer radiation; pulse proportional counting is commonly used and is particularly appropriate for radiation energies in the range 1-20 KeV characteristic of iron NGR studies with a ^{57}Co source. The high impedance of the detector necessitates the use of a preamplifier coupled to the linear amplifier of the system. The peak voltage of the generated pulse is proportional to the gamma-ray energy detected. This signal is modulated using a single channel analyzer (SCA) to provide a preset band pass or 'window' for the selection of gamma-ray energies of interest.

A multichannel analyzer (MCA) coupled with a Doppler spectrometer is a common method used to record NGR data. Synchronization between the source drive unit and the MCA is achieved by means of a control computer or function generator (MFG), which effectively acts as an interfacing mechanism responsible for storage, storage

clearance and memory addressing of the MCA. The MFG produces a sawtooth or triangular waveform from the switching of the MCA subgroups which in turn drives the power amplifier of the linear transducer^S to the source. The motion of the resultant source drive describes a double parabola producing a double mirror image spectrum for display on a cathode ray screen.

The instrument used in this study was essentially modular in design with a Nuclear Data 2200 Multichannel Analyzer and Elscint Mössbauer Function Generator MFG3. A constant acceleration spectrometer was used with a ^{57}Co source mounted on an Elscint Linear Velocity Transducer Model MVT-3 and driven by an Elscint Transducer Drive Unit Model MD-3. A Reuter Stokes RSG-61-M9 pulse proportional counter was used in the detection system with a Canberra 1406 preamplifier and Canberra 816 Pulse Amplifier. A high voltage supply to the preamplifier was provided by a Nuclear Data HVS Model ND-537, and the single channel analyzer used in this system was the Nuclear Data SCA Model ND-522. A Ricor TC4B Temperature Controller was used during this study and the cryogenic spectra were obtained using a Ricor MCH5 Cryostat.

4.3 PREPARATION OF FULVIC ACID-IRON COMPLEXES FOR MÖSSBAUER SPECTROSCOPY

Mössbauer experiments are almost invariably carried out using solid sources and absorbers in order to optimize the signal to noise ratio, the resonance effect which can be observed being related to the recoil-free fraction of the emission. The magnitude of the

observed effect is a negative, exponential function of the mean square amplitude of the Mössbauer atom about its lattice site. In liquids the vibrational amplitude is sufficiently large as to obscure the Effect.

A fulvic acid fraction was extracted from a sample of Dozmary Pool water. The particulate and colloidal material was removed by filtration through a glass-fibre filter followed by ultrafiltration with an Amicon PM-10 membrane. The sample was finally extracted and dialyzed using an Amicon UM-05 membrane. The concentrated dialyzate was freeze-dried.

Fulvic acid extracts were obtained from two peat samples and used to provide reference spectra. A 500 g sample of Bodmin peat taken at Dozmary Pool was extracted with a 1M solution of Analar sodium hydroxide. The extract was centrifuged and filtered to remove particulate debris (Overstreet, 1966). The filtrate was acidified with 1M hydrochloric acid to precipitate humic and hymatomelanic acids which were recovered by centrifugation and filtration. This procedure was repeated to ensure purification of the supernatant fulvic acid solution and complete precipitation of all other humic fractions. The final supernatant was filtered through a rinsed, acid-washed GF/C filter and concentrated and dialyzed using an Amicon UM-05 ultrafiltration membrane. The concentrated dialyzate was freeze-dried. A second fulvic acid sample was obtained from a Lake District peat in a similar manner. With this extract the final supernatant was filtered, acidified to pH 2.2 and concentrated on a macroreticular

resin - Amberlite XAD-2. The concentrated fulvic acid was eluted in ethanol which was allowed to evaporate before the residue was freeze-dried.

0.27 g cm⁻² absorbers of each peat extract were obtained but an absorber density of only 0.055 g cm⁻² could be provided with the material available from the UM-05 dialyzate of Dozmary Pool water. The Mössbauer parameters of each sample were determined on the solid extracts. Each complex was packed into the hole of a Plexi-glass slide wafered between strips of 'Scotch' tape. All spectra were recorded at a temperature of 80 K but cryogenic spectra were also obtained for the Dozmary Pool dialyzate at 180 and 230 K. A commercial source of ⁵⁷Co in copper was used. The spectra were computed using an iterative, least-squares fitting program with a non-linear function of Lorentzian peaks and parabolic baselines. Only a low percentage absorption was recorded for each complex which did not permit the use of any back-ground correction. The values of the Mössbauer parameters measured in these experiments are given in Table 4.1, the corresponding spectra are shown in fig. 4.1 and fig. 4.2. Peak high and half width values for a natural iron calibrant (absorber density 10 mg cm⁻²) are also given for comparison.

4.4 DISCUSSION AND CONCLUSION

Few NGR spectra of humic or fulvic acid complexes have been published, but the technique has been used to study the nuclear quadrupole relaxation (NQR) of ⁸⁵Rb in aqueous solutions of humic acids (Lindman and Lindquist, 1969; Lindquist and Lindman, 1970). An appreciable

TABLE 4.1
Mössbauer Parameters of Fulvic Acid-Fe(III) Complexes

SAMPLE	Peak Position mm sec ⁻¹	Half Width mm sec ⁻¹	Absorption %	Temperature K
Lake District Fe(III) - Fulvic Acid Peat Extract	0.295 ± 0.026	0.3159 ± 0.481	0.17 ± 0.01	80
Dozmary Pool Fe(III) - Fulvic Acid Peat Extract	0.283 ± 0.064	0.4016 ± 0.128	0.14 ± 0.02	80
Amicon UM-05 Dialyzate (Molecular Weight <10,000 >500) Extracted from Dozmary Pool Water	0.258 ± 0.040	0.3587 ± 0.077	0.13 ± 0.02	80
	0.184 ± 0.037	0.3548 ± 0.071	0.15 ± 0.02	180
	0.311 ± 0.052	0.4057 ± 0.05	0.15 ± 0.02	230
Natural Iron Standard (Absorber density 10 mg cm ⁻²)	-3.053 ± 0.001	0.1237 ± 0.0015	8.53 ± 0.06	80
	-0.838 ± 0.002	0.1187 ± 0.0027	4.39 ± 0.06	80
	0.838 ± 0.002	0.1244 ± 0.0028	4.39 ± 0.06	80
	3.050 ± 0.001	0.1245 ± 0.0014	8.38 ± 0.05	80

MOESSBAUER SPECTRA OF PEAT MATERIALS - FULVIC ACID/FE EXTRACTS AT 80K

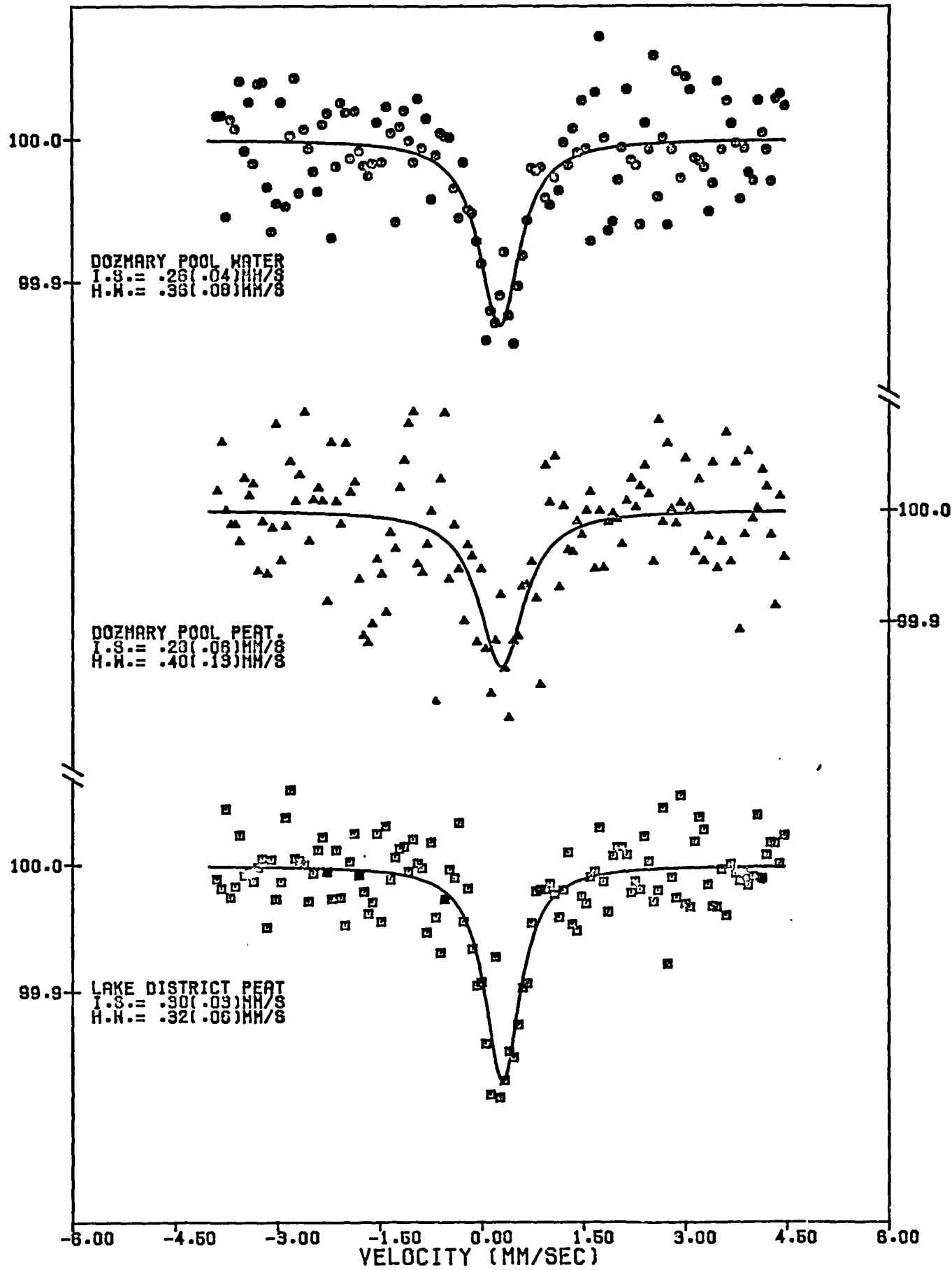


Fig. 4.1

MOESSBAUER SPECTRA - FULVIC ACID/FE (DOZMARY POOL WATER EXTRACT UM-05)

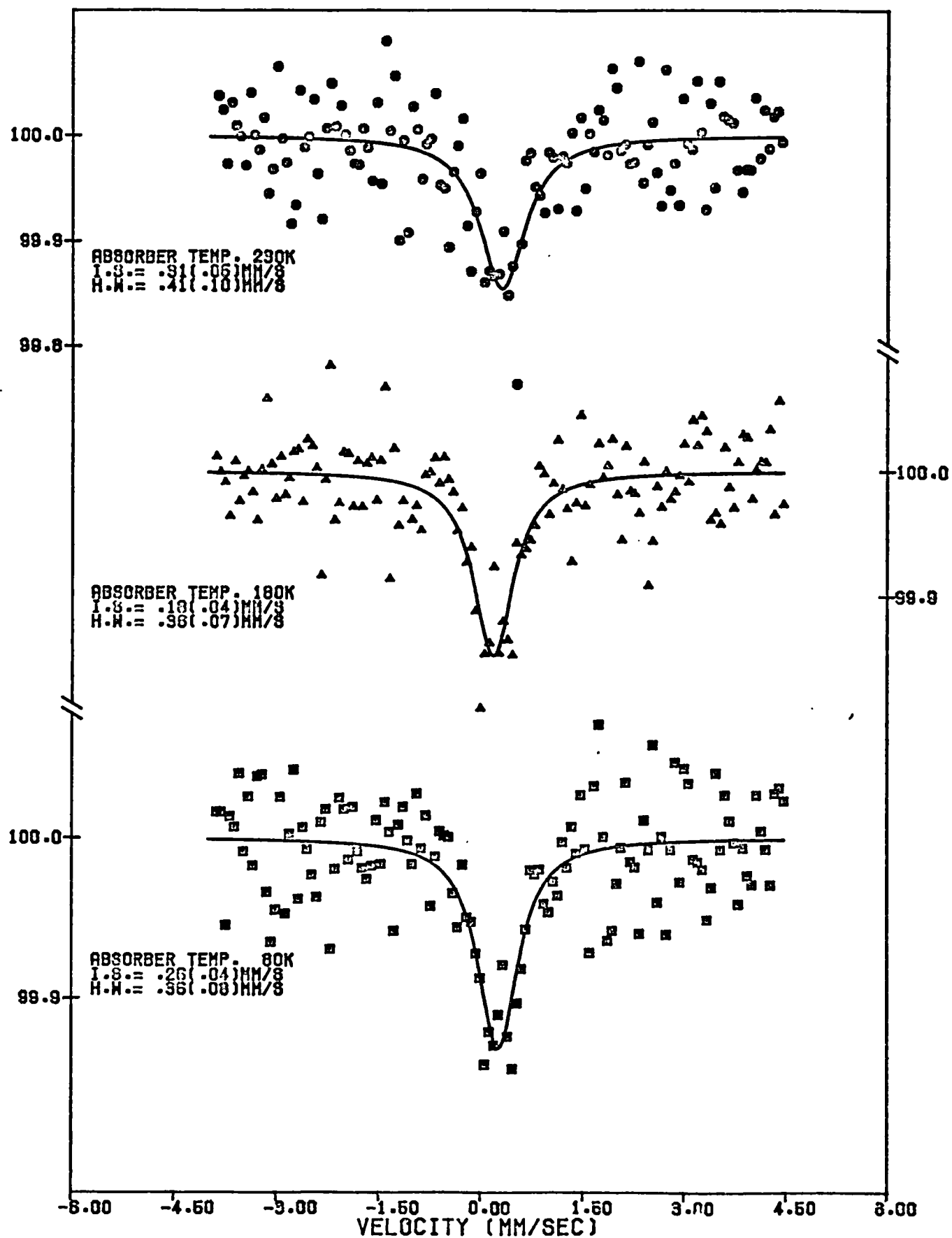


Fig. 4.2

line-broadening is observed in the presence of humic acids and humic acid fractions extracted from different soils produce varying degrees of broadening which provide a useful means of comparing different types of complex.

Hansen and Mosbaek (1970) investigated an Fe(III)-fulvic acid complex by classical Mössbauer spectrometry, however, all of these spectra were obtained at a temperature of approximately 298 K. From experiments performed on a variety of trinuclear Fe(III) chelates, believed to have an analogous stereochemistry, these workers concluded that the Fe(III)-fulvic acid complex is also trinuclear. The 6% absorption spectrum produced by Hansen and Mosbaek (1970) was broad with an isomer shift relative to sodium nitroprusside of 0.64 mm sec^{-1} . A large quadrupole splitting of 0.80 mm sec^{-1} was also clearly discernible in this spectrum which is not a feature of mononuclear iron coordination compounds. Hansen and Mosbaek (1970) conclude that:

"Stereochemically the trinuclear complexes are formulated with a trimeric cation in which two carboxylate groups bridge each of the sides of the equilateral triangle, whose vertices are the metal atoms and whose centre is an oxygen atom"

The complex formation is believed to be associated with specific structures which form part of a well organized molecular system,

The spectra obtained in the present study are weak with a percentage absorption never greater than 0.2% and show no obvious signs of quadrupole splitting. Nevertheless, the peaks are relatively broad with half-widths of 0.3 to 0.4 mm sec^{-1} which are comparable with the published precedent and in accordance with the broad carboxyl

peak of infra-red spectra (Stevenson and Goh, 1971a, 1971b, 1972). The isomer shift relative to natural iron for the five recorded spectra varies from 0.258 to 0.311 mm sec⁻¹; relative to the SNP standard the range of values obtained here are from 0.515 to 0.568 mm sec⁻¹. In contrast, Hansen and Mosbaek (1970) found the isomer shift to vary from 0.64 to 0.67 mm sec⁻¹ using this standard. The Mössbauer data in Table 4.1 are not sufficiently accurate for a precise qualitative interpretation of the complex structure to be inferred. The computed curves are the best fit that can be obtained with scattered points which approximate to background values but the fit may also obscure a developing 'wine bottle' shape which would be characteristic of quadrupole splitting.

Schnitzer (1971) has reviewed the chemistry of metal-fulvic acid complexes and distinguishes two categories of iron complex: water soluble complexes formed when molar metal-fulvic acid ratios are 1:1 or less and water-insoluble complexes which form when the molar ratio exceeds 1:1. Among these water insoluble forms Schnitzer (1971) differentiates two types of complex: a 2:1 or medium complex and a 6:1 high complex. In the lower and medium Fe(III) complexes the iron appears to be present as $\text{Fe}(\text{OH})^{2+}$ but in the high complex the hydroxylated form $\text{Fe}(\text{OH})_2^-$ is apparently complexed (Schnitzer, 1971; Schnitzer and Khan, 1972).

A hierarchy of complexes would be expected with such a mixed polyelectrolyte as fulvic acid. The water of hydration of each type of complex will clearly vary considerably and exert a profound

influence upon the readily hydroxylated Fe(III) ion. The low percentage absorption obtained with the water soluble complexes prepared in this study reflects the relatively low concentration of iron present in the compounds (Chapter 5). The spectra obtained are perhaps indicative of the molecular environment of the iron in 1:1 molar Fe(III)-fulvic acid complexes. The spectrum obtained by Hansen and Mosbaek (1970) is perhaps more characteristic of the partially hydroxylated or polymeric forms of complexed Fe(III) (Schnitzer and Hoffman, 1967; Schnitzer, 1974). Such a conclusion would also account for the unusually high percentage absorption obtained by these workers. This interpretation is compatible with the findings of Lindqvist and Lindman (1970) who concluded that the dominant quadrupole relaxation for ^{85}Rb complexes takes place through a coupling between nuclear quadrupole moment and electric field gradients at the nucleus site and not via interactions between the nuclear magnetic moment and electron spins in the humic acid.

The exact nature of complexation in these extracted compounds may be further complicated by the presence of fulvic acid-metal phosphates (Schnitzer and Khan, 1972). Chemical, spectroscopic and thermogravimetric analysis indicate that in the 'low' and 'medium' Fe(III) complexes phosphorus occurs as an orthophosphate bonded through the metal ion to the fulvic acid. In the 'high' complexes metal phosphates are mixed physically with fulvic acid-metal phosphates.

The experimental data presented here are insufficient for sound qualitative conclusions to be made regarding the Stereochemistry

of the complexes. The computed curves may in fact represent an integration of either single lines or narrow splitting or both. Nevertheless, the broad peaks of these spectra indicate that a wide range of molecular environments is occupied by the iron nucleus. A more systematic analysis of titrated fulvic acid, possibly in conjunction with studies of ^{57}Fe complexes would undoubtedly provide valuable information about the structure of fulvic acid and its complexes.

C H A P T E R 5

5. ORGANO-METALLIC TRACE ELEMENT SPECIATION IN NATURAL WATERS

5.1 INTRODUCTION

Organic interactions are known to predominate in the transport, mobilization and diagenesis of trace elements within soils and sediments (Swanson et al, 1966; Ong, Swanson and Bisque, 1970; Rashid, 1972a, 1972b, 1974; Ferguson and Bubela, 1974; Cline, Hillson and Upchurch, 1973; Saxby, 1969, 1973; Schindler, Alberts and Honick, 1972; Lindberg and Harriss, 1974). And the enigmatic behaviour of many trace metals in natural waters has prompted considerable speculation as to the influence of speciation and the possible role of organic complexing agents. The fate of anthropogenic sources of heavy metals, as well as the effect of organic complexation upon the primary productivity, toxicity of effluents and the aquatic ecosystem as a whole, provides a pragmatic objective to the study of organo-metallic speciation (Helz, Huggett and Hill, 1975; Bender, Matson and Jordan, 1970; Murry^a and Meinke, 1974; Fukai, 1969).

Much experimental evidence for the existence of soluble organic complexes stems from the evaluation of optimum condition for algal culture and the assay of toxic thresholds. Johnston (1964) has demonstrated that the addition of chelated trace metals to culture media promotes primary productivity and that even an addition of 'free' chelating agents may prove equally effective in this respect. An optimum concentration of $10^{-5}M$ has been established for EDTA; an indication, perhaps, of an adequate source of minor elements in general but unavailable for bio-assimilation. Apparent trace

element deficiency, manifest as a latent potential productivity has been observed in nutrient enriched areas of oceanic upwelling (Barber and Ryther, 1969). The general levels of copper, manganese, lead and zinc within a phytoplankton population are seen to fluctuate sympathetically with ambient levels occurring in sea water (Knauer and Martin, 1973). However, a copper induced suppression of algal growth is also found to vary with both season and locality (Erickson, 1972). The toxicity of copper to phytoplankton may be partially or wholly negated with the use of chelating agents such as nitrilotriacetic acid (NTA) or ethylene diamine tetra-acetic acid (EDTA) (Erickson, Lackie and Maloney, 1970; Erickson, Maloney and Gentile, 1970; Steeman-Nielsen and Wium-Anderson, 1970; Barber and Ryther, 1969; Lewis, Ramnarine and Evans, 1971; Lewis, Whitfield and Ramnarine, 1972, 1973). Bacteriocidal effects of copper are also diminished in the presence of both EDTA and humic acids (Milanovich, Wilson and Yin-Yeh, 1974) but in contrast, Albright and Wilson (1974), failed to observe similar effects with amino acids and mercury. The use of nitrogenous compounds in these experiments presents an additional complication to the interpretation of results since it may prove difficult to distinguish between the effect expected with the addition of a nutritious substrate and the activity of a chelating agent. Nevertheless, analogous phenomena, interpreted in terms of trace metal chelation, are observed with the inoculation of extra-cellular polypeptides, protein digests, zooplankton extracts or humic acids to toxic culture media (Prakash et al, 1973; Doig and Martin, 1974).

Extracellular synthesis of natural chelating agents (Fogg, 1966) is suggested as a mechanism regulating the availability of micro-nutrients under conditions of deficiency or toxicity. This apparent sensitivity to organic complexation has led to the suggestion that 'complexing capacity' of natural waters may be a parameter amenable to bio-assay (Davy^e, Morgan and Erickson, 1973). An analytical determination of 'complexing capacity' in conjunction with physiological observations may provide useful information as to both the mechanisms of organic sequestration and bio-accumulation of heavy metals. 'Complexing capacity' as defined by chemical means, however, has not proved to be a reliable index of heavy metal tolerances in phytoplankton (Gächter, Lum-Shue Chan and Chau, 1973).

A number of mechanisms involving complexation, colloidal adsorption or precipitation may result in a growth stimulation or detoxification effect. A given trace element species in any heterogeneous system, such as that of natural waters, will retain a dynamic equilibrium with not only the total concentration of the element and co-ordinating ligand, but an equilibrium which is also a function of the distribution, nature and biological activity of the other elements and available ligands present within the system. Multi-element - multi-ligand systems of culture media prove impossible to predict in terms of an equilibrium or steady state model (Pollock and Norman, 1972). The subjective observations outlined above inevitably yield equivocal results open to ambiguous interpretation in terms of ionic interactions or changes in adsorption equilibria as well as biological phenomena.

The chemical evidence for non-ionic species of heavy metals in natural waters is derived from two principal lines of experiment and analysis (Fukai, 1973; Fukai and Huynh-Ngoc, 1975; Wilson, 1976):

- I. solvent extraction of trace element fractions, apparently unextractable by conventional chelation/concentration techniques without the use of organic oxidation procedures.
- II. the determination of a non-labile or non-dialysable, bound fraction of the 'dissolved' trace elements.

Laevastu and Thompson (1958); Corcoran and Alexander (1964); Alexander and Corcoran (1967); Slowey and Hood (1966)

have demonstrated the release of unreactive and apparently organically bound concentrations of iron and copper upon the oxidation of organic solutes in sea water. The mean concentration of dissolved copper in sea water is approximately $3 \mu\text{g l}^{-1}$ of which 10 to 30% is thought to be associated with dissolved organic matter (Foster and Morris, 1971). A high degree of accuracy in analysis is therefore required to detect these complexes which range in concentration from 0.2 to $0.8 \mu\text{g l}^{-1}$ for copper. The results of such work are perhaps best regarded as a good qualitative illustration of organic complexation of trace metals in sea water. The actual results are subject to inherent inaccuracies arising from blanks and the use of oxidising agents such as perchloric or persulphuric acid. Nevertheless, these observations have been confirmed using photo-oxidation techniques *Foster and Morris (1971)* to destroy refractory organic complexes (Fukai, 1970; Williams, 1969; Gardiner and Stiff, 1975). Chloroform extraction of organo-metallic copper species has also served to demonstrate the occurrence of complexes in sea water. These trace levels of copper are possibly

associated with phospho-lipid, amino-lipid or porphyrin fractions of the soluble lipids (Slowey, Jeffrey and Hood, 1967).

Filterable but non-dialyzable fractions of manganese, zinc and copper have been reported in sea water (Rona et al, 1962; Slowey and Hood, 1966; Hood, 1967). Similarly, radio-tracer techniques, used in conjunction with semi-permeable membranes, have shown an apparent association of cobalt, manganese and zinc with lacustrine, macromolecular and colloidal constituents (Barsdate, 1968). In such observations ionic equilibria and isotopic exchange are assumed which may limit the validity of results (Benes and Steinnes, 1975). Reported exchange rates (2 h to 35 d) are inconsistent (Barsdate, 1968; Fukai, 1970; Benes and Steinnes, 1975) but have been put forward as evidence of both refractory, non-labile complexes or negligible complexation. In considering the transport of metal complexes, Langford and Gamble (1974) point out the philosophical problems of considering the processes in terms of equilibrium position and speculate as to the significance of rates of complex formation. Inorganic complexes of all the trace elements are formed and dissociate on time scales faster than the millisecond. Qualitatively none of the experiments considered by Langford and Gamble (1974) indicate slow equilibration between metal ions and organic ligands such as fulvic acids. The estimated first order rate constant for Fe^{3+} - fulvic acid equilibrium, for example, is approximately $7 \times 10^{-3} \text{ sec}^{-1}$ indicating that the dissociation of the Fe^{3+} - fulvic acid complex is slow but that the complex is relatively labile for a large molecule. Nevertheless, in the presence of organic ligands such as porphyrins

slower complexation reactions may be expected to occur and it is unlikely that the products would be in equilibrium with other species in aqueous solution. Humic ligands may also play a role in rendering metal ions non-labile in aqueous systems but this effect probably does not arise from simple complex formation-dissociation reactions (Langford and Gamble, 1974).

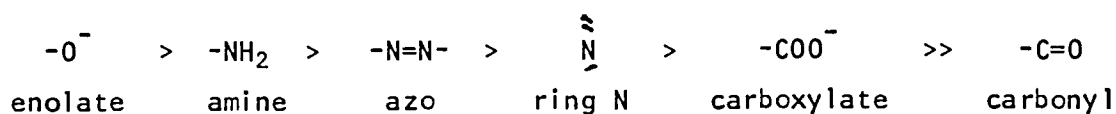
Longerich and Hood (1968, 1969) employed several techniques including ultrafiltration and gel-permeation chromatography in combination with dialysis to define the molecular weight of organically bound species of zinc. The molecular size distribution of copper, iron, lead and manganese species occurring in the Rideau River, Canada was also obtained in a similar manner (Guy and Chakrabarti, 1975). However, estimates of the percentage of trace elements organically bound in sea water vary from 0 to 50% for copper (Slowey, Jeffrey and Hood, 1967) and up to 50% for cobalt, manganese and zinc in certain fresh waters (Barsdate, 1968). Similarly, the dithizone extractable fraction of zinc in estuaries may represent less than 20% of the total 'soluble' concentration which apparently occurs largely in the form of organo-metallic species (Fukai, Murray and Huynh-Ngoc, 1975).

The apparent 'complexing capacity' of natural waters has been determined electrochemically by differential pulse anodic stripping voltammetry (Chau, 1973; Chau, Gachter and Lum-Shue-Chan, 1974; Chau and Lum-Shue-Chan, 1974) and by ion-selective electrodes (Ramamoorthy and Kushner, 1975a, 1975b). In addition to the predominant amino-cation

and carbonate species occurring in polluted waters, a hexanol-extractable species of copper, apparently in the form of a humic acid complex, has also been found using an ion-selective electrode (Stiff, 1971).

The determination of soluble trace element chelates or complexes by any direct method of analysis or isolation presents considerable practical problems at low concentrations. Many methods of trace metal analysis assume the elements of interest to occur in a reactive form or as species which may be determined after suitable pretreatment (Fukai and Huynh-Ngoc, 1975). The possible occurrence of refractory organic complexes and the occlusion of trace metals within colloidal and macromolecular structures may complicate analysis.

The levels of dissolved organic matter in natural waters typically lie in the range of 0.1 to 10 mg l⁻¹. Any scheme of analysis drawn up for the study of organic complexes should be made with some consideration of the type of ligands and species which may occur (Forsberg, 1971). The functional groups which may serve as effective electron donors in the binding of metal ions to organic compounds have been outlined by Martell (1971) and include the following categories: aliphatic and aromatic amino, carboxylate, enolate, alkoxide, phenolic, mercaptide, phosphate and phosphonate groups. A number of other groups of the amide, thioether and hydroxylalkyl type, which exhibit weak affinities for metal ions, do not form stable complexes in solution but in conjunction with strongly co-ordinating groups may augment chelation. The most important chelating groups in the order of decreasing affinity for metal ions are (Chaberek and Martell, 1959):



The functional groups such as $-NH$, $-OH^-$, $-COOH^-$ and $-SH^-$, which predominate in the dissolved organic matter of natural waters, show little specificity of complexation. The co-ordination of the alkaline earth metals, calcium and magnesium, may therefore be expected to predominate (Stumm and Bilinski, 1973). It is therefore necessary to consider the influence of possible organic chelation in terms of a competitive ionic regime within a multi-ligand system. Within such a system the co-ordination of metal ions by organic ligands will be governed, at least to some extent, by the degree of polarizability or 'hardness' of the ligand atoms. The complexity of such an intricate system makes generalization difficult but oxygen co-ordinating sites within a ligand molecule may be expected to preferentially take up the 'hard' cations such as calcium and magnesium. The more polarizable transition metal cations may be selectively co-ordinated by the 'soft' ligand groups with nitrogen donor atoms. In addition steric factors will also govern the complexation of any given cation by specific ligands and molecules.

Chelation by organic molecules is frequently postulated to explain the occurrence of soluble metal concentrations greater than those predicted from solubility products of known species. The expected complexation of trace elements might therefore be demonstrated by experiments designed to observe their solubility within a natural medium. However, in the presence of potential ligands, of both synthetic and natural origin, Duursma and Sevenhuysen (1968) did not

find any significant organic chelation of iron, zinc, nickel, cobalt or copper in sea water. Quantitative information is clearly required on the stability of all the cationic constituents with all the available ligands of such a system since competitive equilibria prove an important factor determining trace element speciation (Duursma, 1968). However, as Fukai (1970) has pointed out, the results of dissociation experiments indicate that organic complexes represent a refractory group of non-labile species which may be independent of ionic forms. Hypotheses which are based upon or assume an equilibrium between ionic and non-labile organic forms may therefore be of questionable validity (Fukai, 1970).

Numerous empirical studies have been made with regard to the metal complexing properties of humic and fulvic acids. Conditional stability constants of these complexes for some elements are available from the literature and have been compiled in Table 5.1. In the case of fulvic acid complexes the acid metal ratio is affected by both pH and ionic strength (Schnitzer and Hansen, 1970; Schnitzer and Khan, 1972). These ratios are found to fall with increasing pH; the rate of this decline bearing an inverse relationship to ionic strength (μ). All the $\log K_1$ values determined by Schnitzer and Hansen (1970) were found to decrease rapidly within a range of ionic strength (μ) from infinite dilution to 0.15. Clearly such factors must be taken into account in a consideration of the behaviour of humic and fulvic acid complexes in natural waters. The order of stabilities reported for the transition elements and the major cations complexed by fulvic acids is: $\text{Fe}^{3+} > \text{Hg}^{2+} = \text{Al}^{3+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+} > \text{Co}^{2+} > \text{Pb}^{2+} > \text{Ca}^{2+} > \text{Zn}^{2+} > \text{Mn}^{2+} > \text{Mg}^{2+}$ (Table 5.1). This order of stabilities

TABLE 5.1

Conditional Stability Constants for Metal-Fulvic Acid Complexes

Log K ₁												Conditions			Method	Reference	
Al ³⁺	Ca ²⁺	Cd ²⁺	Co ²⁺	Cu ²⁺	Fe ³⁺	Hg ²⁺	Mg ²⁺	Mn ²⁺	Ni ²⁺	Pb ²⁺	Zn ²⁺	ionic strength	pH	temperature	medium		
5.3	-	-	-	-	7.6	-	-	-	-	-	-	μ = 0.00	2.0	-	(1.5M KCl)	Schnitzer (1971)	
4.6	-	-	-	-	6.9	-	-	-	-	-	-	μ = 0.05					
3.7	-	-	-	-	6.1	-	-	-	-	-	-	μ = 0.10					
2.9	-	-	-	-	5.4	-	-	-	-	-	-	μ = 0.15					
-	3.6	-	4.3	4.7	-	-	2.7	2.9	4.5	3.6	3.2	μ = 0.00	3.0	-	(1.5M KCl)	C.V.	Schnitzer and Hansen (1970)
3.7	2.6	-	2.9	3.3	6.1	-	1.9	2.1	3.1	2.6	2.4	μ = 0.10					
-	2.1	-	2.2	2.6	-	-	1.6	1.7	2.4	2.1	2.0	μ = 0.15					
-	-	-	2.7	-	4.5	-	-	-	-	-	-		3.0	-	(1M KCl)	i.e.	Malcolm, Jenne and McKinley (1970)
-	-	-	4.0	-	5.4	-	-	-	-	-	-		4.5				
-	-	-	6.6	-	5.6	-	-	-	-	-	-		6.0				
-	-	-	-	3.76	-	-	-	-	-	-	-	μ = 0.05	3.3	25°C	(0.1M KCl)	i.e.	Gamble <u>et al.</u> , (1970)
-	-	-	-	-	-	5.12	-	-	-	-	-	μ = 0.02	3.1	25°C	(0.04M NaNO ₃)	i.s.e.	Cheam and Gamble (1974)
-	-	-	-	3.22	-	4.86	-	-	-	-	-	μ = 0.10	3.0	25°C	(0.1M NaNO ₃)	i.s.e.	
-	-	-	-	3.72	-	5.08	-	-	-	-	-	μ = 0.10	4.0	25°C	(0.1M NaNO ₃)	i.s.e.	
-	-	3.64	-	-	-	-	-	-	-	-	-	μ = 0.10	4.9	25°C	(0.1M NaNO ₃)	i.s.e.	
-	-	3.64	-	-	-	-	-	-	-	-	-	μ = 0.10	5.95	25°C	(0.1M NaNO ₃)	i.s.e.	
-	-	-	-	4.35	-	-	-	-	-	-	-	μ = 0.10	5.0	25°C	(0.1M NaNO ₃)	i.s.e.	
-	-	-	-	2.57	-	-	-	-	3.19	-	2.99	μ = 0.10	4.0		(1M NaCl)	i.e.	Adhikari and Hazra (1972)
-	-	-	-	3.93	-	-	-	-	3.23	-	4.16	μ = 0.10	5.5				
-	-	-	-	8.42	-	-	-	-	5.14	-	5.14	μ = 0.01	8.0	20°C	(0.01M NaCl & 0.01M TRIS)	g.c.	Mantoura and Riley (1975)
-	-	-	-	7.85	-	-	-	-	4.98	-	4.83						
-	-	3.67*	-	3.7*	-	6.9*	-	-	-	3.94*	-				*	i.s.e.	Ramamoorthy and Kushner (1975)
-	-	3.25	-	3.5	-	11.7	-	-	-	3.6	-						

* determined for river water using ion-selective electrodes

- not determined

C.V. Continuous variance i.e. Ion Exchange i.s.e. Ion selective electrode g.c. zonal analysis using gel chromatography

clearly does not follow the Irving-Williams series but is in approximate agreement with the empirical observations of Van Dijk (1971) on the bond strength of divalent metal ion complexes with humic acids.

Despite the formation and obvious importance of humic and fulvic acid complexes in soils, the occurrence and behaviour of these apparently ubiquitous species in natural waters remains poorly understood. The unknown degree of competition in complexation for non-specific organic ligands is, perhaps, the major speculative parameter in any mathematical computation of organic complexation in natural waters. Often the choice of ligands in a model must be limited and homologues for humic acids used to rationalize the problem. As a basis for practical observation and experiment, hypothetical considerations may nevertheless provide a useful guide in the study of speciation (Stumm and Morgan, 1970). The choice of model 'humic' compounds in computations and experiments necessitates difficult assumptions, however, the weak acids (eg. acetic, citric and sulphosalicylic acids) are commonly regarded as suitable for this purpose (Theis and Singer, 1974; Stumm and Morgan, 1970; Langford and Gamble, 1974). Under conditions of organic enrichment there is good evidence to suggest that complexed species of trace metals do occur in fresh waters. However, it is unlikely that any of the fulvic acid complexes are stable at an ionic strength greater than 0.3 and that many are unstable at 0.2 (Schnitzer and Hansen, 1970). The degree of complexation may also be pH dependent as demonstrated for cadmium complexes with humic substances in river water (Gardner, 1974). Since the ionic strength of sea water, assuming a salinity of 35‰, is

approximately 0.7, under estuarine conditions a de-complexation or precipitation of trace metal and major ion organic complexes would be expected as a logical extrapolation of the known chemistry of fulvic acid complexes. Evidence for such a process can be seen from the recent work of Smith (1976) in which the 'complexing capacity' of river water was shown to diminish rapidly with increasing salinity. The results of flocculation studies also clearly indicate that precipitation of organic and inorganic matter does occur with the mixing of river and sea water; the iron and aluminium 'humates' are particularly unstable in such a strong electrolyte (Bondarenko, 1972; Sholkovitz, 1976; Eckert and Sholkovitz, 1976). Organic carbon $^{13}\text{C}/^{12}\text{C}$ variations in estuarine muds indicate that terrestrial organic carbon is limited to sediments within a relatively short distance from the river mouth and provides oblique evidence for the instability of humic substances and their complexes in the estuarine environment (Shultz and Calder, 1976).

Stumm and Brauner (1975) have pointed out that in a consideration of trace element complexation by organic matter it is convenient to distinguish between the following constituents:

1. low molecular weight organic substances in true solution;
2. polymeric substances which contain sufficient numbers of hydrophilic functional groups (eg. $-\text{COO}^-$, $-\text{NH}_2$, $-\text{R}_2\text{NH}$, $-\text{RS}^-$, $-\text{ROH}$, RO^-) and retain their macromolecular structure in solution;
3. colloidal organic material either of high molecular weight compounds, such as humic acids, or of organic substances sorbed or chemically bound with inorganic colloids (eg. polymeric hydrolysis products of iron, aluminium and silica).

As a practical procedure, a distinction of this nature is difficult to achieve but could form the basis of an empirical scheme of analysis designed to evaluate and identify organically complexed trace element species. Indeed, it was this premise that initiated the present investigation to exploit the concentration and fractionation potential of ultrafiltration.

5.2 THE USE OF ULTRAFILTRATION IN THE STUDY OF ORGANIC COMPLEXES IN NATURAL WATERS

Ultrafiltration provides a means of organic concentration and fractionation, based upon molecular size, which is suitable for the isolation and recovery of polydispersed dissolved and colloidal material in dilute concentrations. The technique has proved successful in the study of the formation and distribution of organic complexes in the interstitial water of sediments (Schindler, Alberts and Honick, 1972; Lindberg and Harriss, 1974) and also in the non-denaturant concentration of organic 'chelates' in lake, river and sea water (Chau, 1973; Gjessing, 1970, 1975; Andren and Harriss, 1975; Guy and Chakrabarti, 1975; Ramamoorthy and Kushner, 1975a, 1975b). The superimposition of membrane retention curves published by Sheldon (1972) and Amicon Ltd. on a speculative size distribution of metallic species in natural waters (Stumm and Bilinski, 1973) provides an illustration of the 'soluble' size classes which may be differentiated by this facility (Figure 5.1). In the present study of the molecular size distribution of 'soluble' trace metal species, ultrafiltration has provided a direct means of studying the colloidal and macromolecular

FREE METAL IONS	INORGANIC ION PAIRS AND COMPLEXES	ORGANIC COMPLEXES AND CHELATES	METAL SPECIES BOUND TO MACRO - MOLECULAR ORGANIC SOLS	METAL SPECIES IN THE FORM OF HIGHLY DISPERSED COLLOIDS	METAL SPECIES SORBED ON COLLOIDS AND IN OR UPON VIRUSES	PRECIPITATES, CLAYS, ORGANIC DEBRIS, MICRO ORGANISMS AND ALGAE
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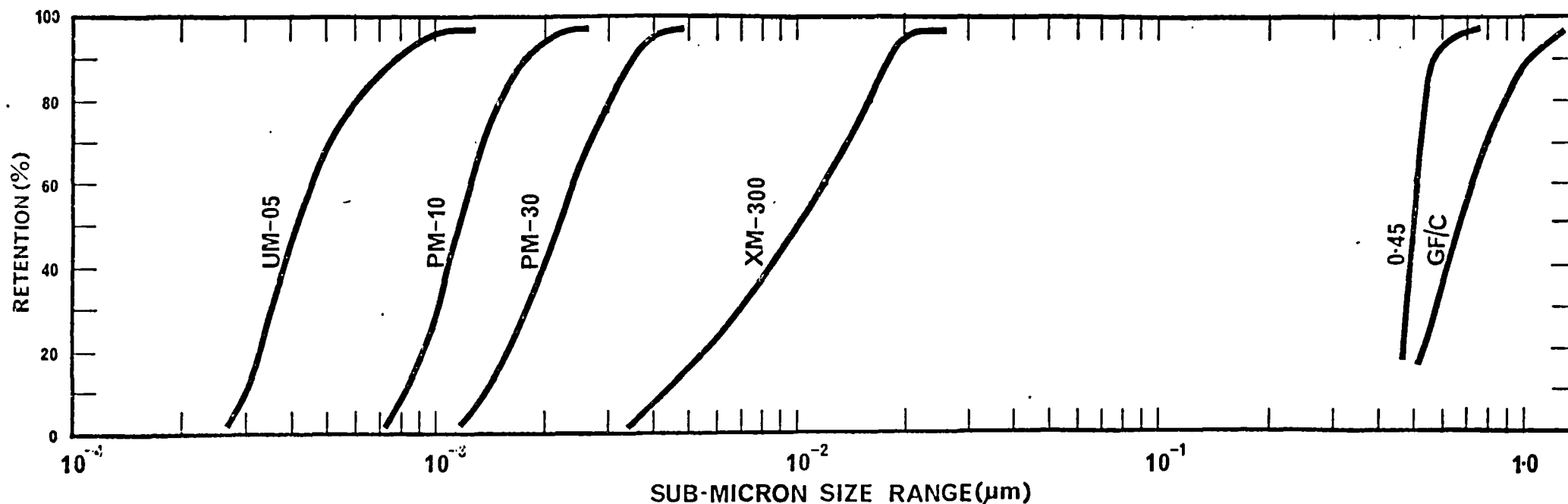


Fig. 5.1 Molecular size fractions of trace element species in natural waters (after Stumm and Bilinski, 1973)

material in Cornish rivers (Chapter 1). The distribution of heavy metals within two non-dialyzable fractions has been investigated using ultrafiltration and indirect methods of analysis; analysis of isolated natural complexes has also been used to confirm observed complexation.

5.2.1 Methods

In the present study Amicon PM-10 ultrafiltration membranes (nominal molecular weight cut-off, 10,000) were used principally in conjunction with simplified, 'plexiglass' ultrafiltration cells. Sampling, and the preparation of filtration apparatus, filters and membranes was carried out as described in Chapters 2 and 3. The cells and rinsed filters were flushed with deionised water followed by a 500ml sample aliquot. Each bulk water sample (10l) was initially filtered through a Whatman (GF/C) glass-fibre filter and then through a PM-10 ultrafiltration membrane. Aliquots of both the primary (GF/C) filtrate(s) and the ultrafiltrate(s) were acidified to pH 2 and retained for analysis of trace metals and selected major ions. The concentrated, non-dialyzable fraction remaining in the cell as a residue after ultrafiltration was collected and freeze-dried for identification and characterization. A multi-element chelation-solvent extraction technique using diethyl dithiocarbamate and chloroform was employed in the trace element analysis of all filtrates (Appendix A). Calcium and potassium concentrations of these fractions were determined by direct atomic absorption spectrophotometry.

During a later period of this study, selected river waters

(Chapter 1) were fractionated by means of sequential ultrafiltration using a PM-10 and a UM-05 membrane (nominal molecular weight cut-off, 500) in cascade. Aliquots of these ultrafiltrates were analysed for the selected major ions and trace metals. Two non-dialyzable residues from each sample were isolated and freeze-dried to provide two molecular fraction: 1. the PM-10 dialyzate (molecular mass >10,000) and 2. the UM-05 dialyzate (molecular mass <10,000 but >500). The UM-05 membrane merely provides a means of concentration by reverse-osmosis; a 500ml volume requiring some 30 hours to permeate the membrane even under a pressure of 50 p.s.i. Dialyzates of this lower molecular weight fraction were concentrated from 5 to 8 litres of the remaining PM-10 ultrafiltrates.

5.2.2 Results

The distributions of 'soluble' trace metals in the monthly samples taken from four rivers in the S.W. of England (Chapter 1, 1.5): the Helford, Carnon, Fowey and the Tamar are presented here as histograms. The results of these analyses are tabulated and appended in this thesis (see Appendix B). Broadly, the results can be considered in terms of the three types of catchment that the four rivers represent, thus the Helford and Tamar waters may be taken as examples of soft rural drainage in a lightly mineralized catchment. The Fowey and Carnon river waters are exceptional in that the former is enriched with humic substances from a moorland environment while the latter is heavily polluted by acid-mine drainage (Aston et al, 1974; Aston and Thornton, 1975; Thornton, Watling and Darracott, 1975).

With the exception of the Carnon river the 'soluble' concentrations of cadmium, cobalt, nickel and lead found in the waters of the Fowey, Helford and Tamar rivers were rarely greater than the limits of sensitivity of the method used to determine these elements (Applied Geochemistry Research Group, Technical Communication Number 62, see Appendix A). Only the levels of iron, manganese, zinc and copper occurring in the total 'soluble' fraction and as species of nominal molecular weight less than 10,000 are presented here. For the purposes of this investigation the filtrate of a Whatman (GF/C) glass-fibre filter was taken to represent the total 'soluble' fraction of each sample. The low molecular weight species (<10,000 and <500 molecular mass) were taken to occur in the ultrafiltrates of the Amicon PM-10 and UM-05 membranes. In the histograms presented in this Chapter, the total 'soluble' concentration is represented as a solid line while the <10,000 molecular weight fraction is shown as a broken line.

5.2.2.1 Distribution of Metal Ions in Helford River Water after Ultrafiltration

The waters of the Helford catchment were found to be relatively 'soft' during the period sampled, with concentrations of calcium ranging from 10 to 30 ppm (Tables 5.2 and 5.3). At Constantine and Gweek Quay concentrations varied little from month to month. However, levels of calcium at Trelowarren were generally higher than those found on the northern side of the Helford valley catchment. With the exception of samples taken from the estuary

TABLE 5.2

Distribution of Calcium and Potassium in Helford River Water after Ultrafiltration

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION (mol. wt.)	CONCENTRATION (mg l ⁻¹)	
			Ca	K
CONSTANTINE	31/1/75	TOTAL	14.0	3.9
		<10,000	13.7	3.8
	25/2/75	TOTAL	15.0	3.6
		<10,000	15.0	3.6
	25/3/75	TOTAL	14.0	3.7
		<10,000	15.0	3.7
TRELOWARREN	31/1/75	TOTAL	13.2	4.3
		<10,000	13.2	4.3
	25/2/75	TOTAL	28.0	5.0
		<10,000	27.0	4.7
	25/3/75	TOTAL	27.0	4.1
		<10,000	24.2	4.2
GWECK QUAY	31/1/75	TOTAL	13.8	4.4
		<10,000	10.0	2.0
	25/2/75	TOTAL	13.5	3.7
		<10,000	12.8	4.0
	25/3/75	TOTAL	13.8	3.7
		<10,000	11.3	3.5

TABLE 5.3

Distribution of Trace Metals in Helford River Water after Ultrafiltration

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION	CONCENTRATION ($\mu\text{g l}^{-1}$)								CONCENTRATION (mg l^{-1})	
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb	Ca	K
GWEK QUAY	29/4/75	TOTAL	10.0	26.5	3.5	<1.0	0.25	1.5	7.5	<5.0	10.0	3.3
		<10,000	9.5	19.5	2.2	<1.0	0.30	0.5	5.5	<5.5	10.0	3.3
		<500	4.6	21.5	2.3	<1.0	0.20	1.5	6.0	<5.0	-	-
CONSTANTINE	29/4/75	TOTAL	31.5	29.5	11.5	<1.0	0.1	1.0	6.5	<5.0	14.5	3.3
		<10,000	21.5	28.5	13.0	<1.0	0.4	1.6	6.25	<5.0	13.2	3.0
		<500	20.0	30.0	11.0	<1.0	0.3	2.5	6.5	<5.0	14.0	3.3
	1/7/75	TOTAL	21.0	49.1	36.5	1.0	0.25	3.0	5.1	<5.0	13.7	5.0
		<10,000	14.6	47.5	35.5	1.0	0.20	2.0	4.5	<5.0	-	-
		<500	12.0	48.0	36.5	1.0	0.20	3.0	4.0	<5.0	-	-
TRELOWARREN	29/4/75	TOTAL	17.0	4.9	1.7	<1.0	0.20	2.0	4.0	<5.0	22.8	5.65
		<10,000	10.5	4.9	1.9	<1.0	0.3	2.0	3.5	<5.0	22.5	5.65
		<500	4.5	3.5	1.6	<1.0	0.20	2.5	3.5	<5.0	22.5	5.5
	1/7/75	TOTAL	7.0	10.1	1.0	<1.0	0.2	1.0	11.5	<5.0	26.5	9.2
		<10,000	5.0	10.3	1.1	<1.0	0.2	1.5	13.5	<5.0	-	-
		<500	3.0	7.6	1.5	<1.0	0.2	1.5	9.0	<5.0	-	-

low tide, ultrafiltration retained little of the total 'soluble' calcium of these waters. At Gweek Quay 15 to 30% of the total 'soluble' fraction was retained by the PM-10 membrane with a *corresponding* reduction in the calcium concentration in the <10,000 molecular mass fraction occurring in the ultrafiltrate. The concentration of potassium in these waters ranged from 2 to 9 ppm (Tables 5.2 and 5.3) and is typical for river water (Hem, 1970). The levels of potassium varied little with locality or from month to month but were generally highest at Treloarwarren; no significant reduction in concentration was observed as a result of ultrafiltration. The pH of samples taken from Constantine and Gweek Quay varied little ranging from 5.3 to 5.6, while waters from Treloarwarren were slightly less acidic with pH values of between 5.8 and 6.0. The distribution of iron, manganese, zinc and copper before and after ultrafiltration of waters from Constantine and Treloarwarren is shown in figures 5.2 and 5.3 respectively. The results of sequential ultrafiltration using PM-10 and UM-05 membranes after primary filtration through a GF/C glass fibre filter, are shown in Table 5.3. With the exception of 'soluble' iron, which has been discussed in Chapter 3, there is little evidence for the retention of any colloidal or macromolecular heavy metal species within the two molecular size classes examined. The concentrations of total 'soluble' copper, manganese and zinc were highest at Constantine but the results are all in good agreement with the findings of a previous survey of this catchment (Thornton, Watling and Darracott, 1975).

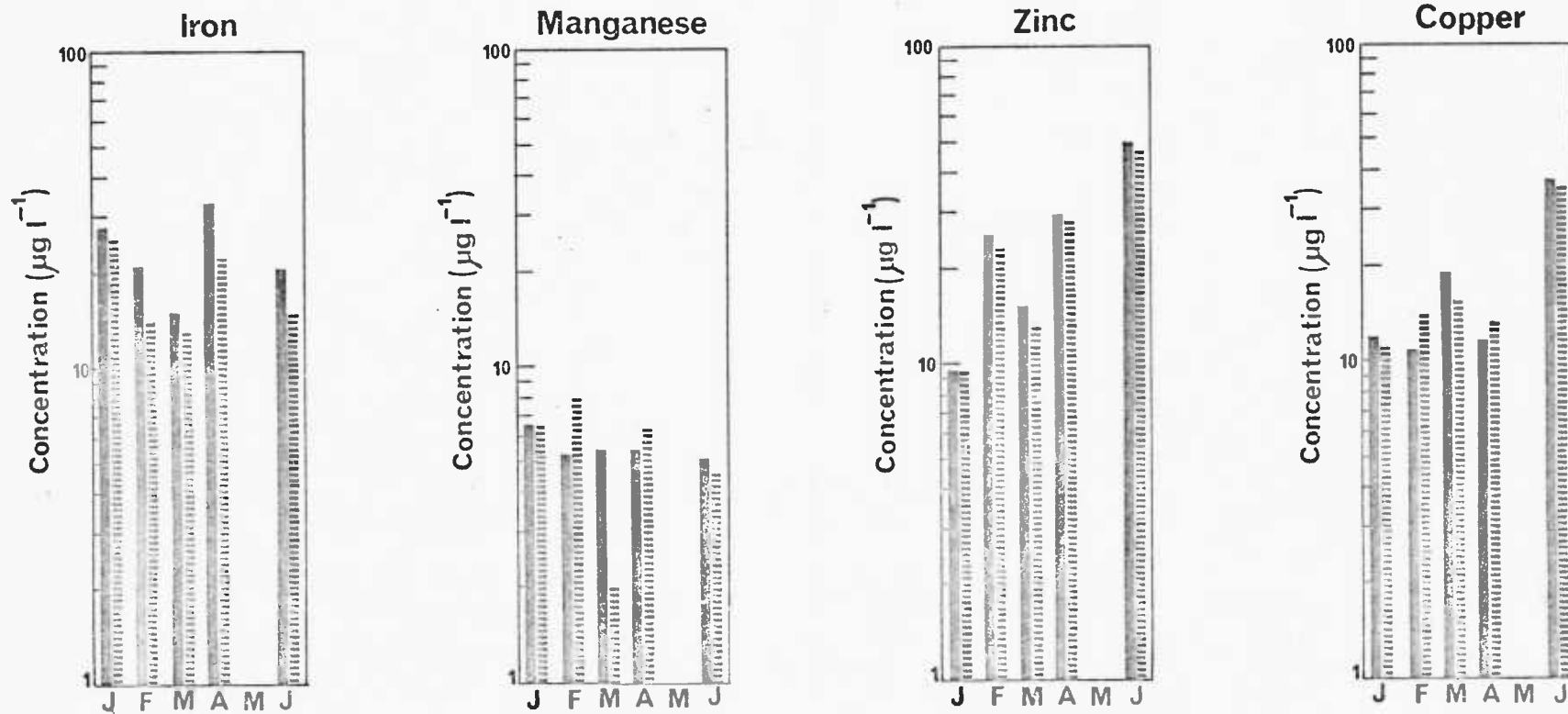


Fig. 5.2 Distribution of 'soluble' iron, manganese, zinc and copper in Helford River Water (Constantine) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

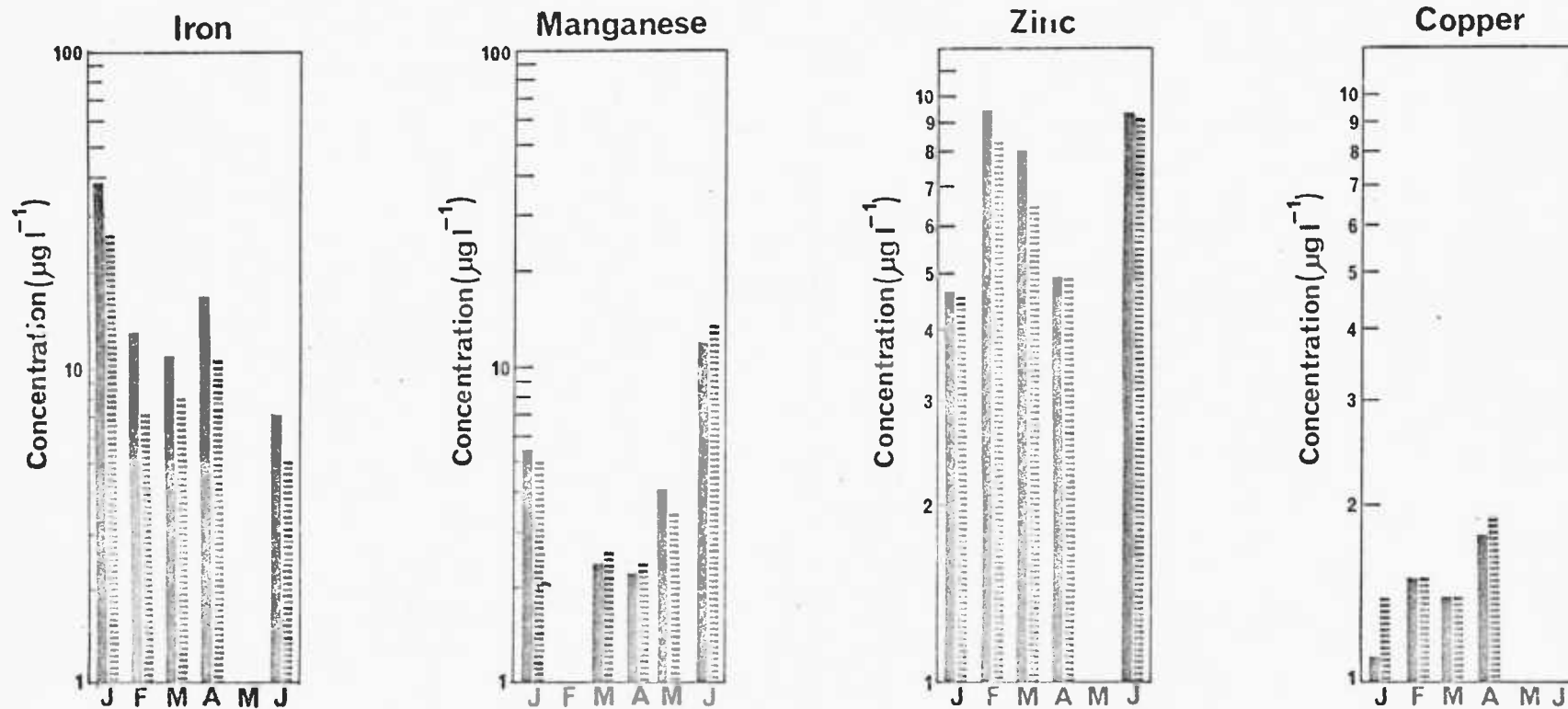


Fig. 5.3 Distribution of 'soluble' iron, manganese, zinc and copper in Helford River Water (Trelowarren) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

5.2.2.2 Distribution of Metal Ions in Tamar River Water after Ultrafiltration

The waters of the Tamar were sampled over an extended period, however, calcium and potassium concentrations were only determined for samples taken during 1975. The drainage is similar to that of the Helford valley as the water is 'soft' (calcium concentrations ranging from 10 to 20 ppm) and acidic (typically pH 5.4 in the main river). A gradual increase in 'hardness' downstream is a discernable trend in this river but no marked variation in concentration from month to month was observed (Tables 5.4 and 5.5). The results suggest that calcium is found in the high molecular weight fractions, particularly in samples taken from Launceston and Gunnislake where 5 to 7% of the total 'soluble' fraction is estimated to occur in the >10,000 molecular size class. Similarly, 7 to 20% of the calcium in the estuary sampled at Calstock at low tide may also be removed by ultrafiltration. In contrast, the concentration of potassium (2 to 4 ppm) in this drainage is found to vary little from month to month or with locality. Only minor indications of the 'entrainment' of potassium in colloidal and macromolecular material can be inferred from the marginal differences in concentration before and after ultrafiltration. The distribution of 'soluble' iron, manganese and zinc after ultrafiltration of waters taken from four stations along the river is shown in figures 5.4 to 5.6 and in Table 5.5. Only small increases in the concentration of copper and zinc were found in the waters draining from the mineralized, mining area of Gunnislake and showed little variation with river flow (see Appendix B).

TABLE 5.4

Distribution of Calcium and Potassium in Tamar River Water after Ultrafiltration

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION (mol. wt.)	CONCENTRATION (mg l ⁻¹)	
			Ca	K
CROSSGATE	25/2/75	TOTAL	10.2	2.0
		<10,000	10.3	2.5
	25/3/75	TOTAL	16.0	3.6
		<10,000	15.4	3.4
LAUNCESTON	25/2/75	TOTAL	15.0	3.6
		<10,000	15.0	2.8
	25/3/75	TOTAL	17.0	3.2
		<10,000	15.8	3.0
GUNNISLAKE	25/2/75	TOTAL	17.5	2.7
		<10,000	16.8	2.6
	25/3/75	TOTAL	15.8	2.2
		<10,000	15.0	2.3
CALSTOCK	25/2/75	TOTAL	17.0	2.6
		<10,000	16.2	2.4
	25/3/75	TOTAL	14.0	2.2
		<10,000	-	-

TABLE 5.5

Distribution of Trace Elements in Tamar River Water after Ultrafiltration

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION	CONCENTRATION ($\mu\text{g l}^{-1}$)								CONCENTRATION (mg l^{-1})	
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb	Ca	K
CROSSGATE	1/5/75	TOTAL	24.5	6.5	1.2	1.0	0.1	3.5	15.0	<5.0	14.0	4.1
		<10,000	6.0	6.2	2.0	1.0	0.1	3.0	9.5	<5.0	13.6	4.3
		<500	5.0	6.6	1.8	<1.0	0.4	2.5	7.0	<5.0	13.0	4.3
	1/7/75	TOTAL	30.5	15.0	2.2	1.0	0.25	4.0	7.0	<5.0	13.0	6.6
		<10,000	6.0	12.5	3.3	<1.0	0.2	3.0	5.0	<5.0	12.0	6.7
		<500	3.5	7.5	2.0	<1.0	0.2	2.5	1.0	<5.0	13.0	6.1
	23/7/74	TOTAL	170.0	28.9	3.8	1.0	0.4	7.0	1.0	<5.0	-	-
		<10,000	33.0	31.0	2.6	1.0	0.6	5.0	1.0	<5.0	-	-
		<500	26.0	29.1	2.6	1.0	1.0	4.0	1.0	<5.0	-	-
LAUNCESTON	1/5/75	TOTAL	19.5	2.1	1.6	<1.0	0.25	2.5	13.5	<5.0	14.0	3.5
		<10,000	9.0	1.8	1.4	<1.0	0.30	2.0	10.5	<5.0	14.0	3.6
		<500	5.5	2.1	2.1	<1.0	0.20	3.0	10.5	<5.0	-	-
	1/7/75	TOTAL	15.0	3.3	1.2	<1.0	0.15	1.5	5.0	<5.0	15.0	6.1
		<10,000	7.5	3.6	1.25	1.0	0.15	1.5	4.5	<5.0	15.0	6.4
		<500	7.0	2.8	0.8	<1.0	0.15	1.0	2.5	<5.0	12.5	6.0

TABLE 5.5 (CONTINUED)

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION	CONCENTRATION ($\mu\text{g l}^{-1}$)								CONCENTRATION (mg l^{-1})	
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb	Ca	K
GUNNISLAKE	1/5/75	TOTAL	18.5	6.2	15.4	1.0	0.4	3.5	26.0	<5.0	16.9	2.3
		<10,000	11.5	7.1	10.3	<1.0	0.2	4.0	25.0	<5.0	16.5	2.8
		<500	5.5	6.5	12.6	<1.0	0.35	6.0	25.0	<5.0	16.9	2.8
	1/7/75	TOTAL	12.0	15.5	31.0	1.0	0.15	5.0	12.0	<5.0	18.0	4.2
		<10,000	3.5	13.5	25.0	<1.0	0.15	4.0	11.5	<5.0	17.9	4.3
		<500	3.0	12.5	21.0	<1.0	0.30	3.5	5.5	<5.0	16.9	3.5
	1/5/75	TOTAL	14.5	4.5	4.1	<1.0	0.2	1.0	1.0	<5.0	14.0	4.0
		<10,000	5.5	4.0	3.5	<1.0	0.2	1.0	1.0	<5.0	13.0	3.5
		<500	5.0	4.0	4.0	<1.0	0.2	2.0	1.0	<5.0	13.0	3.5
CALSTOCK	1/7/75	TOTAL	40.5	12.5	10.5	2.0	0.5	2.5	2.0	<5.0	32.0	30.0
		<10,000	7.5	13.0	11.5	<1.0	0.15	1.5	1.0	<5.0	25.0	28.5
		<500	3.5	12.0	10.0	<1.0	0.3	3.0	1.5	<5.0	26.2	26.0

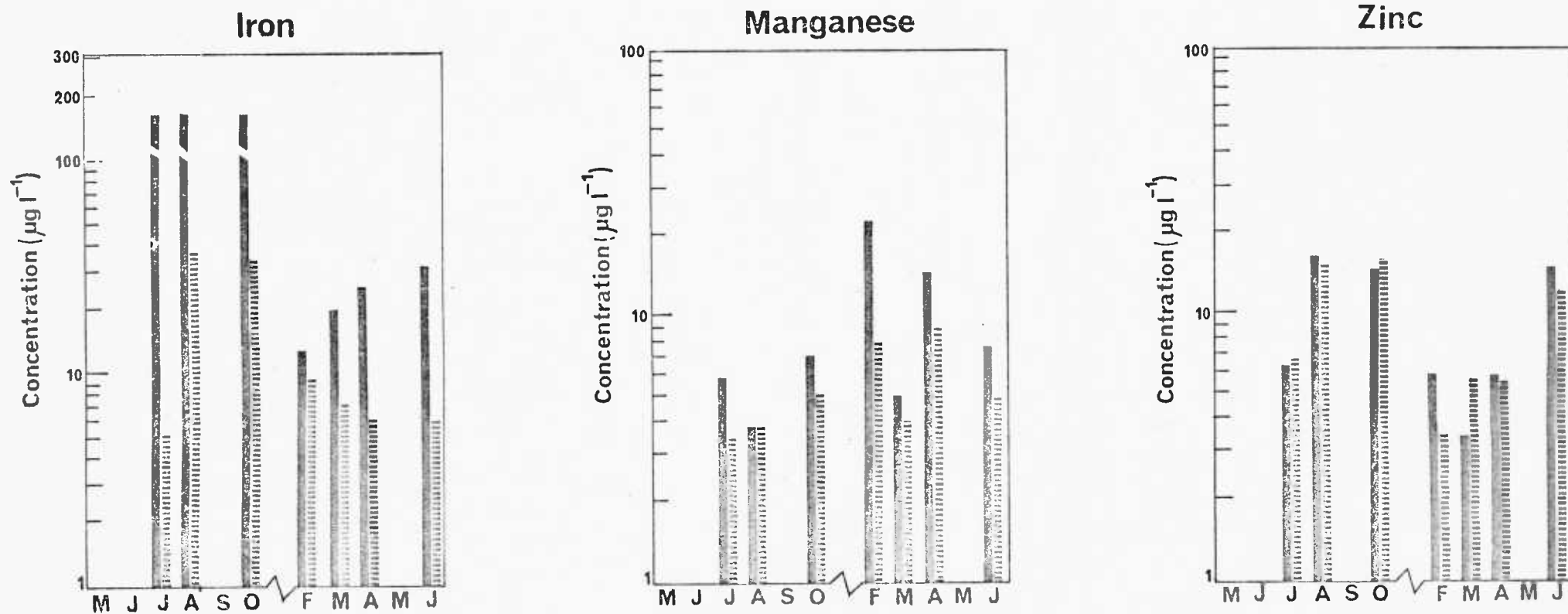


Fig. 5.4 Distribution of 'soluble' iron, manganese and zinc in Tamar River Water (Crossgate) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

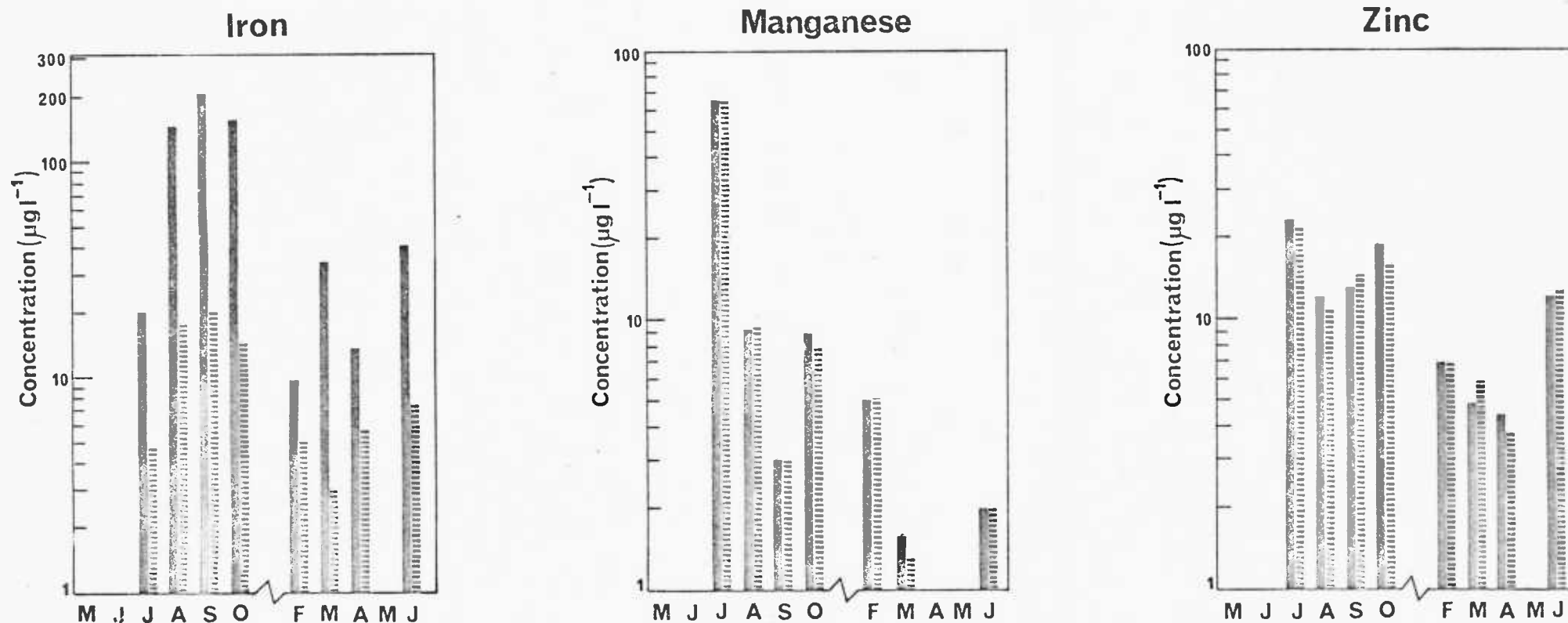


Fig. 5.5 Distribution of 'soluble' iron, manganese and zinc in Tamar River Water (Calstock) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

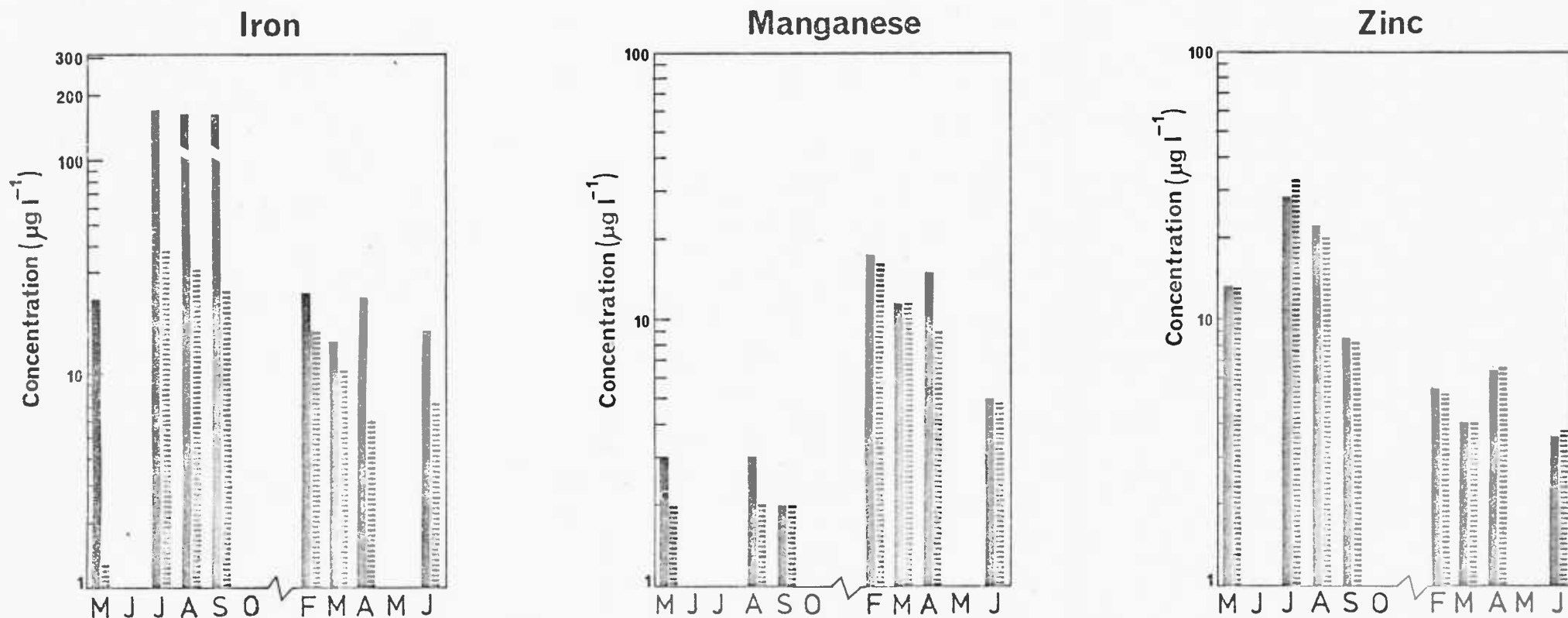


Fig. 5.6 Distribution of 'soluble' iron, manganese and zinc in Tamar River Water (Launceston) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

5.2.2.3 Distribution of Metal Ions in Fowey River Water after Ultrafiltration

The waters taken from the northern, moorland drainage of the River Fowey were all particularly 'soft'; concentrations of calcium were never greater than 10 ppm and commonly less than 5 ppm (Tables 5.6 and 5.7). Similarly, the levels of potassium were found in the range of 2 to 3 ppm; again, typical for river water (Tables 5.6 and 5.6). These waters were also acidic with pH values ranging from 5.0 to 5.5, and generally lower than found for the other rivers sampled. The combination of a low calcium concentration and enrichment with humic acids from the moorland peat probably accounts for the characteristic acidity of this drainage. The concentration differences before and after ultrafiltration provide little evidence for the complexation of calcium or the entrainment of potassium, within the colloidal or macromolecular fractions of the River Fowey. The distribution of iron, manganese, zinc and copper after ultrafiltration is shown in figures 5.7, 5.8 and 5.9 and in Table 5.7. With the exception of iron, the major proportion of soluble trace metals in Fowey river water is found in the low molecular mass fraction of <500. With the exception of iron, the levels of 'soluble' heavy metals found in the Fowey during the present study largely confirm previous findings (Aston et al, 1974; Aston and Thornton, 1975).

TABLE 5.6

Distribution of Calcium and Potassium in Fowey River Water after Ultrafiltration

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION (mol. wt.)	CONCENTRATION (mg l ⁻¹)	
			Ca	K
DOZMARY	31/1/75	TOTAL	2.9	0.9
		<10,000	2.7	1.0
	25/2/75	TOTAL	-	-
		<10,000	-	-
	25/3/75	TOTAL	4.0	1.5
		<10,000	3.5	1.5
EAST COLLIFORD	31/1/75	TOTAL	2.5	0.8
		<10,000	3.5	1.7
	25/2/75	TOTAL	3.5	1.3
		<10,000	3.1	1.3
	25/3/75	TOTAL	5.1	1.2
		<10,000	4.0	1.2
QUARRY WOOD	31/1/75	TOTAL	5.2	2.0
		<10,000	5.0	2.1
	25/2/75	TOTAL	6.4	2.2
		<10,000	6.0	2.3
	25/3/75	TOTAL	-	-
		<10,000	-	-

TABLE 5.6 (CONTINUED)

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION (mol. wt.)	CONCENTRATION (mg l ⁻¹)	
			Ca	K
CUTMADOC	31/1/75	TOTAL	4.0	1.9
		<10,000	4.0	1.9
	25/2/75	TOTAL	8.2	1.9
		<10,000	7.5	1.7
	25/3/75	TOTAL	-	-
		<10,000	-	-
LOSTWITHIEL	31/1/75	TOTAL	5.0	1.7
		<10,000	4.0	1.2
	25/2/75	TOTAL	9.0	1.9
		<10,000	9.0	2.1
	25/3/75	TOTAL	8.8	1.7
		<10,000	8.5	2.4

TABLE 5.7

Distribution of Trace Metals in Fowey River Water after Ultrafiltration

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION	CONCENTRATION ($\mu\text{g l}^{-1}$)								CONCENTRATION (mg l^{-1})	
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb	Ca	K
QUARRY WOOD	29/4/75	TOTAL	7.5	5.5	2.2	<1.0	0.25	1.5	1.5	<5.0	5.0	2.0
		<10,000	5.0	5.4	2.3	<1.0	0.10	1.0	1.5	<5.0	6.0	1.8
		<500	3.0	4.8	1.7	<1.0	0.10	<1.0	1.5	<5.0	6.0	1.7
DOZMARY POOL	29/4/75	TOTAL	32.5	17.0	1.4	<1.0	0.4	1.5	17.5	<5.0	-	-
		<10,000	21.0	18.0	2.0	<1.0	0.2	2.0	16.0	<5.0	-	-
		<500	19.6	17.0	3.0	<1.0	0.2	1.0	17.7	<5.0	-	-
	1/7/75	TOTAL	46.0	14.0	2.9	<1.0	0.2	<1.0	28.5	<5.0	3.0	3.5
		<10,000	21.5	15.0	3.0	<1.0	0.3	<1.0	24.0	<5.0	4.0	3.9
		<500	16.0	14.5	3.3	<1.0	0.4	<1.0	22.5	<5.0	4.0	4.0
EAST COLLIFORD	29/4/75	TOTAL	31.0	6.6	1.6	<1.0	0.15	2.0	17.5	<5.0	4.2	1.1
		<10,000	12.5	8.3	1.0	<1.0	0.10	<1.0	16.0	<5.0	3.0	0.8
		<500	8.5	8.0	1.8	<1.0	0.20	<1.0	17.7	<5.0	3.2	-
	1/7/75	TOTAL	33.5	12.0	2.5	1.0	0.2	2.0	18.0	<5.0	3.1	-
		<10,000	18.0	11.0	3.0	<1.0	0.2	1.5	16.0	<5.0	3.0	-
		<500	15.0	12.0	2.0	1.0	0.1	1.5	16.5	<5.0	-	-

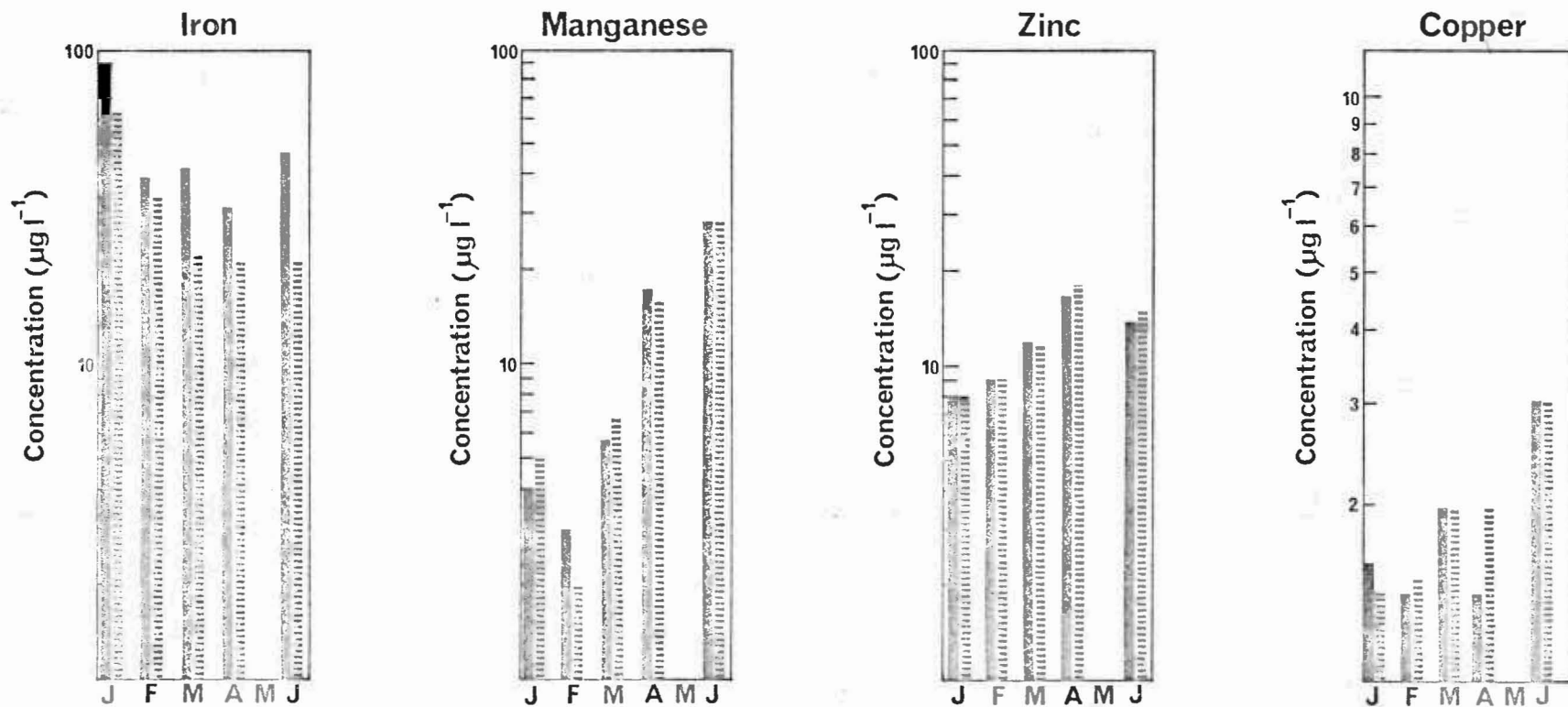


Fig. 5.7 Distribution of 'soluble' iron, manganese, zinc and copper in Fowey River Water (Dozmary Pool) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

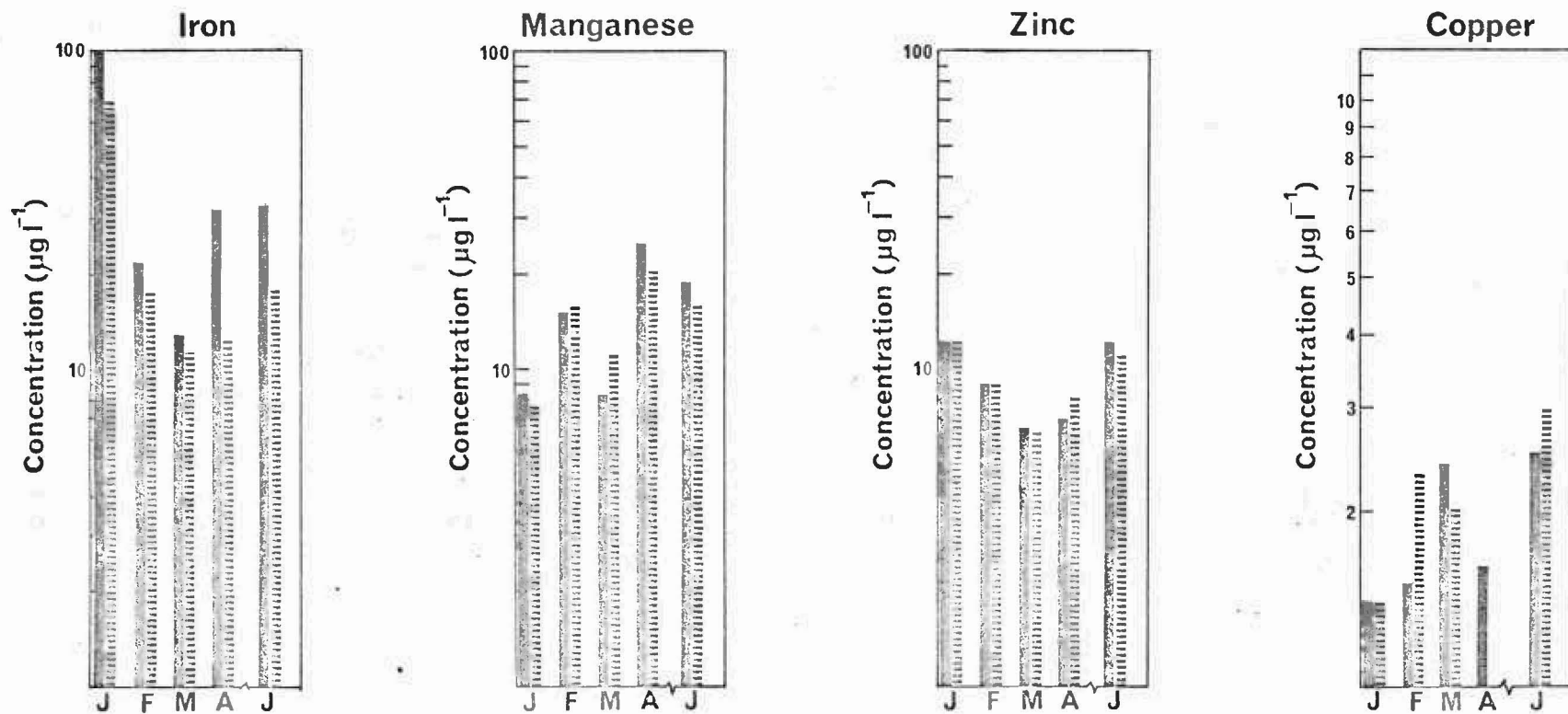


Fig. 5.8 Distribution of 'soluble' iron, manganese, zinc and copper in Fowey River Water (East Colliford) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

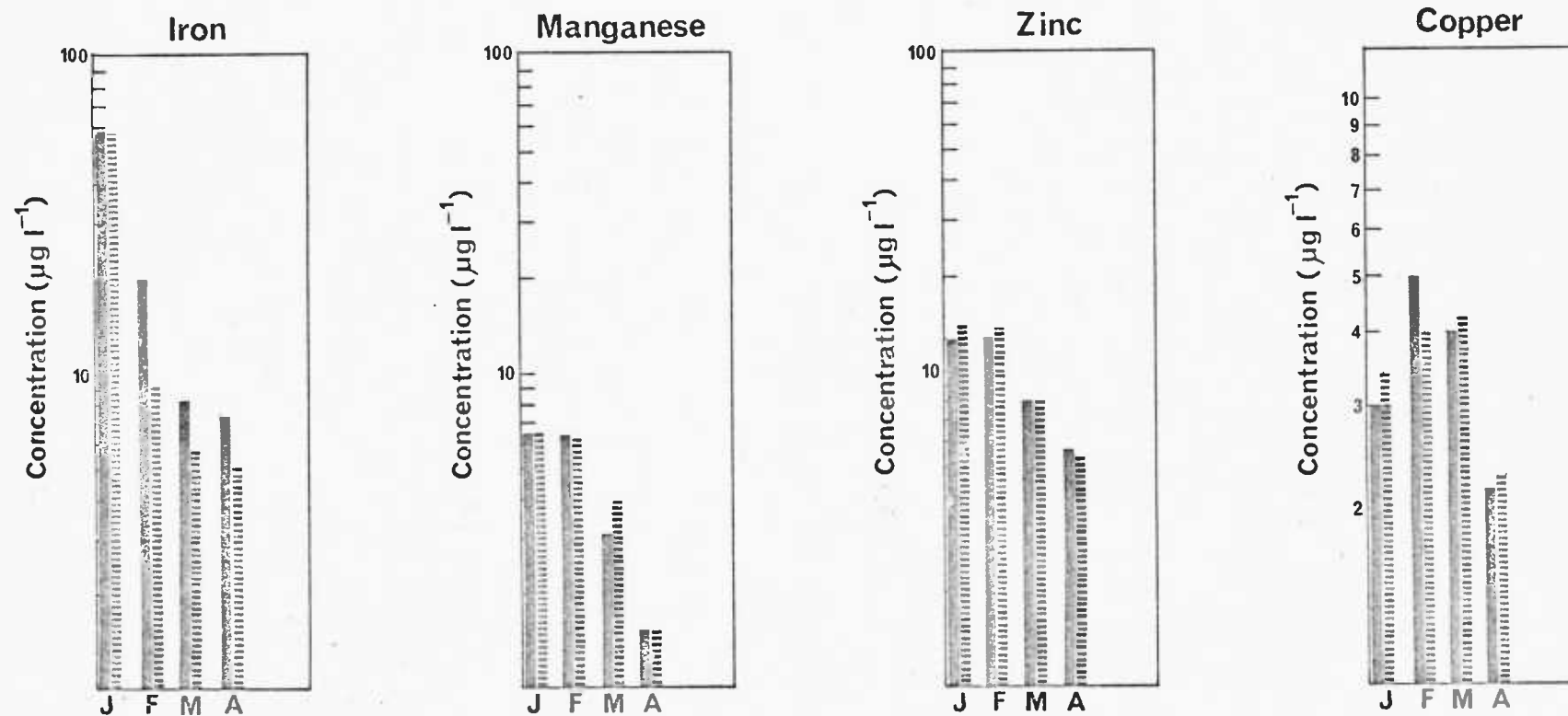


Fig. 5.9

Distribution of 'soluble' iron, manganese, zinc and copper in Fowey River Water (Quarry Wood) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

5.2.2.4 Distribution of Metal Ions in Carnon River Water after Ultrafiltration

The waters of the Carnon river were sampled at three points: Twelve Heads, Bissoe and Devoran (Chapter 1, Figure 1.1) and are relatively 'hard' with concentrations of calcium commonly in excess of 50 ppm below the Great County Adit, which lies between Twelve Heads and Bissoe (Tables 5.8 and 5.9). However, the calcium levels found in this drainage also vary strikingly with location, high concentrations are associated with mining waste waters flowing into the stream. The pH of these waters is typically 5.0 to 5.3 and comparable with values found in the other Cornish rivers sampled. The potassium concentrations were found to vary over a wide range from 3 to 25 ppm with the highest concentrations again localized around areas of current mining activity at Bissoe and Devoran. With the exception of lead and iron, all the measured concentrations of cadmium, cobalt, copper, manganese, nickel and zinc were high (Table 5.9, Figures 5.10 to 5.15); clearly the waters from Bissoe and Devoran are polluted. The major proportion of all 'soluble' heavy metals in these waters was found in the low molecular weight fractions in the ultrafiltrates of the PM-10 and UM-05 membranes. A broad range of concentrations for the soluble heavy metals in the Carnon River has been reported (Bryan and Hummerstone, 1973; Aston *et al*, 1974; Aston and Thornton, 1975; Thornton, Watling and Darracott, 1975). The results of the present study are broadly in agreement with these previous findings, however, generally lower concentrations of iron and copper were found. A mean concentration of $1080 \mu\text{g l}^{-1}$ for copper

TABLE 5.8

Distribution of Calcium and Potassium in Carnon River Water after Ultrafiltration

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION (mol. wt.)	CONCENTRATION (mg l ⁻¹)	
			Ca	K
TWELVE HEADS	31/1/75	TOTAL	18.0	3.1
		<10,000	-	-
	25/2/75	TOTAL	18.0	4.5
		<10,000	16.5	4.5
	25/3/75	TOTAL	-	-
		<10,000	-	-
BISSOE	31/1/75	TOTAL	23.0	4.7
		<10,000	22.5	4.7
	25/2/75	TOTAL	50.6	8.5
		<10,000	51.5	7.5
	25/3/75	TOTAL	74.2	14.5
		<10,000	73.8	14.0
DEVORAN	31/1/75	TOTAL	50.0	7.0
		<10,000	49.8	4.4
	25/2/75	TOTAL	75.0	9.2
		<10,000	76.3	9.6
	25/3/75	TOTAL	86.0	13.0
		<10,000	83.0	13.0

TABLE 5.9

Distribution of Trace Metals in Carnon River Water after Ultrafiltration

SAMPLE LOCATION	DATE	'SOLUBLE' FRACTION	CONCENTRATION ($\mu\text{g l}^{-1}$)								CONCENTRATION (mg l^{-1})	
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb	Ca	K
TWELVE HEADS	29/4/75	TOTAL	2.5	380	19.5	7.5	0.9	11.0	61.0	<5.0	15.4	-
		<10,000	1.5	385	18.5	8.0	0.9	11.0	59.5	<5.0	15.5	-
		<500	2.0	375	18.5	8.0	0.85	10.0	59.0	<5.0	15.0	-
BISSOE	29/4/75	TOTAL	35.5	1800	175	30.0	3.5	47.0	450	<5.0	-	-
		<10,000	27.0	1750	150	25.0	3.5	42.0	450	<5.0	-	-
		<500	26.0	1800	125	28.0	4.0	45.0	445	<5.0	-	-
	1/7/75	TOTAL	375	1350	145	25.5	2.25	42.5	650	6.5	210	24.0
		<10,000	350	1750	200	34.0	3.1	55.0	850	10.0	152	19.0
		<500	330	1760	196	33.0	3.4	54.0	830	10.0	174	19.0
DEVORAN	29/4/75	TOTAL	10.0	4550	180	40.0	3.5	120	500	<5.0	83.5	19.0
		<10,000	6.0	4850	150	27.5	3.7	140	480	<5.0	-	-
		<500	5.5	4750	177	30.0	3.7	140	465	<5.0	-	-
	1/7/75	TOTAL	215	3000	380	43.5	4.25	75.0	1400	7.5	160	22.0
		<10,000	225	2550	330	51.0	4.45	80.0	1370	8.0	154	18.0
		<500	232	2600	365	52.5	4.75	80.0	1410	7.5	151	19.0

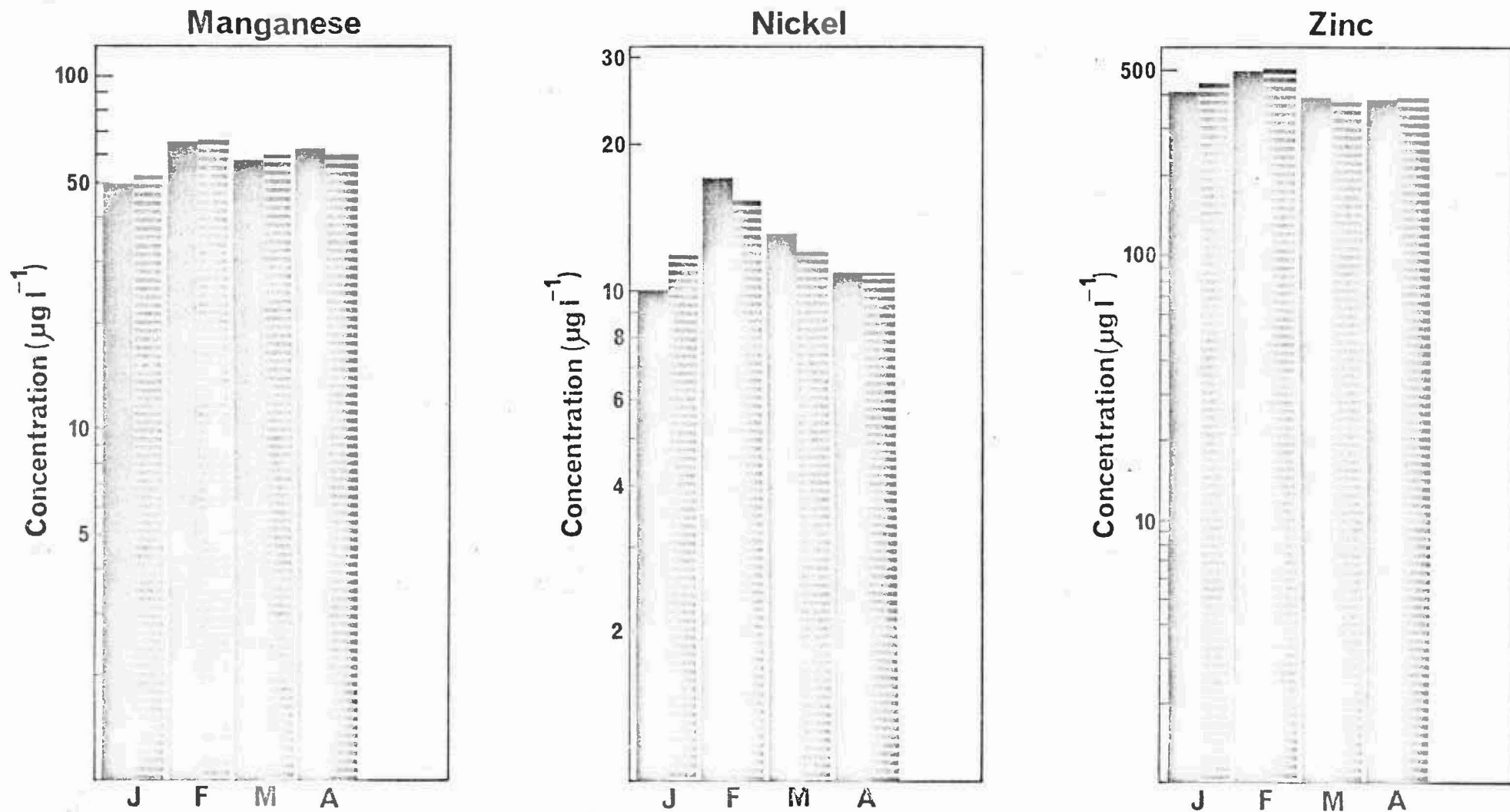


Fig. 5.10 Distribution of 'soluble' manganese, nickel and zinc in Carnon River Water (Twelve Heads) after ultrafiltration (— primary filtrate ▨ secondary ultrafiltrate)

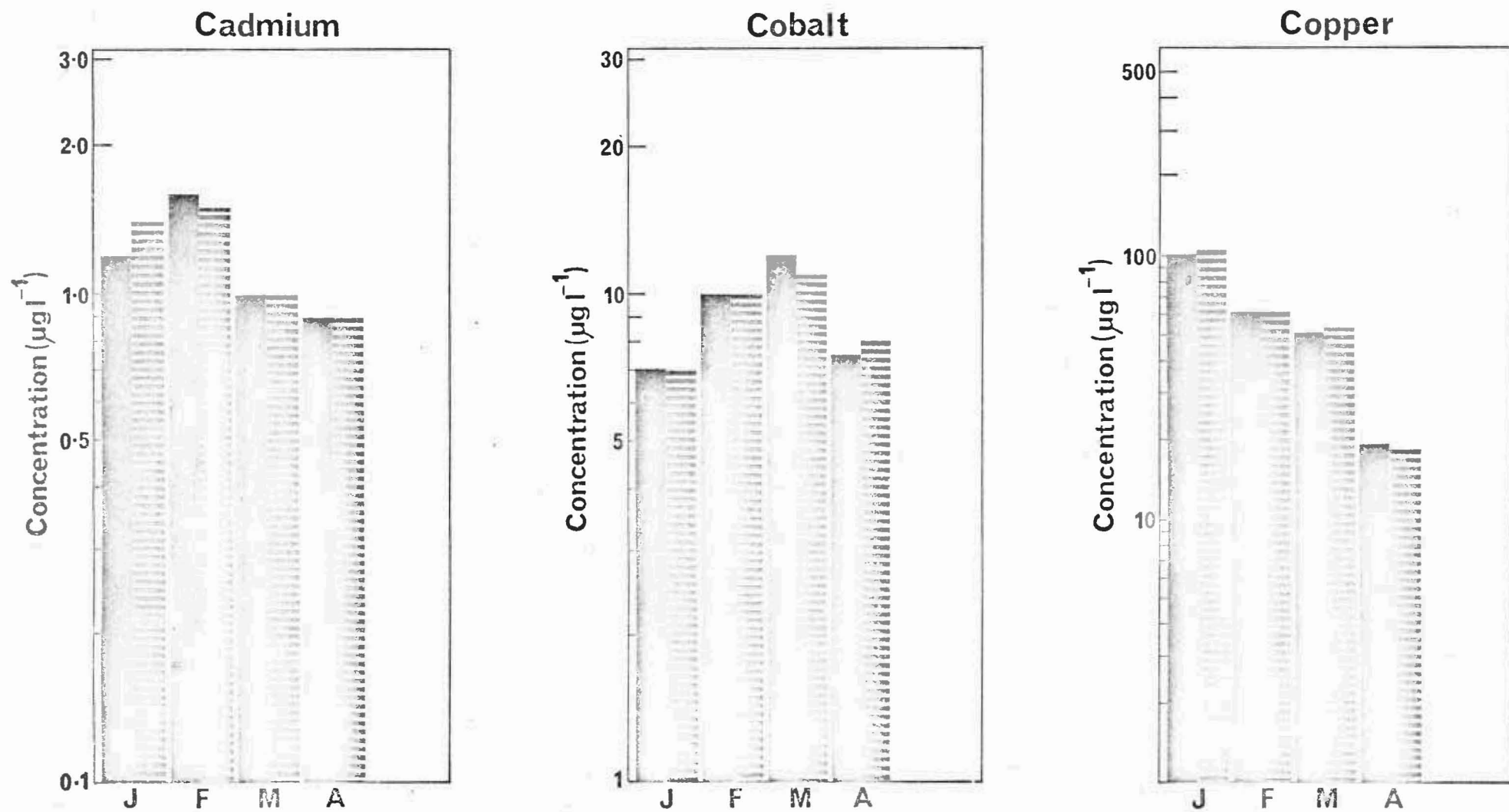


Fig. 5.11 Distribution of 'soluble' cadmium, cobalt and copper in Carnon River Water (Twelve Heads) after ultrafiltration (— primary filtrate ▨ secondary ultrafiltrate)

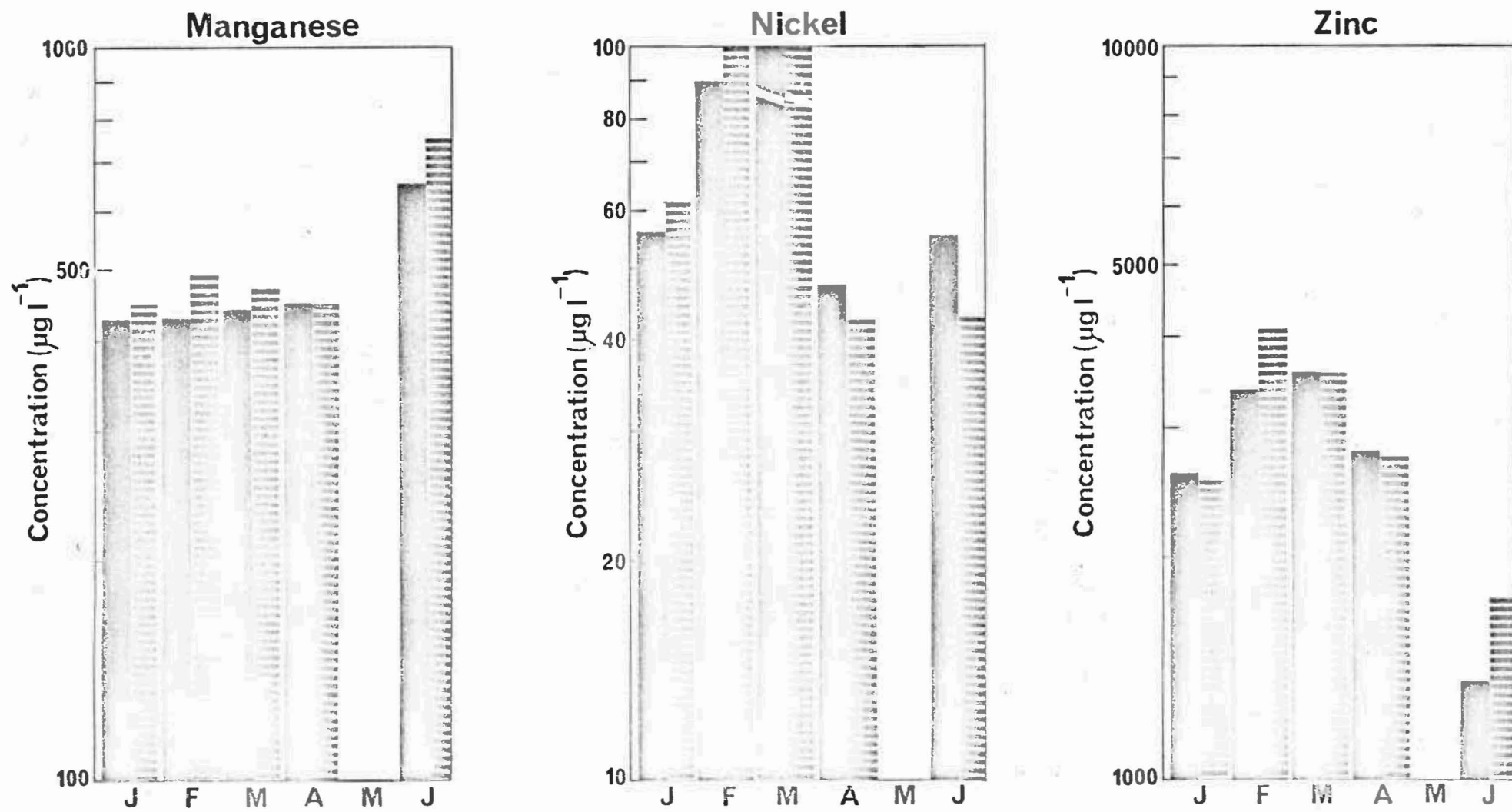


Fig. 5.12 Distribution of 'soluble' manganese, nickel and zinc in Carnon River Water (Bissoe) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

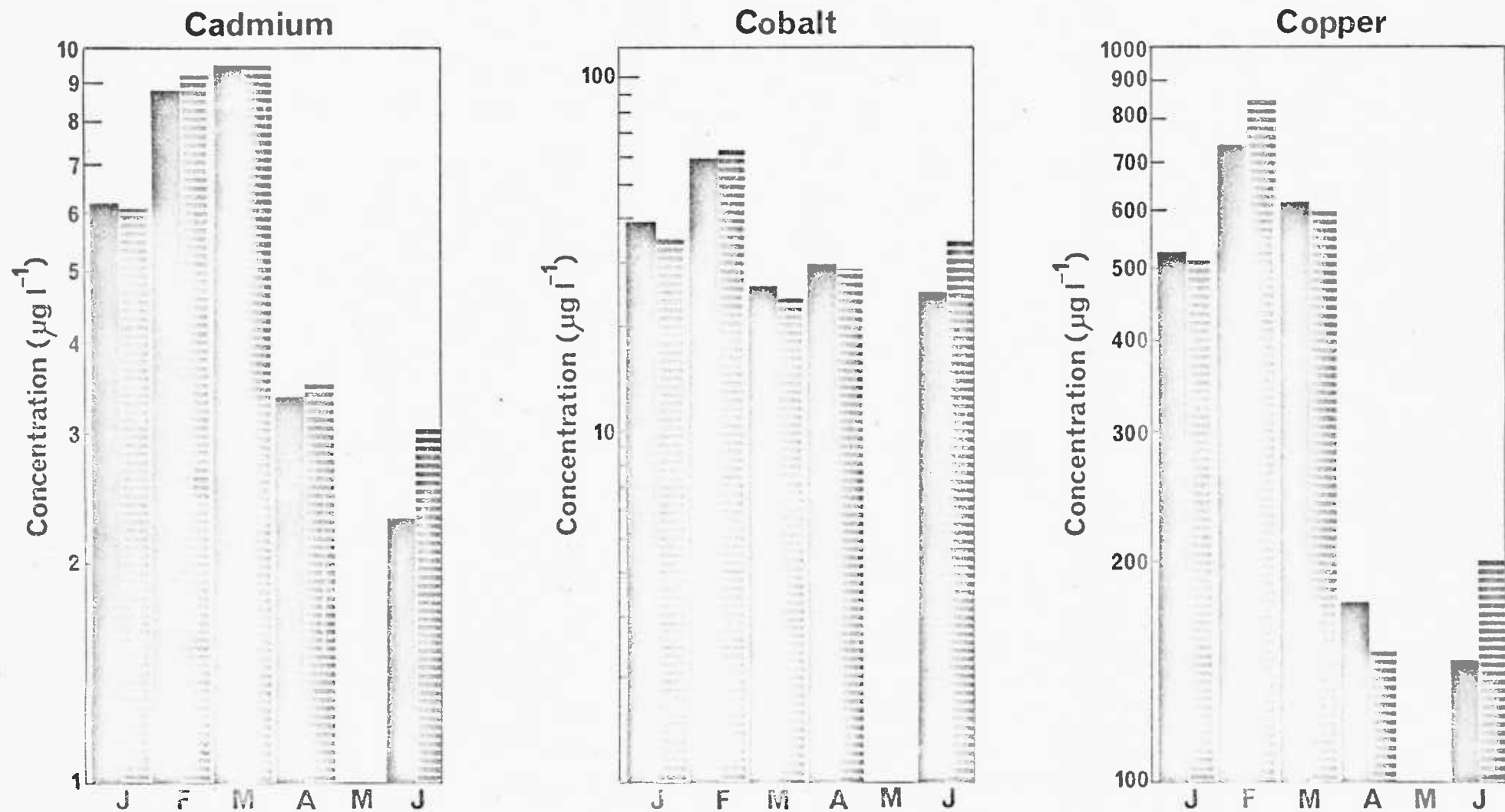


Fig. 5.13 Distribution of 'soluble' cadmium, cobalt and copper in Carnon River Water (Bissoe) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

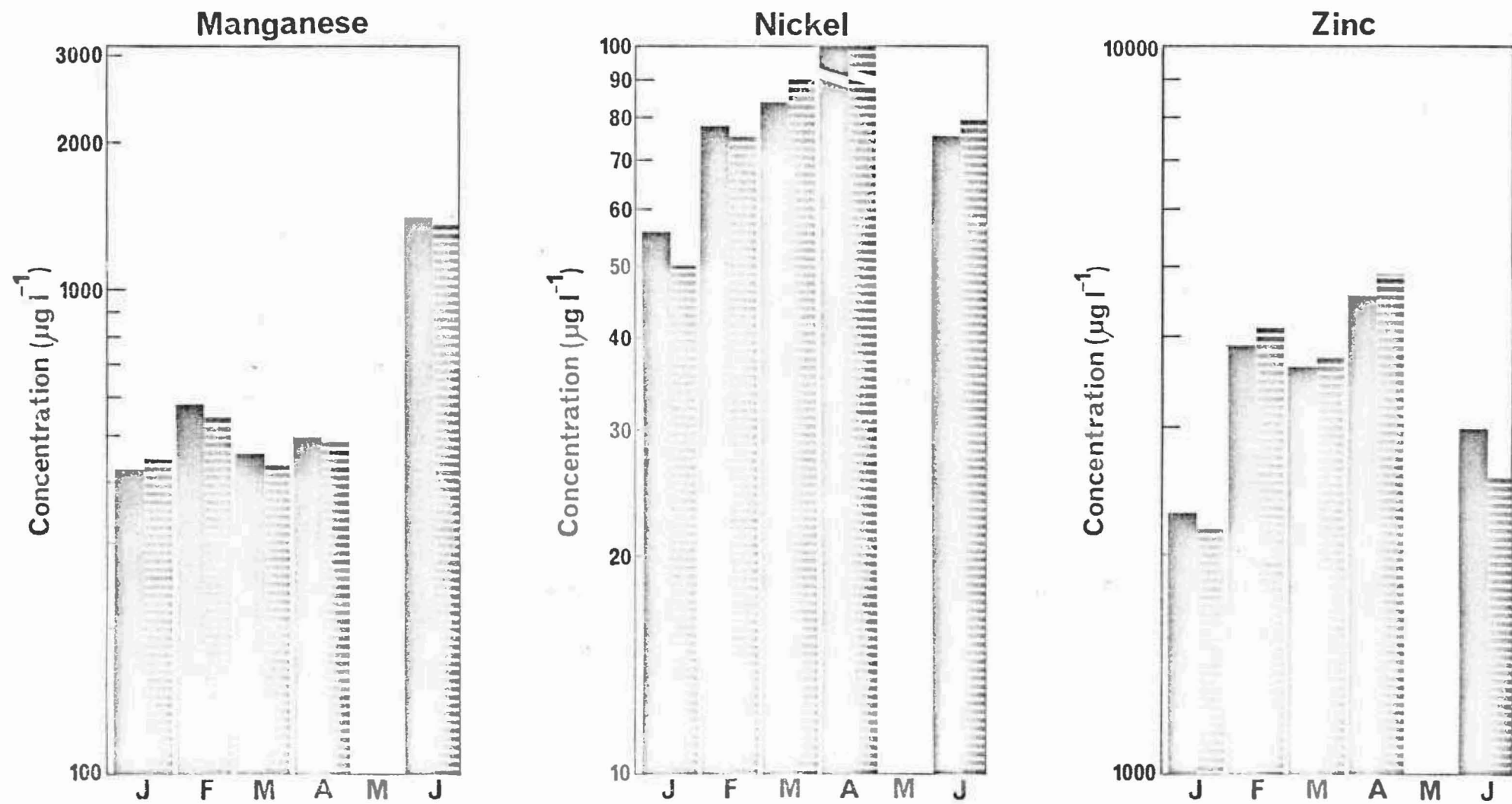


Fig. 5.14 Distribution of 'soluble' manganese, nickel and zinc in Carnon River Water (Devoran) after ultrafiltration (■ primary filtrate ▨ secondary ultrafiltrate)

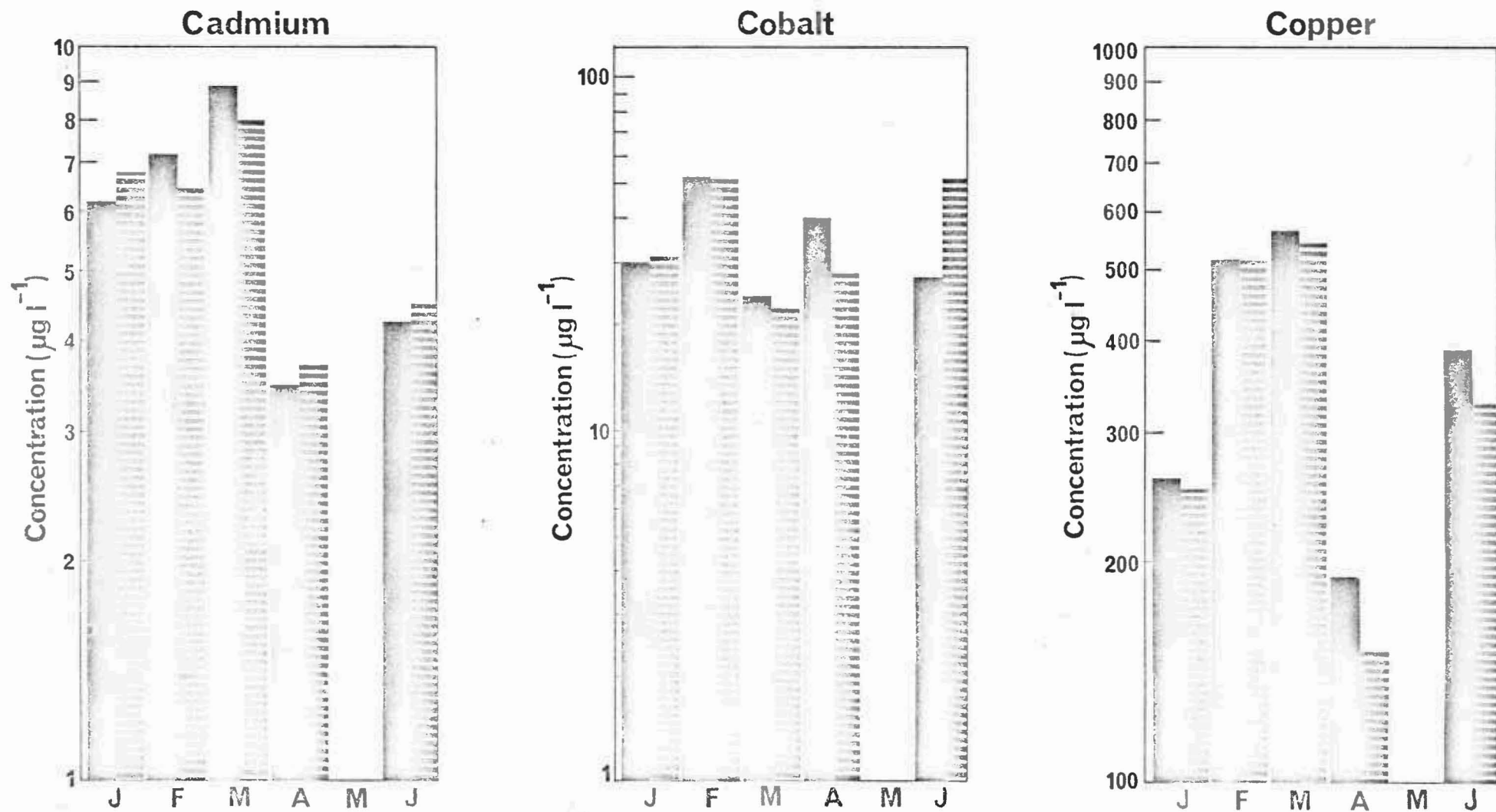


Fig. 5.15 Distribution of 'soluble' cadmium, cobalt and copper in Carnon River Water (Devoran) after ultrafiltration (▒ primary filtrate ▨ secondary ultrafiltrate)

in the Carnon has recently been reported (Aston et al, 1974; Aston and Thornton, 1975). In the present study levels of copper were never found to exceed $900 \mu\text{g l}^{-1}$ and the range of concentrations are more in agreement with the findings for Bryan and Hummerstone (1973) and the previous survey of Thornton, Watling and Darracott (1975). Nevertheless, these results largely substantiate the findings of the previous work.

5.3 DISCUSSION

The principal objective of this investigation has been to evaluate the significance of the colloidal and macromolecular constituents of surface waters, both as a medium for trace element transport and as a factor governing heavy metal speciation. As a preliminary step in this study of colloidal and organo-metallic complexes, the total concentration of 'soluble' trace elements and selected major ions in solution has been determined. This total 'soluble' fraction has been arbitrarily defined as the concentration determined in the filtrate of a glass-fibre filter. Using ultra-filtration, the colloidal and macromolecular fractions included in the primary filtrate are removed and the concentration of the minor and selected major elements in the secondary and tertiary ultrafiltrates are also determined. In the absence of any contamination or adsorption losses, the differences in concentration before and after ultrafiltration represent the levels of colloidal species isolated with any dialyzate. However, this inference includes a number of important assumptions which require justification.

The total 'soluble' fraction is practically defined in the present study and taken to include all the soluble and colloidal constituents occurring in the filtrate of a Whatman (GF/C) glass-fibre filter. All particles and polymers of dimensions less than $0.5\ \mu\text{m}$ and their associated ions will be included in a gross analysis of the primary filtrate (Sheldon, 1972; Wilson, 1976). The particle retention characteristics of the glass-fibre filter therefore practically define the upper limit of the colloidal size class. The justification for using this primary filter is based upon classical definitions of the colloidal size range discussed in Chapter 1. However, in practice it is also expedient to filter water samples rapidly in order to remove suspended sediment. In addition contamination and adsorption losses of both organic and inorganic constituents, must be kept to a minimum in any study of metal-organic associations in solution. On the basis of such criteria glass-fibre filters are to be preferred to the more conventional $0.45\ \mu\text{m}$ cellulose acetate/nitrate membranes which are commonly used. Nevertheless, the total 'soluble' concentrations of heavy metals, as defined in the present study, agree well with the results of previous investigations in which conventional membrane filtration was used to remove particulate material from waters of the Carnon, Fowey and Helford rivers (Bryan and Hummerstone, 1973; Aston et al, 1974; Aston and Thornton, 1975; Thornton, Watling and Darracott, 1975). Clearly the retention characteristics of the membrane used to isolate colloidal material will similarly define the lower size limit of the colloidal classes. The Amicon PM-10 ultrafiltration membrane provides a practical means

of delineating this lower limit with 'particle' retention characteristic which are compatible with the definition of Rickert and Hunter (1972).

In establishing the concentration of colloidal, heavy metal species in river water from the differences in concentration of 'soluble' metals before and after ultrafiltration it is also assumed that:

- i The colloidal and macromolecular fractions are not denatured by the transport, preservation and manipulation of the water sample.
- ii The concentration of heavy metals found in the primary (GF/C) filtrate is the total concentration of these elements occurring in this fraction.
- iii The differences in concentration before and after ultrafiltration are a true reflection of the concentration of the colloidal and macromolecular species included in the total 'soluble' fraction.
- iv The retention characteristics of the selected ultrafiltration membrane (Amicon PM-10 membrane) provides a realistic lower limit to the colloidal size range used in this study.

In the present study, water samples were transported to London from Cornwall and then refrigerated or stored deep-frozen until required for analysis. No preservatives were added until after filtration when filtrate aliquots were acidified to pH 2. The problems of sample storage and preservation in relation to the study of trace metal speciation in colloidal dispersions of natural waters have been considered in previous studies (Breger, Zubovic and Chandler, 1972; Benes and Steinnes, 1975). The freezing of samples has been rejected by these workers as a method of preservation because of the possibility of destroying the colloidal system, and

refrigeration has been recommended (Breger, Zubovic and Chandler, 1972). Clearly only immediate analysis after sampling would be wholly satisfactory but is impractical for detailed investigation of a large number of bulk samples. In extrapolating the extrinsic findings of any study to the conditions occurring in the river at the time of sampling, it must be assumed a priori that the integrity of the sample has been maintained through out all the manipulations of analysis. The justification of such an assumption is difficult to prove.

The assumption that the concentration of heavy metals found in apparent solution in the primary filtrate is the total analysis of these elements may be invalid if the trace metals occurring in the colloidal fractions are not wholly extractable by means of the chelating/solvent extraction method employed here. Essentially this is a problem due to unreactive, non-labile species which must be taken into account since it is possible that the chelating agent used in these analyses - diethyl dithiocarbamate - is in competitive equilibrium with other naturally occurring complexing agents within the sample. Alternatively, heavy metals occluded within colloidal particles and entrained within organic polymers may be inaccessible to the diethyl dithiocarbamate and hence are not extracted or included in the observed total 'soluble' concentrations. The problem of reactive and unreactive species of zinc in sea water has been considered by Fukai and Huynh-Ngoc (1975). In their investigations only 50% of the 'soluble' zinc in sea water was thought to be extractable with dithizone after cold acid treatment. However, in contrast, the estimation of 'ionic' copper by diethyl dithiocarbamate extraction

has been criticized since computations suggest that only copper species complexed with a stability constant in excess of 10^{30} will be excluded from the total analysis (Kamp-Nielsen, 1972). Clearly many complexed species would also be included in the fraction described as 'ionic' copper. Examination of the known stability constants of metal-fulvic acid complexes indicates that all of these heavy metal species would be included in the diethyl dithiocarbamate extractable fraction. Similar evidence regarding the recovery of trace metal species from sea water using a chelating resin in analysis also indicates that the fulvic and humic acids form only weak complexes with trace elements which may be efficiently extracted from the colloidal and macromolecular material of natural waters by means of a strong chelating agent (Florence and Batley, 1976). Nevertheless, the concentration of heavy metals occurring in the non-dialyzable residue, retained by the ultrafiltration membrane, should also be determined to establish the occurrence of any hetero-ions detected indirectly in this fraction by differences in concentration before and after filtration.

In the Cornish river waters studied many of the heavy metals occur only in trace amounts in the total 'soluble' fraction. The 200 fold concentration achieved with solvent extraction is still insufficient to yield extracts at optimum levels for determination by flame atomic absorption spectrophotometry. Clearly analytical error may obscure small but significant differences in concentration before and after ultrafiltration. In this study the number of samples required to be analysed precluded the use of replicate

analysis and a statistical evaluation of results. In the absence of such information it is difficult, if not impossible, to assess the significance of marginal differences in concentration observed in the primary and secondary ultrafiltrates (Tables 5.3, 5.5, 5.7, 5.9). Interpretation of positive or negative differences in terms of contamination, complexation, or adsorption would be difficult to verify. Nevertheless, it may be argued that the observed trace metal concentration in the ultrafiltrate of a natural water sample determines the concentration of the species occurring in a molecular size class defined by the membrane retention characteristics. In the latter stages of this study two such size classes ($<10,000$ and <500 nominal molecular mass) were examined. With the exception of iron, manganese and calcium, the concentration of metals in the primary (GF/C) filtrate was not apparently reduced by ultrafiltration in many of the waters examined. However, analysis of the non-dialyzable residues retained by the ultrafiltration membranes is still required to confirm the findings of this indirect method of evaluating cation-binding in river water. Isolation of natural complexes remains, perhaps, one of the simplest and most direct methods of confirming complex formation (Martell and Calvin, 1952). The results of qualitative and quantitative analysis of the dialyzates isolated during this study are reported in Chapter 6.

The results of this investigation clearly indicate that the 'soluble' heavy metals in the four rivers studied occur as low molecular mass species which are found in the ultrafiltrates of two types of membrane. With the exception of iron, which has been

considered in Chapter 3, there is little evidence from these findings for the occurrence of humic or fulvic acid-metal complexes in river water. Recent studies on the stability of hydroxo and carbonato complexes of lead, copper, cadmium and zinc indicate that, these together with aquo and chloro complexes dominate the speciation of transition elements in natural waters (Bilinski, Huston and Stumm, 1976; Gardner, 1974; Stiff, 1971). Clearly these results substantiate, in a more direct manner, previous findings in so far as the 'soluble' trace metals were found in the low molecular mass fraction (nominal molecular weight <500).

The heavily polluted waters of the Carnon River flow into the head of Restronguet Creek where classical entrainment and co-precipitation of iron and copper has been reported previously (Bryan and Hummerstone, 1973). Iron coatings are a feature of the stream sediments of this drainage, but no evidence for the formation of a colloidal precipitate or the occurrence of organo-iron complexes were found during the present study. In Carnon river water, high concentrations of both iron and other heavy metals were found in the low molecular weight class of <500 nominal molecular mass (Table 5.9), occurring, probably, in the form of simple organic complexes, free ions and ion pairs. The removal of heavy metal ions from solution due to co-precipitation with polymeric hydroxo iron species would be anticipated from the classical chemistry of Fe(III) at a pH >3.5. The dissolution of iron as a result of increasing pH in Restronguet Creek may govern the concentration of copper in these

waters (Bryan and Hummerstone, 1973) and result in the formation of a heterogeneous precipitate in the particulate phase (Aston and Chester, 1973). Paradoxically, Bryan and Hummerstone (1973) found little evidence for the enrichment of zinc and manganese in particulate matter or for the precipitation of these metals in the Creek. Comparable conservative behaviour of manganese and zinc and the precipitation of iron has been reported in the Beaulieu Estuary (Holliday and Liss, 1976). However, in this instance the flocculation of predominant organo-iron complexes is thought to account for the phenomenon; in the present study there has been no evidence of macromolecular, organic iron complexes in Carnon river water (Chapter 3).

Organic, colloidal and macromolecular species of iron were found in the two non-dialyzable fractions of most of the unpolluted waters studied (Chapters 3, 4 and 6). However, the distribution of heavy metals within the ultrafiltrates of these waters varied little with these forms of iron upon their isolation. Sequestration of iron in waters such as those of the Helford and Fowey, may prevent the formation of ferric oxide precipitates and the entrainment of trace metals. Clearly these organo-iron complexes would appear to have little influence upon the size distribution of the other dissolved heavy metal species. This conclusion is in marked contrast to the demonstrable association of iron and manganese in molecular size fractions of interstitial waters from lake sediments (Schindler, Alberts, and Honick, 1972).

In the organically enriched conditions of eutrophic lakes

and moorland streams, the occurrence of calcium in both high and low molecular weight fractions has been observed (Gjessing, 1970; Schindler and Alberts, 1974). Similarly, Otsuki and Wetzel (1973) have reported that large amounts of lacustrine humic acids are removed from solution with precipitating calcium carbonate. In the present study, calcium was found to remain in the low molecular weight fractions (<10,000 and <500). Nevertheless, at a number of localities on the Fowey, Helford and Tamar rivers, trace concentrations (1 to 2 ppm of the total 'soluble' fraction and representing only 5 to 10% of the calcium concentration) were detected as a colloidal constituent. Gjessing (1970) reported that in the hard 'humic' waters of Norway, 14% of the calcium in 'solution' occurred in a molecular weight class of >10,000 and <20,000 molecular mass, while 17 to 32% was found in the 1,000 to 10,000 molecular weight range. Similarly, 30 to 80% of the calcium in Georgia reservoirs is found in the molecular size range of between 10,000 and 500,000 molecular weight (Schindler and Alberts, 1974). High concentrations of calcium are also bound to the high molecular weight fractions of interstitial waters in marine fjords (Nissenbaum, Baedeker and Kaplan, 1971). Clearly in hard surface waters and in estuarine muds, polymeric organic acids may play an important role in the geochemistry of calcium and perhaps, to some degree, regulate the hardness of a water body. In the four rivers studied in Cornwall, soluble calcium is found predominantly in the low molecular weight fraction (<500 nominal molecular mass).

However, calcium may prove an important naturally occurring

coagulant of humic substances in these and other natural waters (Eckert and Sholkovitz, 1976).

Using ultracentrifugation it has been shown that colloidal particles recovered from Tennessee streams of a mineralized catchment, and occurring in the 0.01 to 0.15 μm size range, consist almost entirely of illitic material with traces of kaolinite and vermiculite. Colloidal fractions recovered in the present study were amorphous and largely organic in composition (Chapter 6). No colloidal clay material was found in the Cornwall streams studied. In addition, potassium in these waters remained in the ultrafiltrate and low molecular weight fractions indicating that illitic, colloidal clays are not a significant constituent of the natural colloids present. The entrainment of larger particles during centrifugation may account for the unexpected observation reported previously (Perhac and Whelan, 1972; Perhac, 1972).

5.4 CONCLUSIONS

1. With the exception of iron 'soluble' heavy metals at both pollutant and trace levels in waters from four rivers in Cornwall occur as low molecular weight species (<500 nominal molecular weight).
2. Colloidal and macromolecular fractions of 'soluble' iron contain no significant fraction of the heavy metals as entrained species or adsorbed heteroions.
3. A small proportion (5 to 10%) of 'soluble' calcium, in the organically enriched streams, is associated with the colloidal and macromolecular fractions in the Helford, Fowey and Tamar waters.
4. No colloidal clays in the form of finely divided illitic material could be detected from the distribution of 'dissolved' potassium within the molecular size classes defined in the present study.

C H A P T E R 6

6. CHARACTERIZATION OF COLLOIDAL AND MACROMOLECULAR MATERIAL IN NATURAL WATERS

6.1 INTRODUCTION

The constituents of natural waters occurring in the colloidal size classes are principally organic with trace inorganic material as amorphous minerals or heteroions. The origin and nature of colloids and polymers in river water have been discussed in Chapter I and a speculative distribution of these size classes (of the submicron range) is presented in Fig. 5.1. Much of the material isolated from sediments and waters, either by means of extractants or by some physical separation will constitute an admixture of heterogeneous organic compounds and minerals (Cooper and Harriss, 1974). Using ultrafiltration it is possible to resolve the colloidal suspension of natural waters into broad molecular size fractions but, clearly further microanalysis is required to determine the composition of the isolated dialyzates and to characterize the material retained. In this Chapter, the results of a preliminary investigation into the nature of colloidal and macromolecular substances in river waters are summarized and include the results of elemental analysis and infrared and x-ray spectroscopy examination.

6.2 RECOVERY OF DIALYZATES CONCENTRATED FROM RIVER WATER USING ULTRAFILTRATION

The high molecular weight fractions (>10,000 nominal molecular weight) in river water from three of the catchments described in the study area (Chapter I) were isolated using Amicon PM-10 ultrafiltration

PLATE I

Electron Micrographs of Amicon PM-10 Ultrafiltration Membranes

- a) Top: Unused membrane surface after Ethanol-Acid Washing (x 10,000)
- b) Middle: Membrane surface after brushing with a soft nylon brush (x 5,000)
- c) Bottom: Damage to membrane surface caused by vacuum suction (x 5,000)

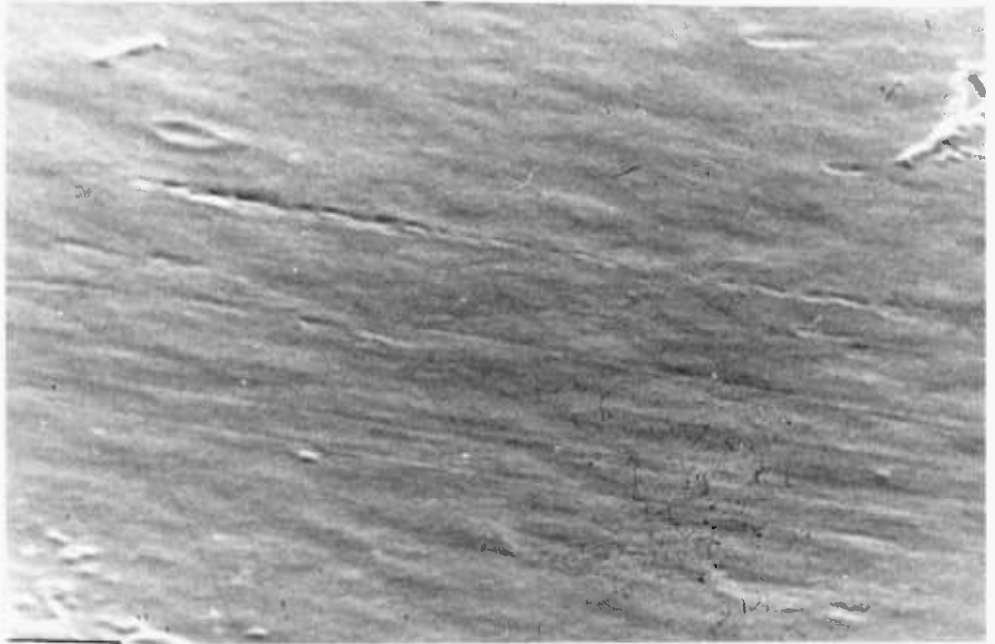
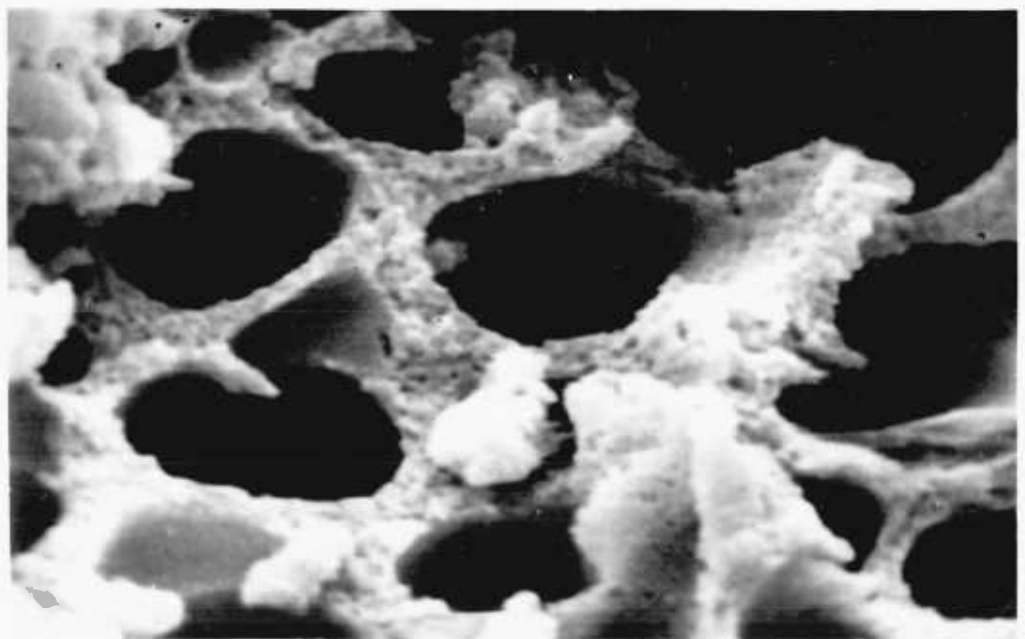
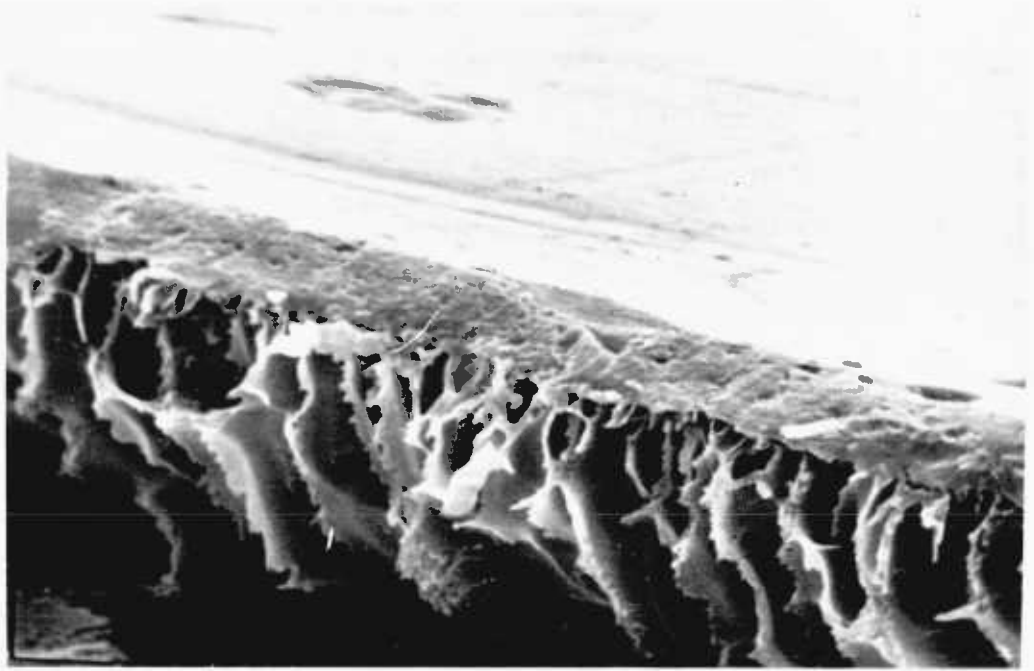


PLATE 2

Electron Micrographs of Amicon PM-10 Ultrafiltration Membranes

- a) Top: Lateral view of unused membrane showing 'active' surface deposit on the labyrinth of the polysulphon support (x 2,000)
- b) Middle: Lateral view of polysulphon support material (x 5,000)
- c) Bottom: Surface of support material with 'active' membrane surface removed (x 10,000)



membranes and a simplified ultrafiltration cell (Chapter 2). The concentrated residues were dialyzed or washed using 100 ml of distilled, deionised water and formed a thin-film coating on the 'active' surface of the membrane. The most convenient means of recovering the dialyzate from the membrane surface has been by mechanical separation using the smooth edge of a polished 'Plexiglass' spatula or a polished PTFE rod. The humic substances of the dialyzates on the membrane surface may be returned into suspension using a dilute (0.5M) sodium hydroxide solution (Briese and Giesy, 1975). However, this practice may denature the dialyzed material since proteinaceous compounds are precipitated and associated hetero-ions may be hydrolyzed or lost under such conditions. After the recovery of dialyzates, membranes were cleaned using a dilute 0.5M NaOH solution prior to acid washing and re-use.

Several alternative means of recovering the high molecular weight dialyzates have been tried; ultrasonic vibration proved ineffective and vacuum suction apparatus using a water pump connected to a PTFE nozzle was found to destroy the durable membrane surface (Plate 1c and 2c). A soft, nylon brush is a satisfactory alternative method of recovery but is found to leave impressions upon the membrane surface (Plate 1b) but with no apparent detriment to the performance of the membrane upon re-use. The dialyzates (<10,000 and >500 nominal molecular weight) retained in a 2 ml volume by the Amicon UM-05 membranes are highly soluble and readily maintained in solution by gentle agitation. The solution is conveniently decanted from the filtration cell with a Pasteur pipette. All the dialyzates recovered

during the present study were freeze-dried to yield brown, tomentous residues (Beck, Reuter and Perdue, 1974; Leenheer and Malcolm, 1973).

6.3 ELEMENTAL COMPOSITION (C, H, N) OF COLLOIDAL AND MACRO-MOLECULAR FRACTIONS IN RIVER WATER

Elemental analysis provides a useful means of preliminary characterization of organic compounds from the distribution and ratio of major elements. The amorphous nature of the dialyzates concentrated from Cornish rivers during the present study suggests a predominantly organic composition of which the humic substances are an important constituent. The elemental composition of humic and fulvic acids from a variety of sources is summarised in Table 6.1. Carbon (35% to 60% (w/w) but typically 56%) and oxygen (27% to 47% (w/w)) concentrations are high but percentages of hydrogen and nitrogen range from 4% to 7% (w/w) and from 2% to 7% (w/w) respectively. The low molecular weight fulvic acids generally contain less carbon and more nitrogen and oxygen than the high molecular weight humic acids (Rashid and King, 1971). Sulphur concentrations ranging from 1% to 2% (w/w) and traces of phosphorus are also found in humic and fulvic acids (Schnitzer and Khan, 1972; Malcolm, 1976). Twenty to fifty percent of the nitrogen in humic substances occurs as amino acids (Schnitzer and Khan, 1972; Beck, Reuter and Perdue, 1974) or in the form of porphyrin groups (Goodman and Cheshire, 1973).

The C, H, N composition of two molecular size fractions, recovered as freeze-dried dialyzates from river waters during the present study, were determined using a Perkin-Elmer Model 240

TABLE 6.1

Elemental Composition of 'Humic Substances'

Humic Acids							Fulvic Acids							Origin	Reference
C%	H%	O%	N%	S%	P%	% Ash	C%	H%	O%	N%	S%	P%	% Ash		
-	-	-	-	-	-	-	49.98	6.76	36.4	6.40	0.46	-	3.4	Sea water	Stuermer and Harvey (1974)
49.70	5.40	-	1.70	-	-	10.00	-	-	-	-	-	-	-	Swamp water	Fotiyev (1968)
39.37	6.84	-	2.92	-	-	54.43	-	-	-	-	-	-	-	Ground water	
-	-	-	-	-	-	-	47.34	5.25	45.49	1.92	-	-	-	Lake water*	Alberts <u>et al.</u> , (1976)
57.12	6.91	27.97	6.19	-	-	-	-	-	-	-	-	-	-	Lake sediment	
15.5	4.6	35.6	1.8	-	-	42.5	-	-	-	-	-	-	-	Sea water	Breger, Zubovic and Chandler (1972)
24.0	3.8	32.4	2.3	-	-	37.5	-	-	-	-	-	-	-	Marine sediment	
59.28	4.90	35.07	4.07	0.73	0.23	1.19	62.28	6.38	43.35	2.77	0.81	0.59	15.00	Soils and sediments	Malcolm (1976)
57.13	3.53	29.73	2.07	0.39	0.10	0.22	48.59	3.53	40.17	1.10	0.39	0.36	1.69		
53.8	3.7	31.9	1.6	1.1	-	-	42.5	3.3	44.8	0.7	0.1	-	-	Soils*	Schnitzer and Khan (1972)
60.4	5.8	36.8	4.1	0.4	-	-	50.9	5.9	47.3	2.8	1.7	-	-	Lake sediments	
53.7	5.8	35.1	5.4	-	-	-	46.2	5.9	45.3	2.6	-	-	-	Fresh water	
43.6	-	-	-	0.39	-	-	-	-	-	-	-	-	-		Swanson <u>et al.</u> , (1966)
40.8	8.5	-	5.3	-	-	-	-	-	-	-	-	-	-		
30.1	6.9	-	5.4	-	-	-	-	-	-	-	-	-	-	Marine sediments*	Nissenbaum (1972)
-	-	-	-	-	-	-	35.50	5.40	-	1.86	-	-	-	Stream water	Black and Christman (1963)
-	-	-	-	-	-	-	43.90	4.75	-	1.96	-	-	-		
59.8	6.6	36.69	6.24	5.9	-	-	-	-	-	-	-	-	-	Marine sediments*	Nissenbaum and Kaplan (1972)
48.89	4.6	27.16	1.50	0.87	-	-	-	-	-	-	-	-	-		
55.0	6.2	33.9	4.9	-	-	5.3	-	-	-	-	-	-	-	Marine sediments	Rashid (1971)
53.8	6.5	35.9	3.9	-	-	4.3	-	-	-	-	-	-	-		
50.2	5.6	40.6	3.6	-	-	5.9	-	-	-	-	-	-	-		
54.1	5.7	34.6	5.6	-	-	5.0	-	-	-	-	-	-	-	Marine sediments	Rashid and King (1971)
58.7	5.5	30.9	4.7	-	-	-	-	-	-	-	-	-	-		
52.8	6.6	35.0	5.6	-	-	-	-	-	-	-	-	-	-		
57.1	7.0	31.8	4.1	-	-	-	-	-	-	-	-	-	-		

* Calculated on ash free basis

TABLE 6.2

Elemental Analysis of Ultrafiltration Dialyzates

DATE	31/1/75			25/2/75			26/3/75			1/5/75			1/5/74			1/7/75			1/7/74		
DIALYZATE	PM-10			PM-10			PM-10			PM-10			UM-05			PM-10			UM-05		
MOLECULAR WEIGHT	>10,000			>10,000			>10,000			>10,000			10,000-500			>10,000			10,000-500		
SAMPLE SITE	C%	H%	N%	C%	H%	N%	C%	H%	N%	C%	H%	N%	C%	H%	N%	C%	H%	N%	C%	H%	N%
Crossgate	-	-	-	42.2	-	3.8	41.0	-	3.2	42.3	12.5	3.7	45.2	-	2.5	40.7	-	4.2	41.5	-	3.5
Launceston	-	-	-	40.6	-	3.3	-	-	-	43.7	15.9	4.4	38.3	16.3	1.8	40.6	-	5.3	40.9	-	3.3
Gunnislake	-	-	-	-	-	-	39.6	-	2.3	40.8	13.4	4.2	42.3	15.8	2.3	41.3	-	4.6	43.2	-	6.5
Calstock	-	-	-	-	-	-	-	-	-	35.5	12.8	2.7	37.3	12.3	2.1	-	-	-	42.2	-	2.5
Dozmary Pool	40.5	4.9	3.5	40.0	-	3.2	39.9	-	2.5	41.7	-	3.6	44.7	-	3.7	39.4	12.9	3.5	43.1	-	2.2
E. Colliford	41.6	-	2.9	38.2	-	2.6	40.6	-	3.3	40.5	-	2.6	38.3	-	2.1	40.5	-	2.9	41.2	11.9	2.1
Quarry Wood	38.6	-	2.7	39.5	-	2.9	40.3	-	2.9	-	-	-	40.5	14.3	3.3	42.3	-	2.2	40.7	-	2.9
Lostwithiel	-	-	-	-	-	-	-	-	-	-	-	-	42.5	-	2.8	-	-	-	39.7	-	2.0
Trelowarren	-	-	-	-	-	-	-	-	-	-	-	-	38.7	-	3.0	-	-	-	37.3	-	3.0
Constantine	-	-	-	-	-	-	38.6	-	2.3	40.4	-	3.2	37.5	-	2.6	-	-	-	40.0	-	2.7
Gweek Quay	-	-	-	-	-	-	-	-	-	-	-	-	42.5	10.7	1.7	-	-	-	-	-	-
Wilake	-	-	-	-	-	-	-	-	-	-	-	-	36.7	10.6	1.0	-	-	-	-	-	-

elemental analyzer. The results of these analyses are shown in Table 6.2. The percentage organic carbon, which is remarkably uniform, is generally lower than that reported for humic and fulvic acids ranging from 35% to 45% (w/w) but the nitrogen concentrations, ranging from 1% to 6.5% (w/w) are comparable with previous findings (Table 6.1).

The C:N ratios of humic and fulvic acids extracted from soils vary markedly with the method of preparation used but are commonly between 10 and 20. A similar variation in the C:N ratios (10 to 15) for the two molecular fractions in river waters is also apparent from the results in Table 6.2. However, the relatively high percentage of hydrogen in some of these residues indicates that the concentration of the other major elements (carbon and nitrogen) may have been diluted by the presence of water of hydration and polymeric hydroxides.

6.4 INFRARED SPECTRA OF COLLOIDAL AND MACROMOLECULAR MATERIAL FROM RIVER WATER

Infrared spectra analysis can provide valuable information about the composition and structure of organic and inorganic molecules in a variety of physical forms. Different functional groups, e.g. COO^- give rise to absorption peaks or bands in the infrared region; the position, intensity and width of these bands may be used to confirm the presence (or absence) of a functional group and to provide information about its environment.

In the present study, infrared spectra have been used to confirm the presence of humic substances within two molecular size classes from river water; a technique commonly used in the characterization of humic substances of diverse origins (Schnitzer and Khan, 1972; Beck, Reuter and Perdue, 1974; Alberts et al., 1976; Stevenson and Goh, 1971_a, 1972, 1974; Midwood and Felbeck, 1968; Lamar, 1968). Infrared spectrophotometry in the range 4000-650 cm^{-1} has also been used in the study of metal-humic acid complexes (Schnitzer and Hoffman, 1967). However, no accepted reference spectra are available for the humic substances and the assignment of absorption bands to particular functional groups from correlation charts remains uncertain. Samples of humic acids are commonly prepared in KBr pellets or in a nujol mull and analyzed under a great variety of instrumental conditions which make direct semiquantitative comparison of published spectra of limited value. Nevertheless, infrared spectra provide some corroboration of other results such as those obtained by Mössbauer spectroscopy (Chapter 4).

The principal infrared absorption bands of humic and fulvic acids have been described and reviewed by Schnitzer and Khan (1972); Table 6.3 summarises some of the characteristic peaks. The spectra reflect the preponderance of oxygen-containing functional groups such as $-\text{COOH}$, $-\text{OH}$ and C=O in humic substances (Rashid and King, 1970) which are largely responsible for the relatively poor resolution of the i.r. spectra reported for humic acids and also observed in the present study. The broad absorption bands of humic acid spectra are in part due to the overlapping characteristic

frequencies of functional groups and in part due to the range of structural environments tending to broaden those band widths. However, humic substances are a polydisperse group of compounds which may be separated into similar molecular size fractions to provide some improvement in the resolution of the spectra obtained (Schnitzer and Khan, 1972).

The conventional preparations of humic and fulvic acids for infrared spectrophotometry have been described by Schnitzer and Khan (1972). The pellet technique is often used and involves mixing a finely ground sample (approximately 1% (w/w)) with a suitable matrix material such as KBr and pressing the mixture into a sintered disc. The matrix material should be pure and dry since structures in the region of OH absorption bands ($3300-3000$ and $1750-1500\text{ cm}^{-1}$) which are characteristic of humic acids may be obscured in the presence of any moisture. Another common technique is to use a mulling agent and form a slurry with the sample and mount the mull as a thin film between KBr plates for analysis. This method is subject to the inherent disadvantage of interferences from the absorption bands of the mulling agent. Similarly sample preparation in KBr discs does not preclude spectral artifacts developing from chemical reactions with the sample, for example by ion-exchange.

Infrared transmission spectra may be obtained by attenuated total reflection (ATR) techniques. Its advantages include the convenient and wide variety of sample preparation for analysis and the elimination of interference fringes present in thin-film, transmission

TABLE 6.3

Principal Infra-red Absorption Bands of Humic Acids (Schnitzer and Khan, 1972)

Wavenumber (cm^{-1})	Assignment
3400	Hydrogen bonded OH stretch
2900	Aliphatic C-H stretch
1725	C=O of COOH, C=O stretch of ketonic carbonyl
1630	Aromatic C=C (?) hydrogen-bonded C=O of carbonyl (?) double bond conjugated with carbonyl, COO^-
1450	Aliphatic C-H
1400	COO^- , aliphatic C-H
1200	C-O stretch or OH deformation of COOH
1050	Si-O of silicate impurity

spectra. Micro-samples may be prepared as a thin smear on the ATR crystal in which there are multiple internal reflections of the i.r. beam. The attenuation of the beam on reflection is characteristic of the sample on the surface of the crystal. The depth of penetration of the sample depends on its refractive index and the beam angle of incidence (commonly 30°, 40° or 60°) and wavelength.

In the present study a thallium bromide-iodide (KRS-5) internal reflection crystal with a 60° angle of incidence was used and the infrared spectra were recorded using a Perkin-Elmer Model 621 double-beam grating spectrophotometer. The 'soluble' dialyzates isolated from river water were prepared as concentrated aqueous solutions in distilled, deionized water. Each sample was applied to the surface of the KRS-5 plate as a thin smear and allowed to dry. The freeze-dried eluates of fulvic acids concentrated from PM-10 ultrafiltrates using a macroreticular resin (Amberlite XAD-2) were also analyzed using infrared spectrophotometry. In this manner, humic and fulvic acids were concentrated from river water to provide samples which were relatively free of metal complexes.

Typical examples of the infrared spectra obtained for the colloidal and macromolecular material concentrated from river water by ultrafiltration and 'ion-exchange' resins are shown in figures 6.1 and 6.2. The principal absorption bands of these spectra and their probable assignments are given in Table 6.4. Many of the absorption peaks are characteristic for humic substances (Table 6.3) however, the 1725 cm^{-1} band was only observed in samples concentrated

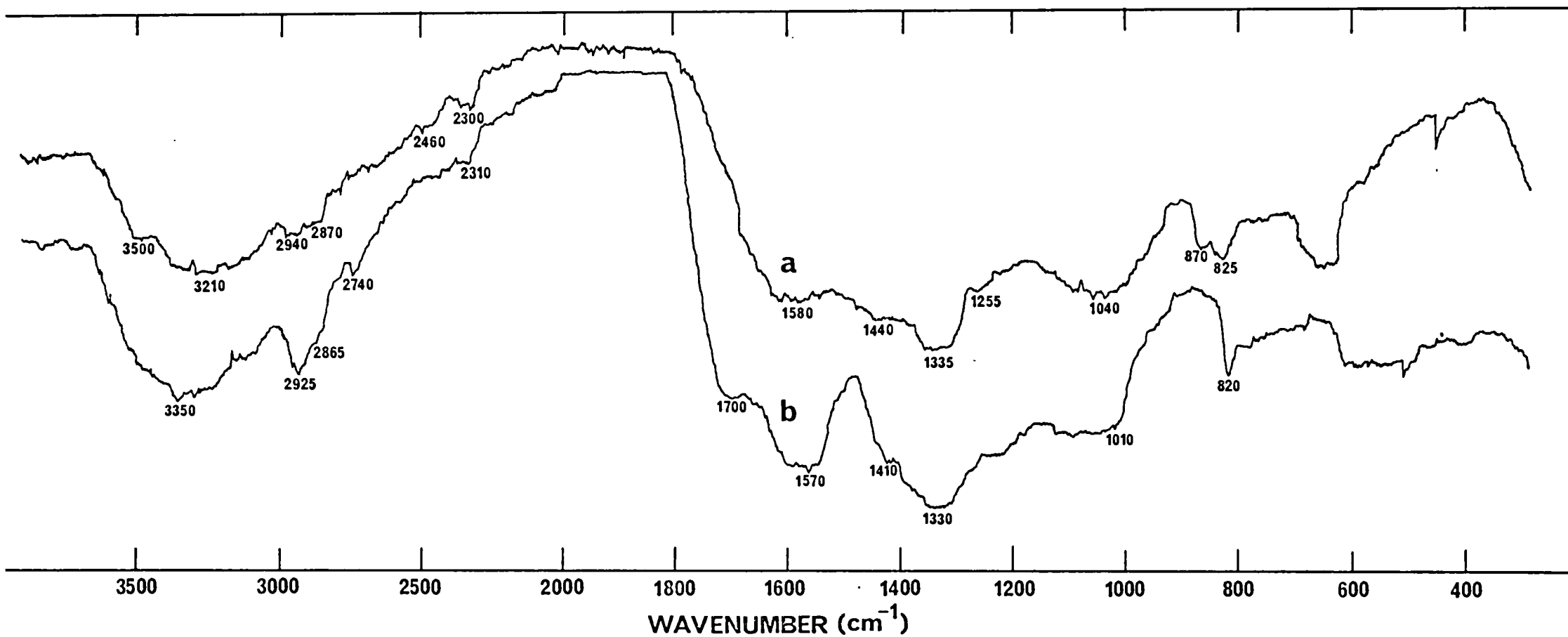


Fig. 6.1 Infrared transmission spectra of humic substances from Fowey river water

- a) Dialyzate (Nominal molecular weight $>10,000$)
- b) Humic substances (Nominal molecular weight $<10,000$) concentrated from ultrafiltered river water

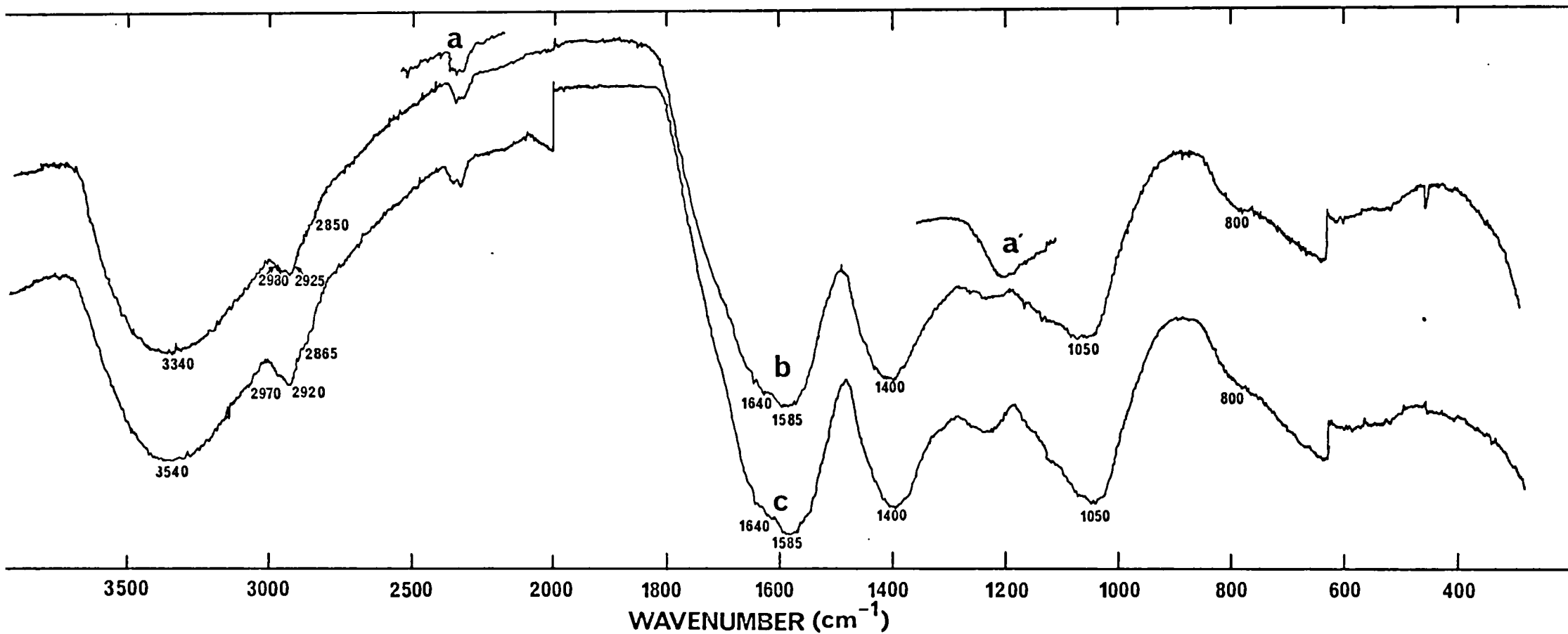


Fig. 6.2 Infrared transmission spectra of humic substances from Tamar river water
 a & a') Blank
 b) Dialyzate (Nominal molecular weight <10,000 >500) from Crossgate
 c) Dialyzate (Nominal molecular weight <10,000 >500) from Launceston

TABLE 6.4

Principal Infra-red Absorption Bands of Selected Dialyzates Concentrated from River Water

Wavenumber (cm ⁻¹)	Assignment
3500-3200	Bonded -OH stretch
2940-2800	Aliphatic C-H stretch, polymethyl groups
1725-1720	C=O of COOH and ketones
1640	C=O of quinones or weakly bonded C=O
1600	Aromatic C=C or strongly bonded C=O
1450-1440	Aliphatic C-H, H-bending
1400	C-O, aliphatic C-H
1250-1200	C=O stretch or OH deformation of COOH
1100-980	Si-O stretch
920-800	Si-O bend

on the macroreticular resin, an example being the material (<10,000 nominal molecular weight) from ultrafiltered Fowey river water - spectrum b in figure 6.1. Sharp but weak peaks in the region 900 to 800 cm^{-1} were also found in samples from the Fowey. In contrast only a shoulder of a broad band centred about 800 cm^{-1} was observed with samples from Tamar river water (fig. 6.2). Peaks at 1600 cm^{-1} and 1050 cm^{-1} are also well defined in dialyzates from the Tamar. Broad bands between 3500 and 3200 cm^{-1} and weaker peaks with shoulders at 2940 and 2800 cm^{-1} , which are characteristic of methyl groups, H-bonded OH and aliphatic C-H stretch vibrations, can also be seen in all the spectra.

The broad, intense bands centred about 3400 cm^{-1} correspond to the stretching frequencies of O-H and N-H bonds although the latter are probably only a minor contribution to the absorption. However, intense infrared absorption in this region is also a characteristic feature of clay impurities in humic acids extracted from soils and sediments (Malcolm, 1976). Weak peaks manifest as shoulders in the region 2950-2800 cm^{-1} result from aliphatic C-H stretching, however, strong absorptions at 1725-1700 cm^{-1} , which are also a feature of humic acid spectra are largely absent from the spectra presented in the present study (fig. 6.1 and 6.2) and are represented by only a slight shoulder of the 1580-1570 cm^{-1} band. The 1640-1575 cm^{-1} band is associated with aromatic C=C stretching or the stretching of hydrogen bonded C=O groups of quinones. The peak at 1400 cm^{-1} is well defined in some spectra (fig. 6.2b and c) and appears as a pronounced shoulder of the 1330 cm^{-1} band in other

spectra (fig. 6.1b).

The characteristic humic acid peak at 1200 cm^{-1} - the C-H stretching vibration - is absent from the spectra shown in figures 6.1 and 6.2. Similar observations have also been reported for lacustrine humic acids (Alberts et al., 1976) and for metal-fulvic acid complexes (Schnitzer, 1971). With the complexation of Fe(III) the 1725 and 1200 cm^{-1} peaks of purified fulvic and humic acids are diminished and may be lost. A shift of the 1620 cm^{-1} peak to a lower wave number accompanied by an increased intensity of the 1400 cm^{-1} band is also observed as a result of heavy metal complexation (Schnitzer, 1971). The broad peaks in this region and the results of Mössbauer spectroscopy (Chapter 4) indicate that Fe(III) is probably complexed by quinoid and carboxyl groups of humic and fulvic acids.

The spectrum of a high molecular weight dialyzate (nominal molecular weight $>10,000$) from the Fowey (fig. 6.1a) shows a marked reduction of absorption in the 1725 cm^{-1} band compared with the low molecular weight fraction ($<10,000$ nominal molecular weight). The differences in intensity of this peak may be due to purification of the low molecular weight fraction by 'ion-exchange' which would largely remove complexed ions. However, the diminished intensity of the 1725 cm^{-1} band with increasing molecular weight may also correspond to a decrease in the number of carboxyl and quinoid groups. The broad band between 1060 and 1010 cm^{-1} is characteristic of Si-O stretching and is a marked feature of these spectra. Silicate

impurities are commonly reported in the ash content of humic substances extracted from soils, sediments and waters and account for the peaks, 1100 to 980 cm^{-1} and at 800 cm^{-1} (Fotiyev, 1968; Schnitzer and Khan, 1972; Malcolm, 1976).

6.5 IDENTIFICATION OF HETERO-IONS ASSOCIATED WITH NON-DIALYZABLE MATERIAL IN RIVER WATER

The non-dialyzable residues concentrated by ultrafiltration yield an admixture of organic and inorganic components. Inorganic ions may be present as discrete minerals or as complexed or adsorbed hetero-ions exhibiting a variety of physical and physicochemical associations. Clearly to confirm the nature of proposed organo-metallic interactions within the colloidal system of river water it is essential to isolate the proposed complexes for characterization and identification by analysis; this task has been a primary objective of the present study.

Direct multi-element analyses of the inorganic colloidal material in sediments and waters have been reported but have been generally restricted to a limited number of semiquantitative spectrographic determinations (Table 6.5). The major inorganic impurities found during the preparation of high purity humic and fulvic acids, extracted from soils and sediments, are reported to be crystalline clay minerals; amorphous minerals of silica, aluminium and various inorganic ions. Silica and chlorides are found to be particularly persistent impurities in these preparations (Malcolm, 1976).

TABLE 6.5

Inorganic Composition of 'Humic Substances'

	CONCENTRATION (ppm)																	SAMPLE
	Al	Ag	Ba	Ca	Co	Cu	Cr	Fe	K	Mn	Mg	Na	Ni	P	Pb	Si	Zn	
*	1000	200	<2000	>2%	<60	200	<300	3000	-	100	6000	>2%	800	-	500	2%	6000	Interstitial water of a marine fjord ¹
**	>10%	-	TRACE	>10%	-	TRACE	-	0.1-1%	1-3%	-	3-10%	1-3%	-	-	-	0.1-1%	-	Humic acid from peaty waters ²
**	ND	ND	ND	0.3%	ND	0.1%	0.03%	0.03%	3%	<0.002%	>30%	>8%	0.03%	-	ND	1.0%	ND	Colloidal non-dialyzable fraction from sea water ³
**	3%	0.01%	0.3%	3%	0.3%	3%	0.03%	0.3%	3%	>10%	3%	3%	0.3%	10%	0.01%	10.0%	ND	Interstitial waters of marine sediment ³
*	-	-	-	7.74%	-	<0.01%	-	0.25%	-	<0.0005	2.41%	-	-	-	-	-	0.1%	Humic acid from fresh water ⁴
*	2.0%	-	0.008%	0.5%	-	0.002%	0.002%	0.5%	-	-	0.3%	0.7%	-	-	-	-	-	Marine sediments 'natural humate
*	1.0%	-	0.002%	0.005%	-	0.003%	0.003%	0.07%	-	-	0.015%	0.7%	-	-	-	-	-	'Extracted humates' ⁵
**	-	-	1100	2.0	190	900	140	9.2	-	0.37	5.0	-	160	-	-	-	-	Algal suspension ⁶

1. Nissenbaum, Baedeker and Kaplan (1971)

2. Chalupa (1966)

3. Breger, Zutovic and Chandler (1972)

4. Midwood and Felbeck (1968)

5. Swanson et al., (1966)

6. Ferguson and Bubela (1974)

* ppm in 'humic substances'

** % in ash from 'humic substances'

ND - not detectable

- - not determined

The ash content of non-dialyzable polymers isolated from the interstitial waters of marine sediments has also been attributed to the presence of minerals bound by high molecular weight organic matter (Nissenbaum, Baedecker and Kaplan, 1971). The qualitative inorganic composition of colloidal material isolated from both marine sediments and waters is similar and includes a great many elements; but mainly aluminium, silicon, alkali and alkaline earth metals as well as trace heavy metals (Swanson et al., 1966; Nissenbaum, Baedecker and Kaplan, 1971; Breger, Zubovic and Chandler, 1972). Few analyses of the inorganic constituents associated with macromolecular material in river water have been reported. However, in peaty streams semiquantitative spectrographic methods have confirmed that aluminium, silicon, calcium, sodium, potassium, iron and magnesium predominate in the colloidal 'humic' fractions (Chalupa, 1966). The occurrence of Na, K, Mg, Ca, Mn, Fe, Al, Si, P and S within colloidal size classes was also found in studies of organic aggregates generated from natural waters by the flocculation of 'dissolved' organic matter (Hirbrunner and Wangersky, 1975; Sholkovitz, 1976).

The characterization of metal ions associated with the colloidal size classes of natural waters requires that a comprehensive method of multi-element analysis is used to determine the elements entrained within organic colloids and colloidal minerals in addition to the ions complexed or sorbed by organic polyelectrolytes. Using a scanning electron microprobe with an energy dispersive x-ray analyzer (Cambridge Scientific Instruments Stereoscan IIA with an

Ortec lithium drifted silicon solid state, x-ray detector) it has been possible to determine qualitatively the inorganic composition of small samples of the non-dialyzable material in river water. Similar methods have also been used previously to characterize heavy metal particles in atmospheric debris (Allen et al., 1974). Examples of the x-ray emission spectra obtained for material in two molecular size fractions in river water are shown in figures 6.3 to 6.6 (see Appendix C). Traces of Al, Si and K were found in all of the microprobe analyses together with Ca, Mg, P, S, Cl and Fe. These findings were also confirmed by qualitative thin-film x-ray fluorescence analysis of all the samples of non-dialyzable material isolated from rivers in the present study area.

Selected samples of the colloidal and macromolecular material isolated from the River Fowey, Helford and the Tamar were digested in Ultragrade nitric acid and analyzed by flame atomic absorption spectrophotometry to determine the concentration of the metals detected by electron probe micro-analysis. These results (Table 6.6a and Table 6.6b) clearly demonstrate that calcium is present in all samples as the predominant inorganic constituent. Similarly the presence of iron in these fractions confirms the complexation of this element which was demonstrated by analysis of successive ultrafiltrates for many of the rivers of the present study area (Chapter 3). The relatively low concentration of iron in the dialyzates concentrated using an Amicon UM-05 membrane (nominal molecular weight <10,000 and >500) also largely confirms previous observations in this study (Chapters 3 and 4) that this element is

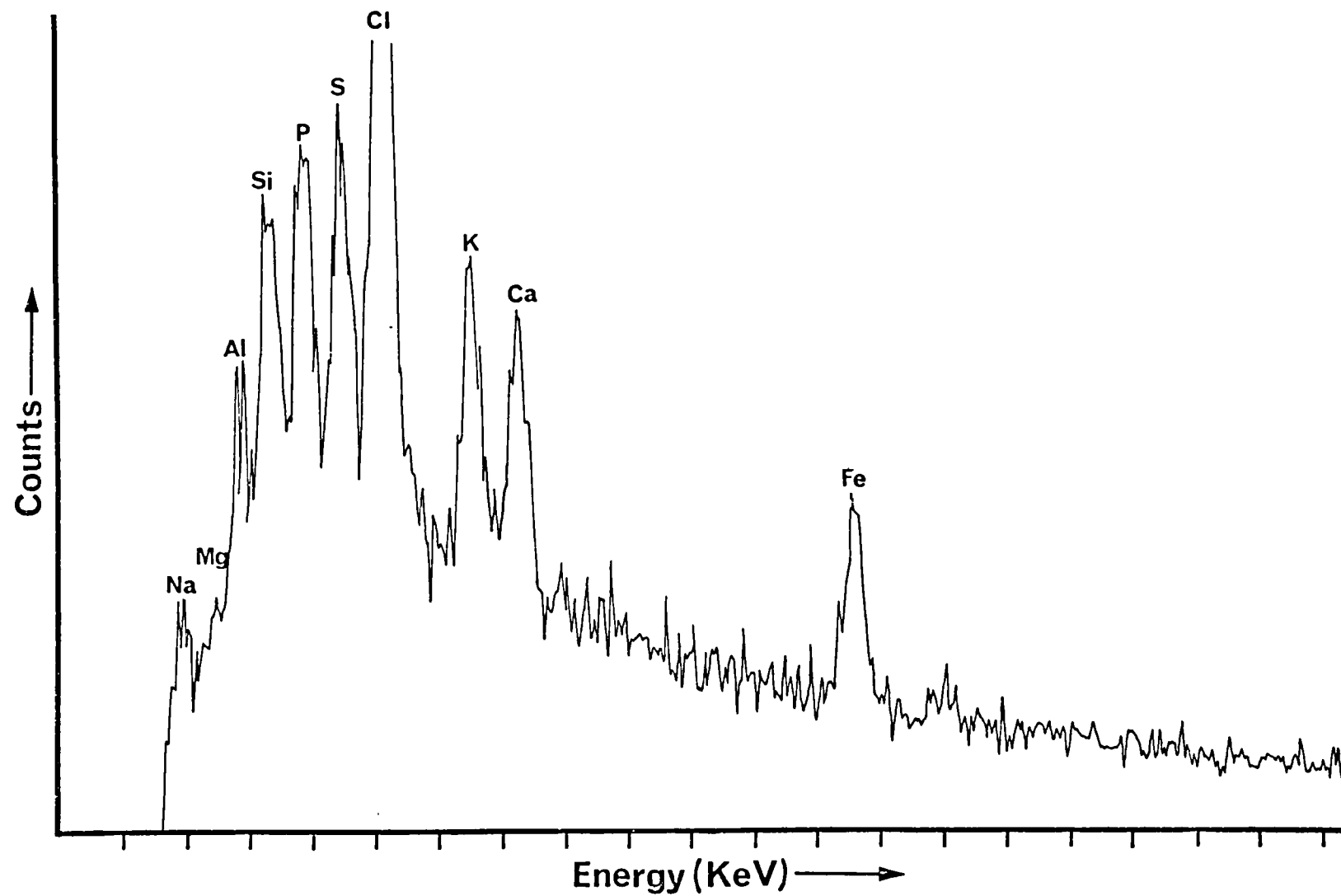


Fig. 6.3 X-ray emission spectrum of typical colloidal material (Amicon PM-10 dialyzate) from Fowey River water (Dozmary Pool)

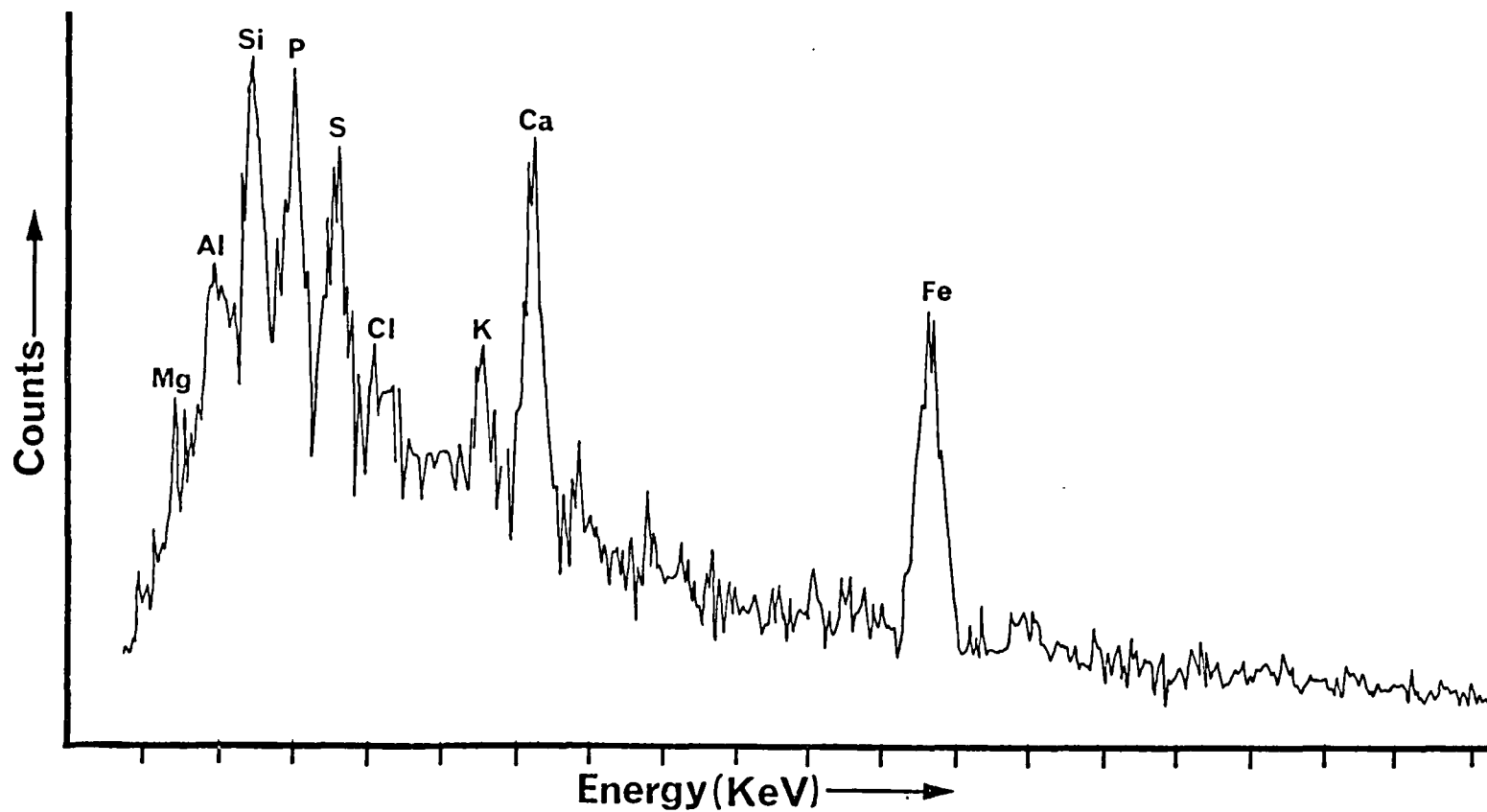


Fig. 6.4 X-ray emission spectrum of typical colloidal material (Amicon PM-10 dialyzate) from Tamar River water (Crossgate)

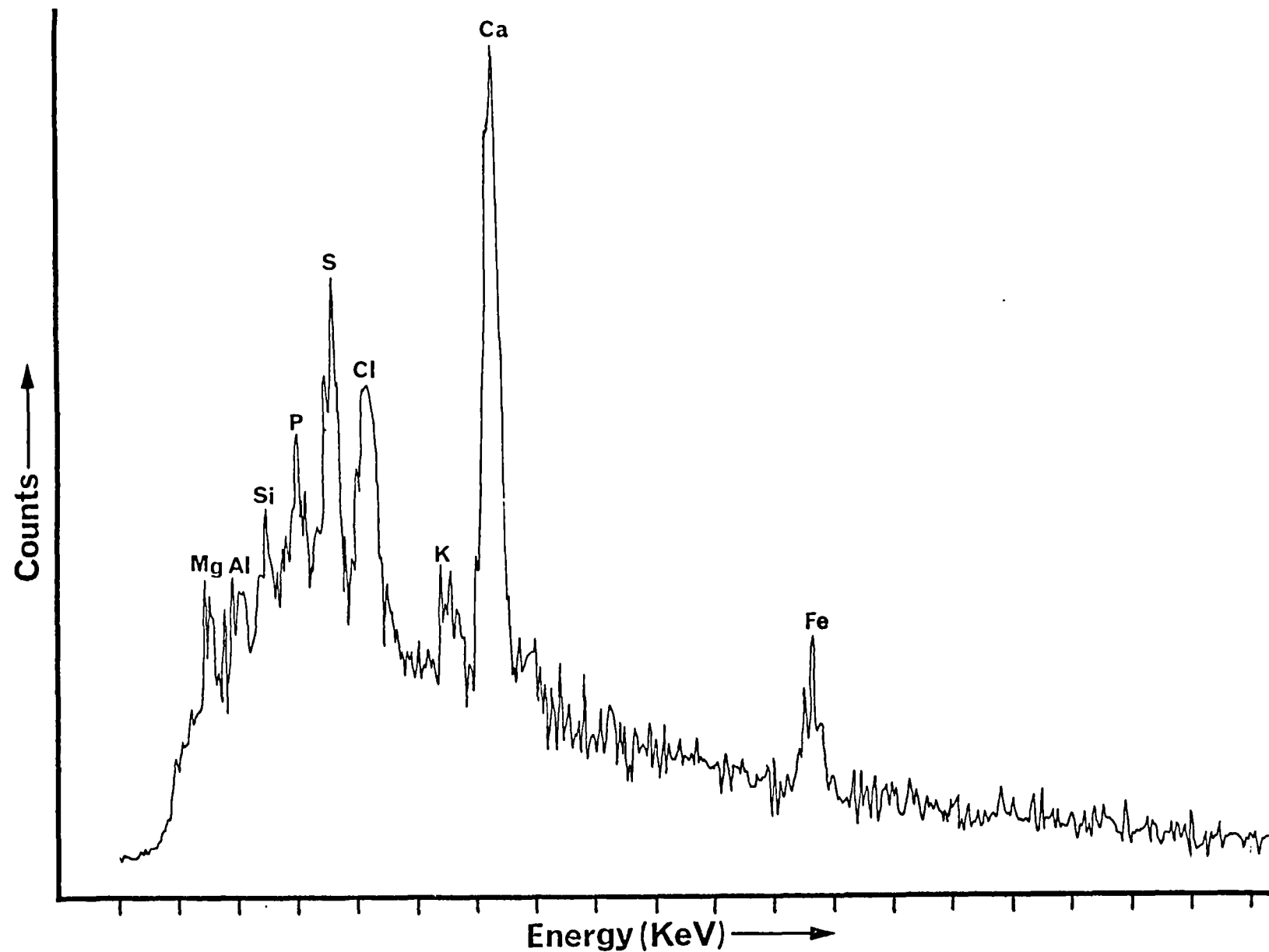


Fig. 6.5 X-ray emission spectrum of typical macromolecular material (Amicon UM-05 Dialyzate) from Tamar River water (Crossgate)

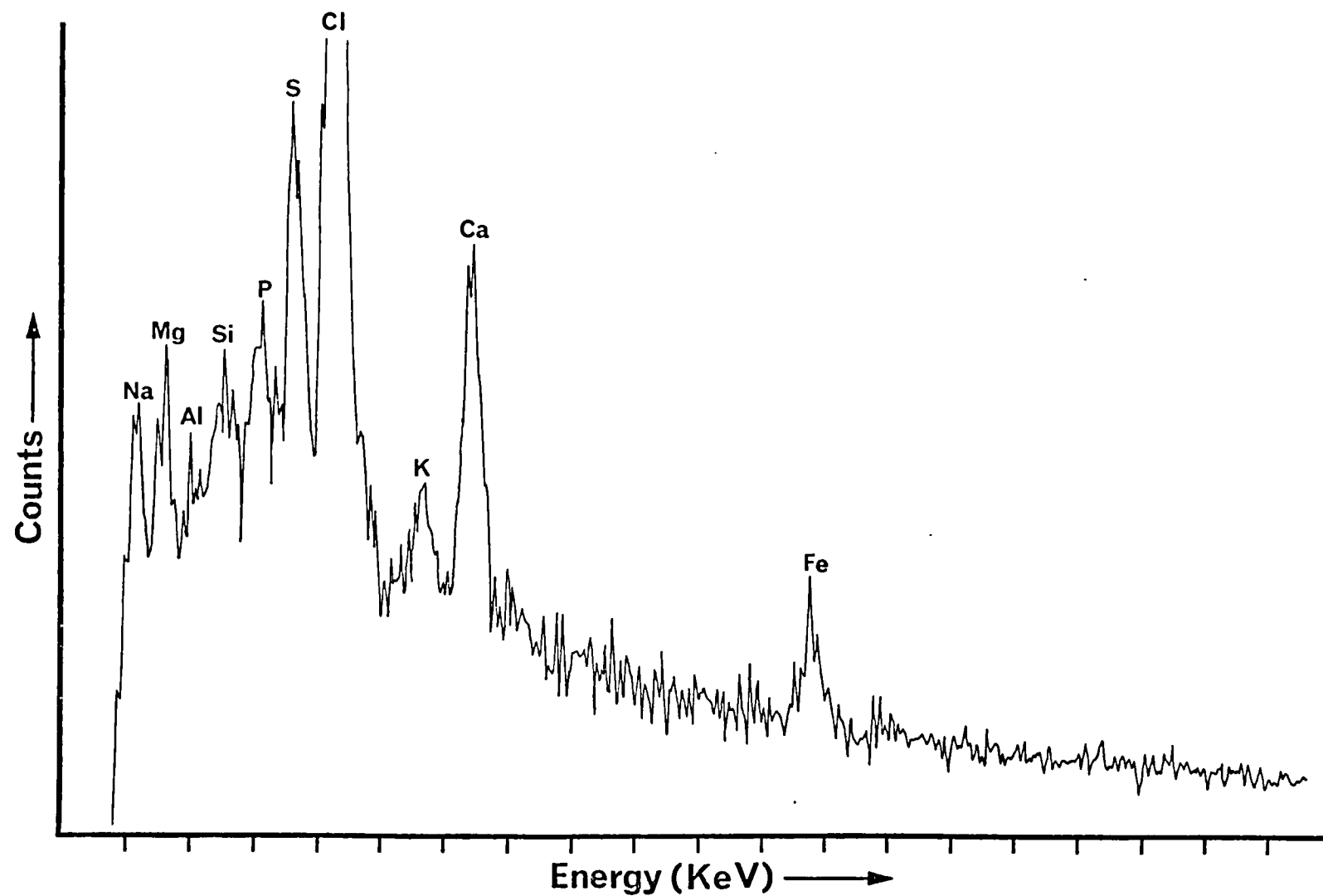


Fig. 6.6 X-ray emission spectrum of typical macromolecular material (Amicon UM-05 Dialyzate) from Helford River water (Trelowarren)

TABLE 6.6(a)

Inorganic Analysis of Colloidal and Macromolecular Material Isolated from Tamar River Water

SAMPLE LOCATION	DATE	MOLECULAR MASS FRACTION	CONCENTRATION ($\mu\text{g g}^{-1}$)										
			Fe	Al	Mg	Ca	K	Zn	Cu	Cd	Ni	Mn	Pb
CROSSGATE	23.7.74	>10,000	68000	ND	ND	ND	ND	920	60	20	<10	600	400
	1.7.75	>10,000	8450	<10000	3600	7890	2390	460	70	ND	ND	ND	ND
	1.5.75	<10,000 >500	1010	<10000	14800	62750	4860	400	400	ND	ND	ND	ND
	1.7.75	<10,000 >500	710	<10000	6400	16100	2840	400	330	ND	ND	ND	ND
LAUNCESTON	21.8.74	>10,000	49000	<10000	ND	ND	ND	1800	600	100	200	300	200
	1.5.75	>10,000	6500	<10000	3400	8700	2200	400	100	ND	ND	ND	ND
	1.7.75	>10,000	8300	<10000	3600	11100	1800	200	300	ND	ND	ND	ND
	1.5.75	<10,000 >500	540	<10000	12600	39600	2700	460	400	ND	ND	ND	ND
	1.7.75	<10,000 >500	1520	<10000	10600	41700	3000	220	400	ND	ND	ND	ND
GUNNISLAKE	23.7.74	>10,000	29000	ND	21000	65500	10000	910	600	<10	<10	600	<10
	21.8.74	>10,000	74600	ND	ND	ND	ND	920	500	<10	<10	100	200
	1.7.75	<10,000 >500	900	<10000	4200	16000	4000	400	2300	ND	ND	ND	ND
CALSTOCK	1.5.75	>10,000	5600	<10000	3800	8000	3600	200	400	ND	ND	ND	ND
	1.7.75	<10,000 >500	100	<10000	30000	15000	ND	100	100	ND	ND	ND	ND

ND - Not Determined

TABLE 6.6(b)

Inorganic Analysis of Colloidal and Macromolecular Material Isolated from River Water

RIVER	MOLECULAR MASS FRACTION	CONCENTRATION ($\mu\text{g g}^{-1}$)						
		Fe	Al	Mg	Ca	K	Zn	Cu
FOWEY	>10,000	11200	<10000	3000	14500	4000	300	200
	>10,000	4000	<10000	2200	5000	5100	300	300
	>10,000	13000	<10000	11500	2300	5500	200	600
	<10,000 >500	1100	<10000	9300	30000	3600	400	200
HELFORD	<10,000 >500	1500	<10000	12200	50000	3800	700	1000
	<10,000 >500	1200	<10000	14500	13700	8500	600	600

bound in the higher molecular size fractions of the PM-10 dialyzate. Only trace concentrations of zinc and copper were found in the dialyzed residues from the Fowey and Helford but higher concentrations of zinc were found in samples from the Tamar. However, these results largely substantiate the conclusion that only traces of the heavy metals are associated with the colloidal and macromolecular fractions of river water.

Suspended particulate material from each monthly water sample was retained for analysis during the present study after primary filtration using a Whatman glass-fibre (GF/C) filter. Each sample was dried, weighed and digested in concentrated Ultra-grade nitric acid for 24 hours at 100°C. These digests were made up in 5ml of 1M Ultragrade hydrochloric acid and analyzed for the transition metals. The results of these analyses are shown in Tables 6.7a, 6.7b, 6.8 and 6.9. The concentrations of trace metals in the suspended sediments from the Carnon, Helford and Tamar rivers are in good agreement with the levels previously reported in bottom sediments from these rivers (Aston et al., 1974; Aston and Thornton, 1975; Thornton, Watling and Darracott, 1975; Bryan and Hummerstone, 1973). Correlations between the levels of heavy metals occurring in sediments and waters can be deduced from these results. High concentrations in the sediments correspond to the composition of the waters which, in turn, reflect the condition of the drainage basin; this relationship is particularly well born out in the Carnon river (Chapter 5) and in the mineralized catchment of the Helford valley which was sampled at Constantine (cf. Table 6.8 and

TABLE 6.7(a)

Trace Metal Concentrations in Suspended Sediment from Tamar River Water (1974/75)

SAMPLE LOCATION	DATE	CONCENTRATION ($\mu\text{g g}^{-1}$)							
		Fe	Mn	Zn	Cu	Co	Cd	Ni	Pb
CROSSGATE	23.7.74	157500	23620	1170	110	160	80	160	390
	21.8.74	142400	10220	720	130	60	60	159	155
	31.10.74	60000	3090	420	60	55	36	55	100
	25.2.75	-	1440	155	45	40	5	80	100
	26.3.75	105740	2350	445	50	50	5	80	105
	29.4.75	82800	1940	-	100	30	6	20	100
	1.7.75	131260	3220	85	12	30	4	36	60
	Average	113280	6550	500	72	60	30	83	145
LAUNCESTON	23.7.74	17320	1000	220	110	26	<2	26	260
	21.8.74	29690	24000	2210	260	156	160	470	390
	31.10.74	83900	4700	1310	85	33	270	100	335
	25.2.75	-	1690	195	55	50	5	87	87
	26.3.75	-	-	-	-	-	-	-	-
	29.4.75	60970	1190	220	40	32	6	55	80
	1.7.75	253100	4600	3130	640	470	47	865	1330
	Average	43400	6200	1210	200	130	80	270	410

TABLE 6.7(b)

Trace Metal Concentration in Suspended Sediment from Tamar River Water (1974/75)

SAMPLE LOCATION	DATE	CONCENTRATION ($\mu\text{g g}^{-1}$)							
		Fe	Mn	Zn	Cu	Co	Cd	Ni	Pb
GUNNISLAKE	23.7.74	-	-	-	-	-	-	-	-
	21.8.74	-	-	-	-	-	-	-	-
	20.9.74	-	-	-	-	-	-	-	-
	31.10.74	42330	5100	980	490	75	<2	150	220
	25.2.75	-	1730	1245	1335	455	11	145	180
	26.3.75	77650	1390	1740	1650	87	14	105	160
	24.4.75	80050	2670	1170	1275	60	11	105	150
	1.7.75	17140	1060	2450	1630	90	12	120	100
	Average	54290	2390	1520	1276	155	10	125	160
CALSTOCK	23.7.74	115040	5400	1630	530	100	6	130	780
	21.8.74	27090	430	460	140	<10	35	35	160
	20.9.74	-	860	380	220	30	20	60	200
	31.10.74	51290	1130	1190	375	115	<2	80	420
	25.2.75	-	-	-	-	-	-	-	-
	26.3.75	41350	870	610	350	90	<2	80	320
	24.4.75	39370	580	480	300	<10	<2	60	250
	1.7.75	38200	1040	590	290	25	<2	40	240
	Average	52060	1470	760	310	54	-	70	340

TABLE 6.8

Trace Metal Concentrations in Suspended Sediment from Helford River Water (1975)

SAMPLE LOCATION	DATE	CONCENTRATION ($\mu\text{g g}^{-1}$)							
		Fe	Mn	Zn	Cu	Co	Cd	Ni	Pb
CONSTANTINE	31.1.75	17150	550	1624	180	<10	-	50	-
	25.2.75	19770	410	1600	580	20	5	20	160
	26.3.75	9760	530	525	300	56	2	35	170
	29.4.75	16330	860	1130	110	34	5	30	135
	1.7.75	18720	710	1060	240	70	3	85	245
	Average	16350	610	1190	280	38	4	45	180
TRELOWARREN	31.1.75	19340	610	180	65	30	5	100	50
	25.2.75	-	-	-	-	-	-	-	-
	26.3.75	17480	520	335	20	185	2	95	125
	29.4.75	36000	1360	400	55	40	5	65	140
	1.7.75	18000	680	200	30	20	3	30	70
	Average	22700	790	280	43	70	4	60	96
GWEEK QUAY	31.1.75	20530	820	495	120	30	-	40	-
	25.2.75	-	-	-	-	-	-	-	-
	26.3.75	18170	370	320	140	110	3	55	110
	29.4.75	19575	1140	420	170	55	4	60	130
	1.7.75	17640	590	260	110	40	-	40	90
	Average	18980	730	370	135	60	3.5	50	110

TABLE 6.9

Trace Metal Concentrations in Suspended Sediment from Carnon River Water (1975)

SAMPLE LOCATION	DATE	CONCENTRATION ($\mu\text{g g}^{-1}$)							
		Fe	Mn	Zn	Cu	Co	Cd	Ni	Pb
BISSOE	31.1.75	140000	350	1750	2900	58	5	75	500
	25.2.75	203000	180	500	620	35	5	25	590
	26.3.75	160000	310	2100	1190	76	4	30	900
	29.4.75	184800	550	5030	9040	63	9	110	670
	1.7.75	285000	1350	2900	2000	45	12	60	1170
Average		194560	550	1586	3150	55	7	60	770
DEVORAN	31.1.75	80400	260	1280	4220	42	8	60	600
	25.2.75	154760	300	4290	5950	48	9	60	1010
	26.3.75	78490	120	4060	650	69	6	33	720
	29.4.75	185000	570	5850	9060	75	13	132	570
	1.7.75	62100	640	1270	375	35	5	45	170
Average		112150	378	3350	4050	54	8	65	610

fig. 5.2). The highest concentrations of heavy metals were found in the hard waters of the Carnon in agreement with previous observations that soluble levels of trace metals are directly correlated with hardness (Andelman, 1973). Iron, manganese, zinc and copper are the major heavy metal constituents of the suspended sediments from the study area. The relatively high concentrations of manganese, zinc and copper are in marked contrast to those levels found in the corresponding colloidal and macromolecular fractions from these waters, particularly in the case of manganese in the Tamar.

These findings clearly indicate that the classical association of iron and manganese which is a feature of many sediments, and can also be seen in the suspended particulate fractions of these waters, does not extend into the colloidal size class. The ratio of iron to manganese in the suspended sediments of these rivers varies considerably ranging from 300 to 400 in the Carnon to 10 to 30 in the Helford and Tamar. However, some indication of an association between iron and manganese in the colloidal and macromolecular fractions in the Tamar can be seen from the size distribution of these elements in Chapter 5 (fig. 5.4). Nevertheless, there is no direct evidence for this association from either the x-ray emission spectra (fig. 6.4) or from the analysis of these fractions (Table 6.6).

6.6 DISCUSSION

The results of this preliminary identification and characterization exercise have confirmed that much of the non-dialyzable material in the waters of the Fowey, Helford and Tamar is organic

with an organic carbon composition ranging from 35% to 45%; generally lower than that reported for humic and fulvic acids (Schnitzer and Khan, 1972). Nevertheless, infrared spectra of the material from two molecular size fractions ($>10,000$ and $<10,000 >500$ nominal molecular weight) have shown humic substances in these macromolecular size fractions and confirmed the presence of silicates. In contrast to previous reports (Perhac, 1972; Perhac and Whelan, 1972), no evidence of substantial amounts of colloidal, illitic material was found in the mineralized regions of the present study area in Cornwall. Traces of aluminium, silica and potassium were found in all the riverine dialyzates isolated during the present study together with a predominance of calcium, magnesium, phosphorus, sulphur, chlorine and iron. These findings were also confirmed by qualitative, thin-film x-ray fluorescence analysis of all the isolated dialyzates. Only traces of the divalent heavy metals were detected in many of these fractions (Table 6.6a and 6.6b). These findings largely confirm the results reported in Chapter 5 from which it was concluded that heavy metal ion binding associated with the colloidal and macromolecular material was only weak and that the major proportion of 'soluble' trace metals occurred in the low molecular size fractions.

The occurrence of macromolecular organic complexes of aluminium and iron in natural waters has been widely reported (Black and Christman, 1963; Shapiro, 1964; Lamar, 1968; Tryland and Gjessing, 1975; Perdue, Beck and Reuter, 1976). These complexes probably arise from the humic acids of soil waters discharged into

rivers since these weak acids are known to form relatively stable complexes with trivalent heavy metals (Table 5.1).

Barsdate (1967) considered that at conditions of pH close to neutrality a substantial proportion of alkali and alkaline earth metals should be present in lake waters as 'humates' and not as free ions. Further indirect evidence for this association with macromolecular fractions in lakes and rivers has also been reported using ultrafiltration to separate various colloidal size classes (Gjessing, 1970; Schindler and Alberts, 1974; Benes, Gjessing and Steinnes, 1976). The results of the present study have clearly confirmed the sequestration of ^{fractions of} alkaline and alkaline earth metals by direct analysis of isolated macromolecular polyelectrolytes. The complexation of major ions such as calcium and magnesium may prove particularly important in the destabilization of humic acid dispersions especially under estuarine conditions. Polycarboxylic acids such as polyacrylic acid are known to form stable complexes with calcium (Travers and Marinsky, 1974) and the formation of soluble and insoluble calcium complexes with fulvic acid has similarly been described (Dupuis, Jambu and Dupuis, 1972). The predominance of potassium with only traces of sodium in the non-dialyzable residues indicates a preferential formation of potassium complexes which suggests that selective steric factors determine the formation of these complexes with humic and fulvic acids. The ionic radius may be a critical factor in this process as in the formation of clathrates with macrocyclic polyethers (Pendersen, 1967, 1970).

An interesting *suggestion* of an inverse relationship between calcium concentration and the percentage nitrogen of the non-dialyzable residues isolated from river waters can also be seen from these results (fig. 6.7). A direct correlation may therefore exist between the number of 'hard' functional groups available in these organic dialyzates and the concentration of 'hard' cations bound in each fraction. Conversely the calcium 'humates' isolated by ultrafiltration may be diluted with proteinaceous material also occurring as a non-dialyzable fraction to give a similar correlation.

With the exception of waters from the Carnon river, which are highly variable in composition, the trace metal levels of waters and their associated suspended sediments from the Helford and the Tamar show a broad interrelationship which has been reported in more extensive surveys (Aston et al., 1974; Aston and Thornton, 1975). For example, the elevated copper and zinc concentrations of both waters and sediments from the northern, mineralized catchment of the Helford at Constantine are in marked contrast to the unmineralized tributary sampled at Treloar Warren and Gweek Quay (Table 6.8 fig. 5.2 and 5.3). A comparison of water samples taken in the winter and summer from the Tamar (Tables 6.7a, 6.7b and Chapter 5, figs. 5.4, 5.5 and 5.6) shows some apparent variation with season, particularly in the case of iron and zinc which is not mirrored in the composition of the suspended sediments of these water samples.

Suspended particulate material in many waters from the present study area show an association of iron and manganese,

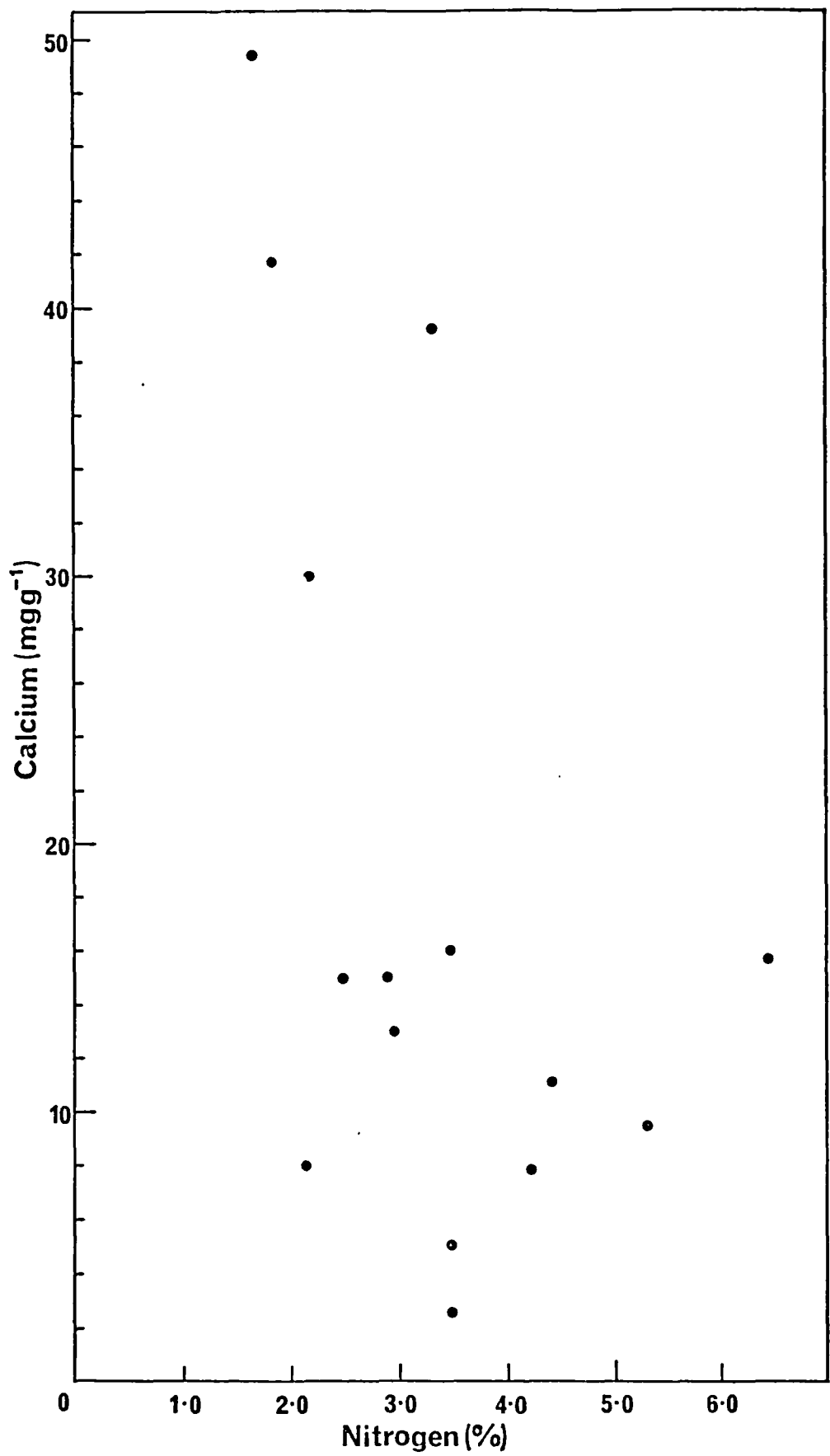


Fig. 6.7 Calcium and nitrogen concentrations of some colloidal and macromolecular non-dialyzable residues from soft river waters in Cornwall

particularly in the Tamar. However, this classical association of lithology is clearly not reflected in the composition of the sub-micron, macromolecular organic fractions of these waters (Table 6.6). Iron in these waters occurs as an organic complex but manganese is present only as a low molecular weight species. Similar observations have been reported by Holliday and Liss (1976); in which they conclude that the destabilization of organo-iron complexes and the maintenance of manganese as a conservative species is thought to account for the non-conservative behaviour of iron during estuarine mixing.

Clearly the relationships between the composition of sediments, colloids and waters in rivers and estuaries are intricate and much remains to be understood before these can be described in terms of a rational physicochemical system. The results of this preliminary investigation have served to characterize the little known colloidal and macromolecular dispersions of tributary drainage in a limited study area. These findings indicate some important relationships between the major alkaline and alkaline earth metals and the ubiquitous humic substances which predominate the non-dialyzable fractions of many natural waters. Using electron probe micro-analysis and ultrafiltration methods, macromolecular polyelectrolytes from rivers in other study areas may also be conveniently characterized to confirm the generality of the observation of the present study.

C H A P T E R 7

7.0 SUMMARY AND CONCLUSIONS

Much of the speciation of trace heavy metals in natural waters remains poorly understood (Stumm and Bilinski, 1973). However, in any consideration of the mass transport and distribution of trace elements an appreciation of the physicochemical forms involved in the intricate processes of mobilization and removal is clearly essential (Gibbs, 1973; Sholkovitz, 1976). The cadre of trace element species is frequently argued to include complexes possibly associated with colloidal and macromolecular organic constituents which are thought to play an important role in determining the temporal and spatial distribution of heavy metals. Humic and fulvic acids, the predominant organic burden of many surface waters, form complexes with heavy metals in soils and sediments which may also prove important in the speciation of trace elements in rivers. Such naturally-occurring polyelectrolytes provide potentially effective transport media as sequestration agents forming polynuclear complexes and chelates in addition to the entrainment of material as simple heteroions in non-labile associations.

Conventionally the concentration of dissolved species in natural water is determined by analysis of membrane filtered samples. Typically a 0.45 μ m cellulosic membrane is used to remove suspended sediment (Chapter 1). Such a gross analysis (Chapter 5) will include many filterable forms, such as colloids and polymers, not in true solution. Stumm and Brauner (1975) point out that in a consideration of trace element complexation by organic matter it is convenient to

distinguish between the following constituents of natural waters:

- (i) low molecular weight organic substances in true solution;
- (ii) polymeric substances which contain sufficient numbers of hydrophilic groups to retain their macromolecular structure in solution;
- (iii) colloidal, organic material either of high molecular weight compounds such as humic acids or of organic substances sorbed or chemically bound with inorganic dispersions (e.g. polymeric hydrolysis products of iron, aluminium and silica).

As a practical procedure a distinction of this nature is difficult to achieve but has formed the basis of the present empirical study into the nature of naturally-occurring organic polyelectrolytes in surface waters.

Martell and Calvin (1952) proposed that complex formation can be demonstrated and confirmed by:

- (a) a change in chemical behaviour;
- (b) absorption spectra;
- (c) electrochemical and polarographic behaviour;
- (d) pH effects in potentiometric studies;
- (e) solubility;
- (f) oxidation potential;
- (g) isolation and identification of natural complexes.

Ultrafiltration may be conveniently employed to isolate naturally-occurring complexes and facilitates simple, non-denaturant concentration of polydisperse organic matter in natural waters and sediments (Schindler, Alberts and Honick, 1972; Schindler and Alberts, 1974; Gjessing, 1970, 1975; Lindberg and Harriss, 1974; Andren and Harriss, 1975). A range of molecular sizes may be recovered and fractionated

into broad molecular classes using ultrafiltration membranes (fig. 5.1). This form of reverse osmosis provides a means of evaluating ion-binding phenomena associated with macromolecular, non-dialyzable material and is particularly appropriate to a preliminary study of such processes occurring in surface waters.

In the present study, trace element contamination arising from the use of ultrafiltration membranes and associated apparatus has been investigated (Chapter 2). A selection of membranes and a 'thin-channel' ultrafiltration cell with accessory fibre-glass reservoir were used in a series of trials. Much of the commercially available apparatus and many of the membranes used proved to be a source of heavy metal contamination. However, selected membranes of the Amicon UM and PM series, after decontamination and thorough rinsing and when used with simplified 'Plexiglass' apparatus do not significantly contaminate ultrafiltrates. High performance membranes of the Amicon PM range facilitate the rapid recovery of colloidal material in dilute concentration. Preliminary adsorption trials indicate that heavy metals in the form of 'free' ions are not significantly sorbed upon this type of membrane or the walls of the apparatus during filtration.

Further investigation is required to assess the magnitude and effect of adsorption losses upon the equilibrium and steady state systems of heavy metals in natural waters; for example Stumm and Bilinski (1973) have demonstrated a preferential adsorption, from solution, of lead carbonate species upon silica. A consideration of the probable first order rate constants for the formation of fulvic

acid-metal complexes (Langford and Gamble, 1974) suggests that such species may be in dynamic equilibrium with other simple organic and inorganic complexes in aqueous solution. Adsorption losses of the type described by Stumm and Bilinski (1973) and also investigated by Benes and Steinnes (1975) may therefore influence the observed molecular size distribution of heavy metal species, as determined by ultrafiltration studies, with the degeneration of these weak organic complexes.

The establishment of ultrafiltration and molecular fractionation by reverse osmosis in routine analysis must await the development of more efficient membranes and further study of the organic and inorganic contamination and adsorption losses associated with these procedures. Nevertheless, with the increasing use of ultrafiltration in the study of natural waters, a pragmatic and more rational approximation to a conceptual definition of material in true solution will be achieved. In conclusion, a further appraisal of the application of ultrafiltration to the study of ion-binding phenomena would perhaps usefully include:

- (i) the use of radio-tracers and labelled heavy ^{metal} species in solution to follow adsorption losses and possible decomposition processes associated with increasing concentration of 'feed' waters (Sourirajan, 1970);
- (ii) the elimination of systematic errors by comparative studies using both ultrafiltration and ultracentrifugation;
- (iii) the study of changes in Donnan equilibria and their effect upon observed molecular size distribution of weak complexes of macromolecular organic acids;
- (iv) a study of observed molecular size classes in relation to membrane retention characteristics as determined by adsorption, temperature, pressure and variation in the composition of 'feed' solutions and dialyzates (Sourirajan, 1970).

In the present study, a method of direct analysis using conventional chelation/solvent extraction techniques has been adopted to determine the distribution and concentration of trace heavy metals in selected molecular size fractions of river water. The colloidal, organic material occurring in rivers of the study area in the South-west of England (Chapter 1) has been investigated to evaluate possible ion-binding phenomena associated with macromolecular polyelectrolytes. Using ultrafiltration, waters from four rivers in Cornwall: the Carnon, Fowey, Helford and Tamar, have been examined and sampled over several months. With the exception of iron in organically enriched streams, the major proportion of all heavy metals in waters from this area was found in the low molecular size classes (nominal molecular weight <500). Only marginal differences in the concentration of dissolved heavy metals were observed after removal of colloidal and macromolecular material from filtered samples (Chapter 5). During the present study it has proved expedient to confirm apparent complexation or ion-binding with colloidal polyelectrolytes by analysis of any concentrated dialyzate isolated during ultrafiltration. The binding of calcium and the occurrence of organo-iron complexes has been established and confirmed by analysis (Chapters 5 and 6).

The nature of colloidal iron and the significance of heteroions associated with macromolecular material in rivers remains an enigma (Chapter 3). However, the results of Mössbauer spectroscopy have confirmed that ^{some of} the iron occurring in the colloidal polymeric assemblages of waters from the River Fowey is in the form of an organic complex (Chapter 4). These polynuclear complexes are similar

to the fulvic acid complexes extracted from peat, in which Fe(III) occupies a broad range of the molecular environments of this multi-dentate ligand. A broad band in the infrared spectrum, associated with the preponderance of carbonyl and carboxyl groups, is a feature of humic and fulvic acids. Similarly a broad peak is a characteristic of the Mössbauer spectra of the isolated organo-iron complexes. It may be deduced that iron is complexed by carboxyl groups to form a polynuclear coordination compound with fulvic acid (Chapters 4 and 6). The structure of humic and fulvic acids remains poorly understood, however, Mössbauer studies of ^{57}Fe labelled complexes, carried out in conjunction with far infrared spectroscopy would undoubtedly provide useful information as to the nature of these complex substances. Trace concentrations of phosphorus have been found in the non-dialyzable material from river water (Chapter 6) but it has been reported that phosphates do not react with humic substances except in the presence of iron which acts as a bridging ion (Schnitzer and Khan, 1972). The results of further Mössbauer studies could provide good evidence for this type of interaction.

The results of preliminary identification and characterization work indicate that a heterogeneous admixture of organic complexes, inorganic minerals and polymeric polyelectrolytes and associated heteroions constitutes the bulk of filterable but non-dialyzable material in the rivers of the present study area (Chapter 6). Macromolecular material recovered from rivers in Cornwall has a remarkable uniformity of composition: particularly with respect to the non-dialyzable ions associated with the two molecular size classes

examined. Elemental analysis has shown that the concentrated material is largely organic, and infrared spectroscopy results indicate that silicates and humic substances are the predominant constituents. Multi-element analysis using electron probe micro-analysis and qualitative, thin-film x-ray fluorescence has confirmed the presence of silicon as well as the occurrence of aluminium, calcium, iron, magnesium and potassium at levels in excess of 1% (w/w). Chlorine, phosphorus and sulphur are also common non-dialyzable heteroelements found in the inorganic 'ash' of many of the isolated residues.

Non-dialyzable material extracted from the waters of three rivers in Cornwall: the Fowey, Helford and Tamar, were found to contain only trace concentrations of heavy metals. However, humic and fulvic acids are the principal macromolecular constituents of these surface waters. These findings are in marked contrast to the reports of humic acid heavy metal complexes occurring in rivers and determined by electrochemical methods (Guy and Chakrabarti, 1975; Ramamoorthy and Kushner, 1975; Smith, 1976). The occurrence of organic complexes of the trivalent metals such as aluminium and in particular iron, has been well established in previous studies (Black and Christman, 1963; Shapiro, 1964; Lamar, 1968; Tryland and Gjessing, 1975; Perdue, Beck and Reuter, 1976). Similarly, the association of alkali and alkaline earth metals with humic substances and the macromolecular fractions of lake waters has also been reported (Barsdate, 1967; Gjessing, 1970; Gamble, 1973; Schindler and Alberts, 1974; Benes, Gjessing and Steinnes, 1976). Phosphorus and sulphur are also common

heteroelements of humic substances and silicates are frequently associated with extracted humic acids (Schnitzer and Khan, 1972). However, the presence of chlorine as a non-dialyzable ion has not been commonly observed ^{although} ~~in~~, a weak association between the halogens and 'humus' in fresh water has been reported (Benes, Gjessing and Steinnes, 1976). It has also been observed that chlorides are a persistent impurity of many humic acid preparations (Malcolm, 1976).

Much of the evidence of this thesis confirms that humic substances, most probably derived from soil water, are the major component of the organic macromolecular assemblage in surface waters. Nevertheless, these findings demonstrate that heavy metals in the rivers of the present study area occur as low molecular weight species. An inevitable conclusion which questions the importance of macromolecular polyelectrolytes in the transport and distribution of many of the transition metals in natural waters. However, the methods of sampling and analysis used during the present investigation may prove inappropriate for the study of weak organic complexes and ephemeral metal-organic species such as those of polycarboxylic acids.

The formation of humic and fulvic acid-heavy metal complexes in natural waters requires that trace concentrations of these organic acids specifically interact with individual transition metal ions. The presence of calcium and magnesium at concentrations many orders of magnitude larger than these potential multidentate ligands may establish competitive equilibria antagonistic to the formation of such organic complexes. The presence of high concentrations of calcium

and trace levels of magnesium in the non-dialyzable material from the rivers studied in the present investigation provides some oblique evidence for this suggestion (Chapter 6). A preferential complexation of 'hard' alkali and alkaline earth metals might also be anticipated from the probable coordination chemistry of humic and fulvic acids and the known chemistry of polycarboxylic acids. Compounds containing O donor atoms are rather general complex formers and hence not selective for Class B cations and transition metals. The preferential uptake of 'hard' Class A cations might be expected from the known functional groups of humic and fulvic acids, which are principally carboxyl, hydroxyl, quinoid and phenolic. The precipitation of polymethacrylic and polyacrylic acids by Ca^{2+} ions (Travers and Marinsky, 1974) provides a homologous example of the uptake of 'hard' cations by a polycarboxylic acid; very similar observations have been reported for fulvic acids (Dupuis, Jambu and Dupuis, 1972).

'Soft' bases, for example ligands of N and S donor atoms, are more selective; selectivity increasing with decreasing electronegativity of the element. In general, compounds of nitrogen and sulphur are much more selective for Class B cations and transition metals. The polyamines and amino acids in natural waters may therefore form strong complexes with heavy metals and evidence for an amino-copper species has been reported by Stiff (1971).

The occurrence of such low molecular weight species may account for the many reports of organic complexing agents in lakes and rivers and in marine coastal waters and would also be consistent with

the findings of the present study. The presence of nitrogen as a heteroelement in humic substances suggests that 'soft' functional groups may also be available in these multidentate ligands. Electron paramagnetic resonance (EPR) studies suggest that traces of copper are complexed in humic acids by the nitrogen of porphyrin groups of humic acids (Goodman and Cheshire, 1973). Investigations of this nature using EPR spectroscopy would provide further useful information about the type of coordination compounds formed by humic and fulvic acids and their form in natural waters. Much of the nitrogen of humic and fulvic acids extracted from soils is considered to occur largely as amino acids of which 1 to 10% is in the form of amino sugars (Schnitzer and Khan, 1972). Thus, a study of the role of nitrogen in the coordination chemistry of naturally occurring polyelectrolytes would be essential in any further investigation of macromolecular heavy metal species in natural waters. The importance of low molecular weight organic ligands remains to be established but may also prove a significant factor determining the form of heavy metals in rivers and their availability to the aquatic fauna of marine and fresh water habitats.

Ultrafiltration facilitates but a preliminary means of preconcentration and fractionation of the admixture of macromolecular material occurring in surface waters. The organic humic substances are ^{the} principal constituent of the non-dialyzable material discharged into rivers from soils and sediments. As polydisperse organic compounds, the humic acids present a continuum of molecular sizes ranging from low molecular weight polyions in true solution to polyelectrolytes

and high molecular weight sols. These also present a continuous range of intrinsic acidity. Associated with this gamut of disperse phases may be a variety of metal-organic interactions. In rationalizing the problem of humic acid geochemistry, it is convenient to distinguish between interactions of colloidal and macromolecular material which are electrostatic in nature and those which are reactions of complexation or chelation. Many of these properties may be differentiated on the basis of molecular size since low molecular weight species will be expected to exhibit colligative properties associated with a true solution and hence may be regarded as polyions and potential multidentate ligands. With increasing molecular mass, electrostatic interactions associated with the more general properties of polymeric material and a macromolecular polyelectrolyte should predominate. Ultimately ion-exchange reactions may be anticipated which are consistent with the behaviour of discrete dispersions of insoluble material. The objective of any further separation of non-dialyzable material of this nature must be to produce fractions which are chemically and physically homogeneous. This requirement is an important prerequisite of any further empirical studies of ion-binding phenomena associated with humic substances (Malcolm, 1976).

The structure of humic polyelectrolytes remains ill-defined. At present it is considered to be a three dimensional array of poorly condensed aromatic rings which are extensively substituted with carboxyl, hydroxyl, phenolic and quinoid groups and side chains; ester linkages, hydrogen and $p-\pi$ bonding are thought to contribute to the stability of the structure. A better understanding of molecular

structure would undoubtedly rationalize much of humic acid chemistry. However, a great deal of attention has been focused upon the complexing properties of these substances with particular emphasis upon the formation of heavy metal complexes. Empirical studies have frequently excluded any consideration of the role of other ions and the properties of humic acids as weak polymeric acids. The present study has demonstrated the existence of macromolecular species of iron and aluminium in natural waters. Also these preliminary results indicate that alkali and alkaline earth metals may play an important role in the geochemistry of humic substances and furthermore ^{supports the view} that some of the major ions govern the mobilization and *precipitation* of the organic colloidal and macromolecular constituents of surface waters. A more objective consideration of the role of major and minor elements in the stability of naturally-occurring dispersions of organic acids will be essential in any further studies. The importance of humic and fulvic acid complexes in the chemical speciation of heavy metals in natural waters remains open to question and many more simple ion pairs such as hydroxo and carbonato species may be of greater significance.

The composition of surface waters is characterized by suspended sediment, discrete colloidal dispersions and dissolved species of cations and anions in dynamic equilibrium or at a steady state. Each of these phases may be closely interrelated as a result of the processes of dissolution, 'sorption' and complexation governing the transition from one phase to another. Coagulation, aggregation and precipitation of dispersed and dissolved constituents determines

to a large degree the composition of the suspended sediments and bottom sediments. Nevertheless, the nature and geochemical fate of much of the organic carbon and trace inorganic species transported by rivers and discharged into the estuarine environment remains unresolved.

In sea water, it has been suggested that hydrous ferric oxides 'scavenge' the ionic species of trace elements (Goldberg, 1954; Krauskopf, 1956; Aston and Chester, 1973; Eisma, 1975). This mechanism of co-precipitation is postulated a priori upon the assumption that 'dissolved' iron forms unstabilized and colloidal hydroxo complexes under natural conditions. The results of the present study demonstrates that organo-iron complexes are a major species of iron in river waters. Much of this colloidal material is in the form of humic and fulvic acid complexes which are unstable under conditions of ionic strength 0.2 (Schnitzer and Hanson, 1970). The non-conservative behaviour of iron during estuarine mixing is in marked contrast to the apparently stable condition of zinc under such conditions (Boyle et al., 1974; Eisma, 1975; Holliday and Liss, 1976). Such evidence suggests that co-precipitation may not govern the concentration and dispersion of trace metals in estuaries. Sequestration through colloidal interactions has been shown to play a role in the removal processes in estuaries (Sholkovitz, 1976) which may account for the removal of iron and also prove an important consideration in the budget of organic carbon in the world oceans.

Ultrafiltration techniques, used in conjunction with electrochemical methods such as anodic stripping voltammetry,

differential pulse polarography and ion-selective electrodes have been used to isolate the principal macromolecular constituents responsible for the apparent 'complexing capacity' of natural waters (Chau, 1973; Chau, Gachter and Lum-Shue-Chan, 1974; Ramamoorthy and Kushner, 1975; Smith, 1976). These methods are capable of yielding useful information as to the distribution of macromolecular complexing agents and the behaviour of natural waters as metal-ion buffers. In this manner, an index of the proportion of 'free' ions in natural waters can be monitored over an extensive period during a general survey. Similar results might also be obtained with the use of in situ dialysis cells using membranes resistant to bacterial attack.

Many of the natural processes involving particulate, colloidal and dissolved material in natural waters remain a matter for conjecture (Eppler, Neis and Hahn, 1975). However, theoretical considerations and the formulation of models to describe the physicochemical processes of transport, distribution and removal present a task with pragmatic objectives allied to the problems of pollution control and the maintenance of water quality. Such models may be of limited application in quantitative predictions but provide a useful guide to the empirical information required from further studies (Boyle et al, 1966; Hahn and Stumm, 1970; Eppler, Neis and Hahn, 1975).

In any study of the organic and inorganic removal processes occurring in estuaries, a number of parameters remain to be evaluated (Bondarenko, 1972; Sholkovitz, 1976; Boyle et al, 1974);

- (i) the stability of naturally-occurring colloidal dispersions;

- (ii) the rate of coagulation of naturally-occurring colloids and polymeric material under specified conditions of pH and ionic strength;
- (iii) the size and composition of agglomerates formed within realistic time periods under defined conditions of the continuous phase;
- (iv) precipitation rates of suspended particulate material (at given flow velocities) and the concomitant rates of co-precipitation;
- (v) the sequence of precipitation of colloidal and polydisperse organic matter from the heterogeneous admixtures of aquatic systems under estuarine conditions.

In order to describe the processes of coagulation and flocculation in natural waters, it is essential to consider the mechanisms of transport in terms of two measurable parameters: particle size and flow pattern. The macromolecular dispersions of humic acids with nominal particle size $< 1 \mu\text{m}$ are subject principally to random Brownian motion or perikinetic transport which is a second-order reaction law (Eppler, Neis and Hahn, 1975):

$$\frac{dN}{dt} = K_p N^2$$

where K_p = a reaction constant $\propto (4k/3\mu)T$

N = number concentration of particles in a flocculating system

α = collision efficiency

k = Boltzmann's constant

T = Absolute temperature

μ = Dynamic viscosity

Particles larger than $1 \mu\text{m}$ diameter are transported principally by velocity gradients under conditions prevailing in natural systems;

under conditions of laminar flow the process is described by a first-order reaction law (Eppler, Neis and Hahn, 1975):

$$\frac{dN}{dt} = K_o N$$

where K_o = a reaction constant: $\propto \frac{4\phi}{\pi} \left(\frac{du}{dz} \right)$

ϕ = volume concentration of the suspended phase

$\frac{du}{dz}$ = linear velocity gradient in a laminar fluid motion

The effectiveness of any coagulation process is determined by the number of successful and permanent collisions or aggregates that occur relative to the total number of collisions in any given system. The temperature dependent ratio α , termed the collision efficiency may assume any value in the range from $\alpha = 0$ for a stable non-coagulating system to unity for destabilized and ^{is an} index of stability which might be appropriately applied to naturally-occurring dispersions to describe the removal processes in an estuary.

In practical terms, these relationships demonstrate that it is essential to define the polydispersity of a natural system in order to quantify the stability of a suspension of a colloidal dispersion. For particles $>1\mu\text{m}$ Coulter counting techniques might reasonably be applied to produce size spectra of suspended sediments and flocculating systems (TeKippe and Ham, 1970). However, Coulter counters facilitate the examination of only a fraction of the size spectrum at any given time. In addition, such measurements may be complicated by artifacts associated with changes in the relative conductivity of the dispersive medium and the disperse phase under

investigation. Single particle scattering counters are now available for particle size analysis which facilitate the examination of charged dispersions using laser-light scattering and photon correlation spectroscopy. Alternatively narrow angle x-ray goniometry may be used to determine the association and dissociation of polydisperse systems such as humic acids (Wershaw and Pinkney, 1972). Perhaps the most promising means of resolving high molecular weight mixtures is provided by high pressure, gas-liquid chromatography; molecular sizes ranging from 2,600,000 to <1,000 molecular weight are separable with the use of modern column packing materials and polystyrene standard calibrants. High pressure g.c. techniques may prove particularly valuable in calibrating molecular separations by gel filtration.

A combination of these techniques would provide a useful means of determining the changes in size spectra of macromolecular material and suspended particles in natural waters under controlled conditions of the continuous phase. In this manner, conditions analogous to those occurring in an estuary could be modelled with a mechanistic approach to the processes of transport and precipitation. The ultimate objective of these investigations would be to define solution stabilities in terms that are well established for simpler colloidal systems. In conjunction with further studies designed to characterize the products of coagulation and precipitation processes in an estuary, such an approach would provide much information about the natural removal processes and form a useful extension of the present study.

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A P P E N D I X A

APPENDIX A

ANALYTICAL METHODS - Applied Geochemistry Research Group,
Technical Communication Number 62

CHELATION/SOLVENT EXTRACTION SYSTEM FOR THE DETERMINATION
OF CADMIUM, COBALT, COPPER, IRON, MANGANESE, NICKEL, LEAD
AND ZINC IN NATURAL WATERS

The metals are solvent extracted from the sample as their diethyldithiocarbamate chelates. After destruction of the organic complexes, and dissolution of the residue in dilute acid, the metal concentrations are estimated by atomic absorption spectrometry. The method was developed for the routine analysis of several trace elements at low concentration in natural water samples, where both separation from possible interfering matrix materials, and a concentration step are required. The technique is similar to that used by Bryan (1973), and has been successfully applied in this laboratory to both saline and fresh water samples.

A. Sampling Procedure

1. Leave the 11 high density polyethylene sampling bottles filled with approximately 2M nitric acid for 24 hours. Empty, and then wash out each bottle three times with deionized water.
2. Wash the bottle out with the water being sampled, and take a 11 sample.

B. Preliminary Treatment - 'Soluble Metal Fraction'

1. Wash a 0.45 μ membrane filter in 1MHCl, and rinse with

deionized water. Filter the sample through this washed membrane as soon as possible after sampling.

2. Add 2ml of 16M nitric acid to the sample and shake for a few seconds to ensure mixing.

C. Preliminary Treatment - 'Total Metal Fraction'

1. Add 2ml of 16M nitric acid to the sample and shake for a few seconds to ensure mixing.
2. Allow the sample to stand for at least four days, giving the bottle a daily shake.
3. Wash a 0.45 μ membrane filter in 1MHCl, and rinse with deionized water. Filter the sample through this washed membrane.

D. Reagents

1. Acetic acid, glacial (A.R.).
2. Ammonia solution. Isothermally distilled, 0.880 S.G., 'low lead' reagent (i.e. the ammonia gas is allowed to equilibrate, in a closed system at room temperature, between the original ammonia solution and a similar volume of deionized water).
3. Chloroform. Ultrapure grade.
4. Deionized water. The water is doubly distilled using a silica element still after passing through an anion/cation exchange resin.
5. Hydrochloric acid, 1M. Make up 89 ml of ultrapure 11.3M hydrochloric acid to 1l with deionized water.
6. Nitric acid, 16M. Ultrapure or redistilled AR grade.
7. Sodium acetate trihydrate (L.G.R.).

8. Sodium diethyldithiocarbamate (A.R.).

9. Sodium diethyldithiocarbamate (SDDC)/buffer solution

Add 250g of sodium acetate trihydrate to 500ml of deionized water, and mix to dissolve. Pour 6ml of glacial acetic acid into the solution and mix thoroughly. Add 50g of SDDC to this buffer solution and mix thoroughly. Make up the SDDC/buffer solution to 1000ml with deionized water and pour this into a 2l separating funnel. If the buffer is prepared correctly, the pH of the solution at this stage is 8-9. Extract any SDDC-metal complexes by successive extractions with 30ml aliquots of chloroform, until consecutive extracts are colourless.

E. Apparatus

1. Cleanliness of apparatus and precautions against contamination at all stages are essential. Soak glassware in 8M nitric acid, followed by 5% ^v/v solution of 'Decon 90' in deionized water. Wash out five times with deionized water before use. For cleaning equipment required immediately, shaking with the SDDC/buffer and rinsing five times with deionized water has proved satisfactory. The separating funnel should be cleaned in this way before beginning a series of extractions.

2. A piston 'Oxford' type pipette proves convenient for dispensing large numbers of hydrochloric acid aliquots at stage F.9.

3. The pH's in sections F.1 and F.2 are checked by removing a drop on a glass rod from the flask and using the appropriate narrow range test paper.

F. Extraction Procedure

1. Place 50ml of the sample in a 1l separating funnel, and by addition of the ammonia solution adjust the pH to approximately 7.
2. Add 20ml of SDDC/buffer solution, and check if the pH is 5.8-6.1. If not, adjust the pH to this range by dropwise addition of either ammonia solution or 16M nitric acid. Shake for c. 10 sec. after each addition to ensure thorough mixing.
3. Shake for five minutes, and then add 30ml of chloroform to the flask.
4. Shake for five minutes, and then allow the phases to separate. Run the chloroform layer into a 50ml Quickfit conical flask.
5. Add 1ml of 16M nitric acid to the extract, and place the flask on a hot plate.
6. Add a further 20ml of chloroform to the separating funnel and shake for five minutes. Add the separated chloroform phase to the initial extract.
7. Gently evaporate the combined chloroform extracts to dryness, without boiling.
8. The residue in the flask should be a white colour. If darker than a pale yellow, add a further 1ml of 16M nitric acid, and evaporate to dryness. Repeat, if necessary, until all the organic matter has been destroyed and a pale yellow/white residue remains.
9. Take up the salt residue in exactly 5ml of 1M hydrochloric acid, stopper, and leave overnight to ensure complete dissolution.
10. Swirl the flask contents to ensure mixing, and determine the metal concentrations by atomic absorption spectrometry.

11. Perform a blank determination, repeating steps 1-10 on 500ml of deionized water, already stripped of any trace metals by the extraction procedure.

G. Remarks

1. If a suitable mechanical flask shaker is available the tedium of manual shaking is removed; as well as allowing more samples to be analysed (with a multi-flask shaker).

2. Where high trace element concentrations are evident, i.e. an immediate very deep colour or a precipitate is formed on the addition of the SDDC/buffer, carry out a 30ml, and then a 20ml, chloroform extraction before continuing at F.2.

3. The SDDC solution is unstable under acid conditions, and rapidly decomposes (Martin, 1953). The pH of the SDDC/buffer on standing a short while after preparation should be approximately 9. At this pH, the SDDC solution has been shown to be stable for at least one month.

4. Acidification of the sample as described has been demonstrated to stabilize the concentration of extractable trace elements for at least a 35 day period (Watling, 1974).

5. It is recommended that a duplicate and a blank be carried out after every tenth sample. Also, to ensure the extraction procedure is being operated satisfactorily, spiked samples should be analysed periodically, particularly at the beginning and end of each batch of SDDC/buffer.

6. In this method an arbitrary criterion has been used to distinguish between 'soluble' and 'total' metal fractions. 'Soluble' refers here to those metal species capable of passing through a 0.45μ membrane (N.B. sample acidified after filtration), whilst 'total' refers to that metal fraction of the particulate solubilized by the dilute nitric acid and capable of passing through a 0.45μ membrane plus the soluble fraction (N.B. sample acidified prior to filtration). Obviously a genuine 'total' fraction would include metals still not leached from the particulate.

As has been shown by Kamp-Nielsen (1972) for copper, which probably also applies to the other metals extracted, the SDDC chelation technique removes both ionic and weakly bound metal forms from the sample. Strongly organically bound metals may well also be removed during the extraction stage, if they are soluble in chloroform.

7. The method has been used in a United States Geological Survey international intercalibration study. As can be seen in table 1, all values showed good agreement with the intercalibration mean values; the values obtained by this technique being consistently just below the USGS mean values.

8. Comparison of direct analysis (atomic absorption spectrometry, using deuterium lamp background correction) and the solvent extraction system, for zinc spikes in aliquots of a bulk seawater sample, was carried out. A good correlation over a wide range of values ($13-620 \mu\text{g l}^{-1}$) was found, with the direct analysis results being slightly higher than the solvent extraction figures (Watling, 1974)

9. Evaluation of detection limits and precision was based on the method of Thompson and Howarth (1973), using 45 duplicate determinations. In the data used, the analysis was repeated for every tenth sample, and thus represent within batch duplicates. The data are presented in table 2.

10. Recoveries were estimated by spiking 500ml aliquots of a bulk seawater sample at varying levels for each metal (table 3). The initial concentrations of trace metals in the seawater were determined using the SDDC technique. Thus it should be noted that the variance of the spike recovery data is increased by the errors in determination of the trace metals in the unspiked seawater. Lead appears to give poor recoveries. The analytical data for this element should be improved by increasing the sample size analysed.

H. Additional References

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TABLE I

Comparison of Solvent Extraction Technique and USGS Intercalibration Study Data

Element	Applied Geochemistry Research Group Solvent Extraction Technique (mean of 2 extractions, ppb)	USGS Intercalibration study (ppb)	
		Mean	Standard Deviation
Co	1.5	ND	ND
Cd	2.2	3.6	1.1
Cu	112	145	38
Fe	920	1008	106
Mn	25	35	13
Ni	4	ND	ND
Pb	12	16.7	9.0
Zn	151	192	29

ND - no data for this element

TABLE 2
Detection Limits and Precision

Element	Detection Limit	Standard Deviation(s)	Range or level at which Standard Deviation estimate applies (ppb)
Cd	0.2	0.1	0.2-5
Co	1.0	0.5	1-5
Cu	0.5	0.2	0.5-20
Fe	1.6	0.8	1.6
		1.9	10.0
		3.3	20.0
Mn	2.0	1.0	2.0-30
Ni	1.0	0.5	1.0-20
Pb	0.4	0.2	0.4-20
Zn	0.4	0.2	0.4
		0.6	10
		4.0	50
		8.0	100

TABLE 3
Analysis of Sea Water Containing Added Metals

Element	Wt. Added (μg)	Wt. Extracted (μg)			No. Observations
		Mean	Min.	Max.	
Cd	1.0	0.7			1
	2.0	1.9	1.7	2.1	6
	4.0	3.8	3.4	4.1	6
	5.0	4.6			1
	10.0	9.3	8.9	9.5	3
	19.9	17.4	17.4	17.4	2
Co	1.0	0.9			1
	2.0	1.9			1
	5.0	3.9			1
	10.0	9.2	8.9	9.4	2
Cu	2.0	1.4	1.2	1.6	6
	2.2	2.8			1
	4.0	3.4	2.8	3.8	6
	5.4	5.6			1
	10.8	10.4	10.4	10.4	3
Fe	10.0	7.9	5.4	11.4	6
	20.0	17.6	15.4	19.4	6
Mn	1.0	0.9	0.7	1.6	6
	2.0	1.7	1.5	1.8	6

TABLE 3 (CONTINUED)

Element	Wt. Added (μg)	Wt. Extracted (μg)			No. Observations
		Mean	Min.	Max.	
Ni	20.0	19.3	18.0	20.0	4
	30.0	29.5	29.0	30.0	4
Pb	2.0	1.1	<0.4	2.4	5
	4.0	1.6	0.4	2.4	6
	5.0	0.4			1
	10.0	7.0	6.0	10.0	3
	20.0	16.0	14.0	18.0	2
Zn	1.1	1.2			1
	2.0	2.0	1.3	2.6	6
	2.2	2.5			1
	4.0	4.5	4.4	4.7	4
	5.6	6.3			1
	11.2	11.1	9.8	11.9	3
	22.4	24.2	23.8	24.5	2

A P P E N D I X B

APPENDIX B

MOLECULAR SIZE DISTRIBUTION OF 'SOLUBLE' TRACE METALS IN
SELECTED SURFACE WATERS IN THE SOUTH-WEST OF ENGLAND

TABLE I

Distribution of 'Soluble' Trace Metals in Tamar River Waters (Sampled at Crossgate 1974/75) after Ultrafiltration

DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
		Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
7/74	GF/C	350.0	6.2	2.4	1.0	1.0	6.0	6.0	<5.0
	GF/C*	310.0	6.7	2.5	2.0	0.2	7.0	7.0	<5.0
	PM-10	10.0	7.0	3.0	1.0	0.5	7.0	3.0	<5.0
	PM-10*	10.0	6.5	3.0	2.0	0.5	6.0	2.0	<5.0
8/74	GF/C	500.0	16.6	2.8	2.0	0.6	4.0	4.0	<5.0
	PM-10	37.0	15.0	3.0	1.0	0.5	3.0	4.0	<5.0
10/74	GF/C	470.0	14.8	3.2	2.0	0.5	5.0	7.0	<5.0
	PM-10	34.0	15.5	3.5	2.0	0.5	3.0	5.0	<5.0
2/75	GF/C	25.0	5.8	1.9	2.0	<0.1	5.0	23.0	<5.0
	PM-10	9.0	3.3	1.0	1.0	0.2	3.0	8.0	<5.0
3/75	GF/C	20.0	3.2	2.0	<1.0	0.4	4.0	5.0	<5.0
	PM-10	7.0	4.9	2.0	1.0	0.2	2.0	3.8	<5.0
4/75	GF/C	24.5	6.5	1.2	1.0	0.1	3.5	15.0	<5.0
	PM-10	6.0	6.0	2.0	1.0	0.1	2.5	9.0	<5.0
	PM-10*	11.0	6.5	2.1	<1.0	0.1	3.0	9.5	<5.0
	UM-05	5.0	6.65	1.8	<1.0	0.4	2.5	7.0	<5.0
6/75	GF/C	30.5	15.0	2.2	1.0	0.25	4.0	7.0	<5.0
	PM-10	6.0	12.5	3.3	<1.0	0.20	3.0	5.0	<5.0
	UM-05	3.0	6.0	1.5	<1.0	0.20	2.0	1.5	<5.0
	UM-05	4.0	8.8	2.2	<1.0	0.20	3.0	1.0	<5.0

* Duplicate Analyses

TABLE 2

Distribution of 'Soluble' Trace Metals in Tamar River Water (Sampled at Gunnislake 1974/75) after Ultrafiltration

DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
		Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
7/74	GF/C	267.0	20.0	21.0	1.0	0.8	6.0	6.0	<5.0
	PM-10*	19.0	19.0	18.6	<1.0	0.7	5.0	5.0	<5.0
	PM-10*	18.2	21.0	19.5	<1.0	0.6	5.0	5.0	<5.0
8/74	GF/C	240.0	34.0	13.8	2.0	0.3	6.0	2.0	5.0
	PM-10*	14.0	32.9	11.8	1.0	0.4	5.0	<1.0	<5.0
	PM-10*	12.0	32.6	11.3	1.0	0.6	5.0	<1.0	5.0
	PM-10*	13.0	38.0	12.4	1.0	0.6	5.0	<1.0	6.0
9/74	GF/C	270.0	8.0	16.3	1.0	0.2	5.0	2.0	<5.0
	PM-10	22.0	7.6	17.0	1.0	0.2	4.5	2.0	<5.0
10/74	GF/C*	250.0	16.0	13.9	2.0	0.3	5.0	1.9	5.0
	GF/C*	280.0	17.8	14.2	1.0	0.3	4.0	2.0	5.0
	PM-10	18.0	16.3	14.0	2.0	0.2	4.3	2.2	<5.0
2/75	GF/C	7.0	7.1	14.0	1.0	0.7	4.0	20.0	<5.0
	PM-10	6.0	7.2	13.7	2.0	0.3	5.0	20.0	<5.0
3/75	GF/C	10.0	6.8	17.0	1.0	0.4	4.0	7.0	<5.0
	PM-10	5.0	4.6	15.0	<1.0	-	4.0	7.2	<5.0

TABLE 2 (CONTINUED)

DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
		Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
4/75	GF/C	18.5	6.2	15.4	1.0	0.4	3.5	26.0	<5.0
	PM-10	11.5	7.1	10.3	<1.0	0.2	4.0	25.0	<5.0
	UM-05	5.5	6.5	12.6	<1.0	0.35	6.0	25.0	<5.0
6/75	GF/C	12.0	15.5	26.0	1.0	0.2	5.0	12.0	<5.0
	PM-10	3.5	13.5	25.0	<1.0	0.2	4.0	11.5	<5.0
	UM-05*	2.0	12.5	22.5	<1.0	0.1	3.0	5.0	<5.0
	UM-05*	4.0	12.0	20.0	<1.0	0.6	3.5	6.5	<5.0

* Duplicate Analyses

TABLE 3

Distribution of 'Soluble' Trace Metals in Tamar River Waters (Sampled at Launceston 1974/75) after Ultrafiltration

DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
		Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
5/74	GF/C	22.0	13.0	1.3	<1.0	0.1	1.0	3.0	-
	PM-10	1.3	12.5	0.8	<1.0	0.1	1.0	2.0	-
	PM-10*	1.3	13.0	0.5	<1.0	0.1	1.0	2.0	-
7/74	GF/C	190.0	28.9	3.8	1.0	0.4	7.0	1.0	<5.0
	GF/C*	144.0	22.0	2.6	<1.0	0.4	4.0	1.0	<5.0
	PM-10	30.0	31.7	2.0	1.0	0.8	6.0	1.0	<5.0
	PM-10	37.0	31.4	2.6	1.0	0.6	5.0	1.0	<5.0
	UM-05	28.0	29.1	3.1	1.0	1.0	5.0	1.0	5.0
	UM-05*	26.0	32.0	2.6	1.0	1.0	4.0	1.0	5.0
8/74	GF/C	380.0	21.9	11.1	1.0	0.4	10.0	3.0	8.0
	PM-10	31.0	20.0	10.5	1.0	0.5	8.0	2.0	7.0
9/74	GF/C	440.0	8.1	2.0	1.0	0.4	5.0	2.0	6.0
	GF/C*	380.0	6.5	1.6	1.0	0.2	4.0	2.0	5.0
	PM-10	250.0	8.0	2.0	1.0	0.2	4.0	2.0	<5.0
2/75	GF/C	13.0	5.6	1.5	1.0	0.4	4.0	17.6	<5.0
	PM-10	9.0	4.2	1.1	2.0	0.3	4.0	16.0	<5.0
3/75	GF/C	14.0	4.0	2.0	<1.0	0.4	3.0	11.0	5.0
	PM-10	11.0	4.0	2.0	<1.0	0.2	3.0	11.0	6.0

TABLE 3 (CONTINUED)

DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
		Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
4/75	GF/C	19.5	2.1	1.6	<1.0	0.25	2.5	13.5	<5.0
	PM-10	9.0	1.8	1.4	<1.0	0.30	2.0	10.5	<5.0
	UM-05	5.5	2.1	2.1	<1.0	0.20	3.0	10.5	<5.0
6/75	GF/C	15.0	3.3	1.2	<1.0	0.15	1.5	5.0	<5.0
	PM-10	7.5	3.6	1.3	1.0	0.15	1.5	4.5	<5.0
	UM-05	7.0	2.8	0.8	<1.0	0.15	1.0	2.5	<5.0

* Duplicate Analyses

TABLE 4

Distribution of 'Soluble' Trace Metals in Tamar River Waters (Sampled at Calstock 1974/75) after Ultrafiltration

DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
		Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
7/74	GF/C	20.0	22.0	7.2	2.0	0.6	3.0	56.0	<5.0
	GF/C*	20.0	24.0	6.7	1.0	0.5	3.0	54.0	<5.0
	PM-10	4.0	23.9	6.7	<1.0	-	3.0	54.0	<5.0
	PM-10*	5.0	22.4	6.4	<1.0	-	3.0	57.0	<5.0
8/74	GF/C	125.0	12.0	15.0	1.0	0.2	3.0	8.0	<5.0
	GF/C*	160.0	10.0	20.0	1.0	0.2	5.0	10.0	<5.0
	PM-10	18.0	11.0	16.0	1.0	0.3	3.0	9.0	<5.0
	PM-10*	19.0	10.5	17.2	1.0	0.2	2.5	10.0	<5.0
9/74	GF/C	210.0	13.2	14.0	<1.0	0.3	4.0	2.0	<5.0
	PM-10	20.5	14.0	16.0	<1.0	0.2	3.0	2.0	<5.0
10/74	GF/C	154.0	19.0	32.5	1.0	0.6	7.0	9.0	7.0
	GF/C*	180.0	16.0	31.0	1.0	0.6	7.0	8.0	7.0
	PM-10	15.0	17.5	29.0	1.0	0.5	6.0	9.0	6.7
2/75	GF/C	10.0	7.1	6.6	1.0	0.3	2.0	5.0	<5.0
	PM-10	5.0	7.0	6.4	1.0	0.3	2.0	5.0	<5.0
3/75	GF/C	17.0	5.0	6.3	<1.0	0.1	3.0	1.5	6.0
	PM-10	4.0	5.9	6.1	<1.0	0.2	2.5	1.3	8.0

TABLE 4 (CONTINUED)

DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
		Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
4/75	GF/C	14.5	4.5	4.1	<1.0	0.2	1.0	1.0	<5.0
	PM-10	5.5	3.8	3.9	<1.0	0.2	1.0	1.0	<5.0
	UM-05	5.0	4.0	4.0	<1.0	0.2	2.0	1.0	<5.0
6/75	GF/C	40.5	12.5	10.5	2.0	0.5	2.5	2.0	<5.0
	PM-10	7.5	13.0	11.5	<1.0	0.2	1.5	2.0	<5.0
	UM-05	3.5	12.0	10.0	1.0	0.4	3.0	2.0	<5.0
	UM-05*	3.5	12.5	9.0	<1.0	0.2	3.0	2.5	<5.0

* Duplicate Analyses

TABLE 5

Distribution of 'Soluble' Trace Metals in Helford River Water after Ultrafiltration (1975)

SAMPLE LOCATION	DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
CONSTANTINE	1/75	GF/C	28.0	9.4	11.8	2.0	0.3	3.0	6.5	6.0
		GF/C*	29.5	9.0	12.3	2.5	0.3	3.0	7.0	6.2
		PM-10	26.0	9.4	11.0	4.0	0.4	3.0	6.5	6.0
	2/75	GF/C	21.0	25.0	11.0	<1.0	0.3	3.0	5.4	<5.0
		PM-10	14.0	23.0	14.0	2.0	0.4	4.0	7.4	<5.0
	3/75	GF/C	15.0	15.0	19.0	<1.0	0.3	2.0	5.5	<5.0
		PM-10	13.0	13.0	16.0	<1.0	0.4	1.0	1.8	<5.0
	4/75	GF/C	31.5	29.5	11.5	<1.0	0.10	1.0	5.5	<5.0
		PM-10	21.5	28.5	13.1	<1.0	0.25	2.5	6.5	<5.0
		UM-05	20.0	30.0	11.0	<1.0	0.30	2.5	6.5	<5.0
	6/75	GF/C	20.8	49.1	36.5	1.0	0.25	3.0	5.1	<5.0
		PM-10	14.6	47.5	35.5	1.0	0.2	2.0	4.5	<5.0
		UM-05	12.0	48.0	36.2	1.0	0.2	3.0	4.0	<5.0
		UM-05*	13.2	46.7	33.8	1.0	0.3	3.0	4.7	<5.0
TRELOWARREN	1/75	GF/C	38.0	4.6	1.1	1.0	0.5	3.0	5.4	<5.0
		PM-10	26.0	4.5	1.4	1.0	0.2	5.0	4.9	<5.0
	2/75	GF/C	13.0	9.5	1.5	<1.0	0.2	2.0	1.0	5.0
		PM-10	7.0	8.4	1.5	<1.0	0.1	3.0	1.0	<5.0

TABLE 5 (CONTINUED)

SAMPLE LOCATION	DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
TRELOWARREN	3/75	GF/C	11.0	8.0	1.4	<1.0	0.3	2.5	2.4	<5.0
		PM-10	8.0	6.4	1.4	<1.0	0.2	3.0	2.6	<5.0
	4/75	GF/C	17.5	4.9	1.7	<1.0	0.2	2.0	4.0	<5.0
		PM-10	10.5	4.9	1.9	<1.0	0.3	2.5	3.5	<5.0
		UM-05	4.5	3.5	1.5	<1.0	0.10	2.5	2.0	<5.0
		UM-05*	4.0	3.5	1.6	<1.0	0.4	2.5	3.5	<5.0
	6/75	GF/C	7.0	10.1	0.9	<1.0	0.2	1.0	11.5	<5.0
		PM-10	5.0	10.3	1.1	<1.0	0.2	1.5	13.5	<5.0
		UM-05	3.0	7.6	1.5	<1.0	0.2	1.5	9.0	<5.0
	1/75	GF/C	19.0	33.1	4.7	<1.0	0.3	<1.0	8.2	<5.0
		PM-10	14.0	32.7	4.4	<1.0	0.3	1.0	8.5	<5.0
GWEK QUAY	2/75	GF/C	7.0	28.0	5.0	1.0	0.2	3.0	7.8	<5.0
		PM-10	6.0	30.0	4.0	2.0	0.3	3.0	8.0	<5.0
	3/75	GF/C	6.0	14.0	2.0	<1.0	0.1	1.0	3.7	<5.0
		PM-10	3.4	13.7	3.7	<1.0	0.4	2.0	8.5	5.0
		PM-10	4.1	14.2	3.0	<1.0	0.2	1.0	9.0	5.0
	4/75	GF/C	10.0	26.5	3.5	<1.0	0.25	1.5	7.5	<5.0
		PM-10	9.5	19.5	2.2	<1.0	0.3	1.0	5.5	<5.0
		UM-05	4.6	21.5	2.3	<1.0	0.2	1.5	6.2	<5.0
	1/75	GF/C	11.0	14.1	7.5	1.0	0.4	2.0	30.0	<5.0
		PM-10	9.0	15.6	7.3	1.0	0.4	4.0	29.0	<5.0

* Duplicate Analyses

TABLE 6

Distribution of 'Soluble' Trace Metals in Carnon River Water after Ultrafiltration (1975)

SAMPLE LOCATION	DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
TWELVE HEADS	1/75	GF/C	5.0	405	100	7.0	1.2	10.0	50.0	<5.0
		PM-10	4.0	415	105	7.1	1.4	11.8	52.0	<5.0
	2/75	GF/C	7.0	500	61.0	10.0	1.6	17.0	65.0	<5.0
		PM-10	3.0	520	61.0	10.0	1.5	15.0	66.0	<5.0
	3/75	GF/C	5.0	380	51.0	12.0	1.0	13.0	58.0	<5.0
		PM-10	2.0	367	54.0	11.0	1.0	12.0	60.0	<5.0
	4/75	GF/C	2.5	380	19.5	7.5	0.9	11.0	61.0	<5.0
		PM-10	1.5	385	18.5	8.0	0.9	11.0	59.5	<5.0
		UM-05	2.0	375	18.5	8.0	0.85	10.0	59.0	<5.0
	1/75	GF/C	48.0	2610	530	38.0	6.2	56.0	430	<5.0
		PM-10	39.0	2600	510	35.0	6.0	61.0	450	5.0
	2/75	GF/C	250	3900	740	59.0	8.8	90.0	430	<5.0
		PM-10	260	4100	850	64.0	9.2	100.0	480	<5.0
BISSOE	3/75	GF/C	1750	3600	610	26.0	9.5	105	440	20.0
		PM-10	1680	3600	600	24.0	9.5	100	480	25.0
	4/75	GF/C	35.5	1800	175	30.0	3.4	47.0	450	<5.0
		PM-10	27.0	1750	150	25.0	3.5	42.0	450	<5.0
		UM-05	26.0	1800	130	28.0	3.9	45.0	445	<5.0

TABLE 6 (CONTINUED)

SAMPLE LOCATION	DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
BISSOE	6/75	GF/C	375	1350	145	25.5	2.25	42.5	650	6.5
		PM-10	350	1750	200	34.0	3.1	55.0	850	10.0
		PM-10*	340	1500	165	27.5	2.5	45.0	750	8.0
		UM-05	330	1760	196	33.0	3.4	54.0	830	10.0
DEVORAN	1/75	GF/C	7.0	2250	260	30.0	6.2	56.0	54.2	<5.0
		PM-10	4.0	2190	245	31.0	6.8	50.0	52.4	<5.0
	2/75	GF/C	11.0	2700	520	52.0	7.2	78.0	580	6.0
		PM-10	10.0	3200	520	51.0	6.5	75.0	550	<5.0
	3/75	GF/C	455	3620	560	24.0	8.7	85.0	460	7.0
		PM-10	425	3700	540	22.0	8.0	90.0	440	7.0
	4/75	GF/C	10.0	4550	180	40.0	3.5	120	500	<5.0
		PM-10	6.0	4850	150	27.5	3.7	140	480	<5.0
		UM-05	5.5	4750	180	30.0	3.7	140	465	<5.0
	6/75	GF/C	215	3000	380	43.5	4.25	750	1400	7.5
		PM-10	225	2550	330	51.0	4.45	80.0	1370	8.0
		UM-05	235	2600	365	52.0	4.75	80.0	1410	7.5
		UM-05*	237	3100	385	51.0	4.75	100.0	1300	7.5

* Duplicate Analyses

TABLE 7

Distribution of 'Soluble' Trace Metals in Fowey River Water after Ultrafiltration (1975)

SAMPLE LOCATION	DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
DOZMARY POOL	1/75	GF/C	87.0	7.6	1.6	<1.0	<0.1	3.0	4.0	<5.0
		PM-10	57.4	8.0	1.4	<1.0	<0.1	2.0	5.0	<5.0
	2/75	GF/C	40.0	9.0	1.4	2.0	0.6	2.0	3.0	<5.0
		PM-10	31.0	9.1	1.5	2.0	0.5	2.0	2.0	<5.0
	3/75	GF/C	43.0	12.5	2.0	1.0	0.3	3.0	5.8	<5.0
		GF/C*	41.5	11.7	2.0	1.0	0.2	3.0	6.2	<5.0
		PM-10	24.0	12.0	2.0	1.0	<0.1	3.0	6.4	<5.0
	4/75	GF/C	32.5	17.0	1.4	<1.0	0.4	1.5	17.5	<5.0
		PM-10	21.0	18.0	2.0	<1.0	0.2	2.0	16.0	<5.0
		UM-05	19.6	17.2	3.0	<1.0	0.2	1.0	17.7	<5.0
	6/75	GF/C	46.0	14.0	2.95	<1.0	0.2	<1.0	28.5	<5.0
		PM-10	21.5	15.0	3.0	<1.0	0.3	1.5	24.0	<5.0
		UM-05	16.0	14.5	3.3	<1.0	0.4	1.0	22.5	<5.0
EAST COLLIFORD	1/75	GF/C	100.0	11.9	1.4	2.0	0.7	3.0	8.4	<5.0
		PM-10	70.0	12.1	1.4	<1.0	0.8	3.0	7.7	<5.0
		PM-10*	63.4	12.5	1.5	<1.0	0.5	3.0	8.0	<5.0
	2/75	GF/C	22.0	9.4	1.5	2.0	0.2	1.0	15.0	5.0
		PM-10	17.0	9.3	2.3	2.0	0.3	2.0	16.0	<5.0

TABLE 7 (CONTINUED)

SAMPLE LOCATION	DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
EAST COLLIFORD	3/75	GF/C	13.0	6.4	2.4	1.0	0.1	3.0	8.4	<5.0
		PM-10	11.0	6.2	2.0	<1.0	0.1	1.0	11.0	<5.0
	4/75	GF/C	31.0	6.6	1.6	<1.0	0.15	2.0	25.0	<5.0
		PM-10	12.5	8.4	1.0	<1.0	0.10	1.0	22.0	<5.0
		UM-05	8.5	8.0	1.8	<1.0	0.20	1.0	21.0	<5.0
	6/75	GF/C	33.5	12.0	2.5	1.0	0.2	2.0	18.0	<5.0
		PM-10	18.0	11.0	3.0	<1.0	0.2	1.5	16.0	<5.0
		UM-05	15.0	12.0	2.0	1.0	0.1	1.5	16.5	<5.0
		UM-05*	16.2	12.3	1.0	1.0	0.3	2.0	15.9	<5.0
	1/75	GF/C	59.0	12.5	3.0	2.0	0.6	4.0	6.3	<5.0
		PM-10	55.0	13.4	3.4	1.0	0.4	1.0	6.5	<5.0
	2/75	GF/C	19.0	12.6	5.0	<1.0	0.5	4.0	6.2	<5.0
		PM-10	9.0	13.6	4.0	<1.0	0.3	2.0	6.2	<5.0
		PM-10*	8.6	13.0	5.5	<1.0	0.3	2.0	6.0	<5.0
QUARRY WOOD	3/75	GF/C	8.0	8.0	4.0	<1.0	0.2	2.5	2.0	<5.0
		PM-10	5.7	8.0	4.2	<1.0	0.2	3.6	3.1	<5.0

TABLE 7 (CONTINUED)

SAMPLE LOCATION	DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
QUARRY WOOD	4/75	GF/C	7.5	5.5	2.2	<1.0	0.15	1.5	1.5	<5.0
		PM-10	5.0	5.4	2.3	<1.0	0.10	1.0	1.5	<5.0
		UM-05	3.0	4.85	2.0	<1.0	0.10	1.0	1.5	<5.0
		UM-05*	4.5	4.7	2.1	<1.0	0.3	1.5	4.0	<5.0
LOSTWITHIEL	1/75	GF/C	49.0	10.0	2.5	<1.0	1.1	2.0	7.0	<5.0
		PM-10	35.0	12.0	3.1	<1.0	1.0	2.0	6.0	<5.0
	2/75	GF/C	11.0	16.4	3.0	<1.0	0.4	4.0	7.0	8.0
		PM-10	9.0	12.6	5.0	1.0	0.5	4.0	6.2	<5.0
		PM-10*	9.6	13.2	3.7	1.0	0.2	3.0	6.6	<5.0
	3/75	GF/C	7.0	22.0	2.0	<1.0	<0.1	1.0	5.6	<5.0
		PM-10	6.0	24.0	2.5	1.0	1.0	3.0	5.0	<5.0
	4/75	GF/C	8.0	15.5	2.0	<1.0	0.3	2.0	2.5	<5.0
		PM-10	4.0	8.2	1.75	1.0	0.1	1.5	2.5	<5.0
		UM-05	4.5	8.3	2.4	1.5	0.3	1.5	2.5	<5.0
CUTMADOC	1/75	GF/C	31.0	6.8	1.2	<1.0	0.5	3.0	8.0	<5.0
		PM-10	28.0	6.6	2.0	<1.0	1.8	2.0	7.0	<5.0
	2/75	GF/C	10.0	11.0	1.8	<1.0	0.2	1.0	9.0	<5.0
		PM-10	7.0	12.0	2.0	<1.0	0.6	3.0	8.0	<5.0

TABLE 7 (CONTINUED)

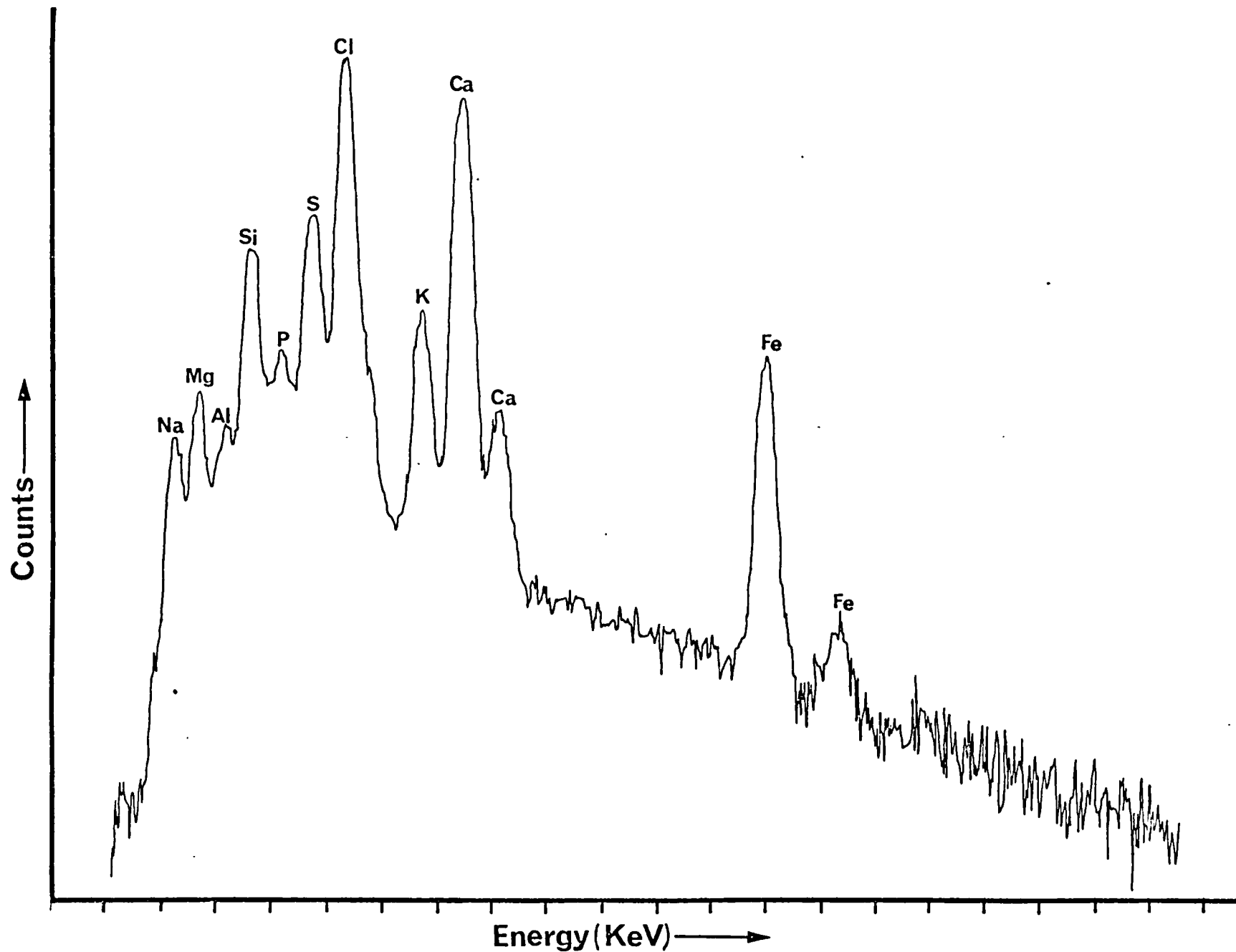
SAMPLE LOCATION	DATE	FILTRATE (FILTER TYPE)	CONCENTRATION ($\mu\text{g l}^{-1}$)							
			Fe	Zn	Cu	Co	Cd	Ni	Mn	Pb
WILAKE	1/75	GF/C	14.0	5.8	1.3	2.0	1.4	3.0	8.7	<5.0
		PM-10	9.0	7.2	1.0	<1.0	1.4	2.0	9.0	<5.0
	3/75	GF/C	4.0	3.5	2.0	<1.0	0.3	2.0	5.2	<5.0
		PM-10	4.0	3.3	1.0	2.0	0.4	3.0	4.5	<5.0
PENPOLL	1/75	GF/C	16.0	5.0	1.1	2.0	0.2	2.0	7.7	<5.0
		PM-10	10.0	7.1	1.4	2.0	0.4	1.0	6.1	<5.0

* Duplicate Analyses

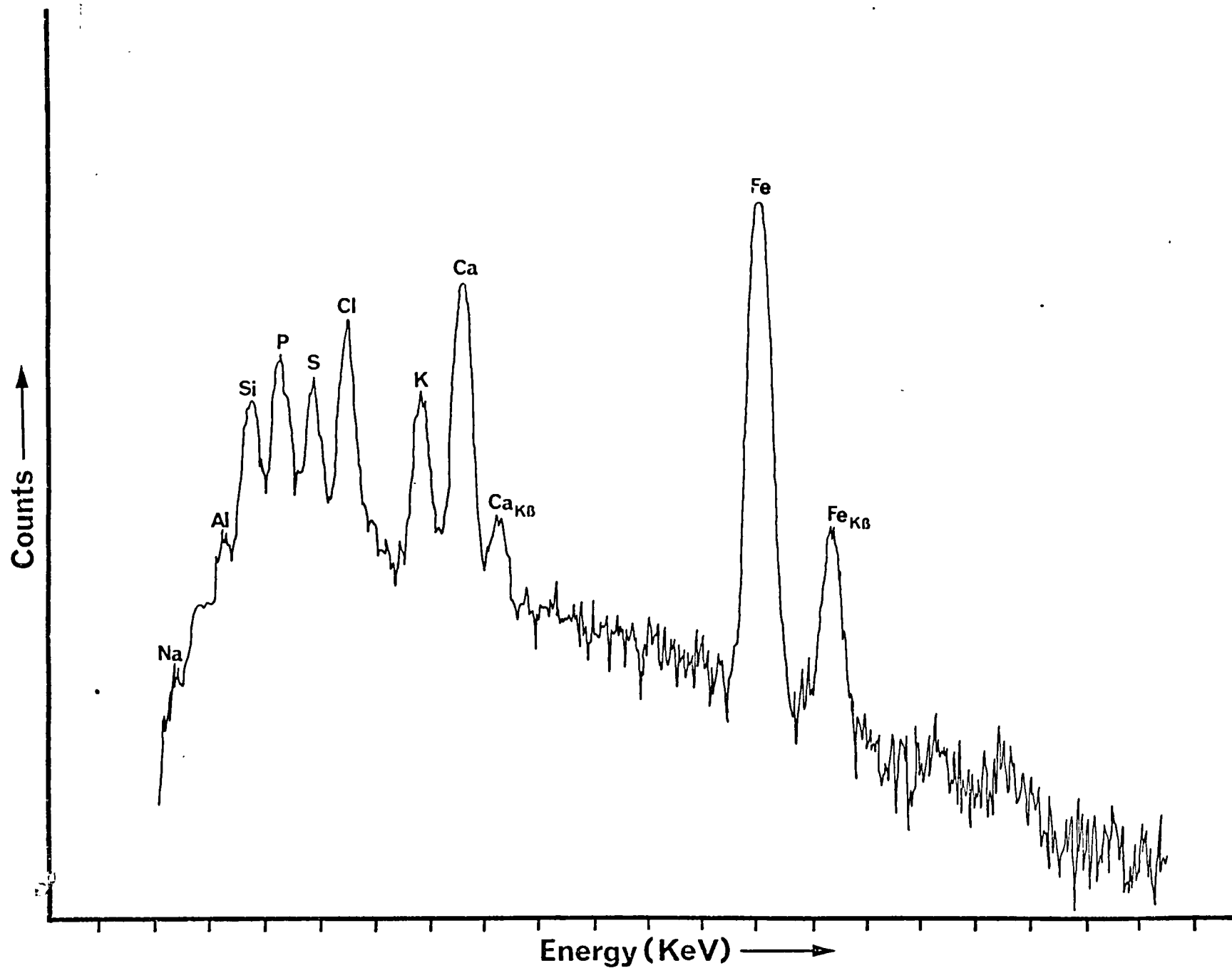
A P P E N D I X C

APPENDIX C

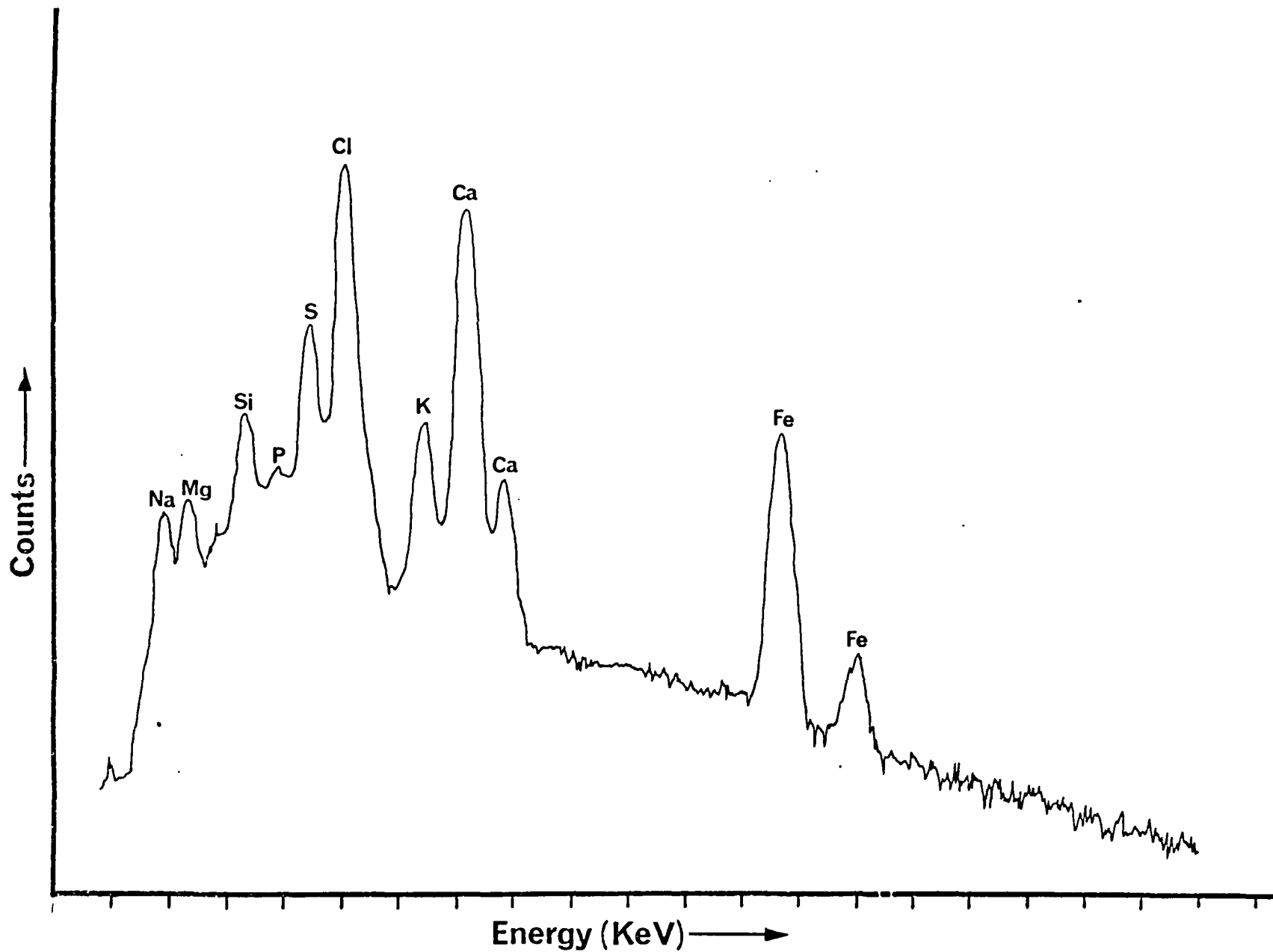
X-RAY EMISSION SPECTRA OF COLLOIDAL NON-DIALYZABLE MATERIAL
(NOMINAL MOLECULAR WEIGHT >10,000) FROM THE RIVER TAMAR



X-ray emission spectrum of typical colloidal material (Amicon PM-10 dialyzate) from Tamar River water (Gunnislake 21/8/74)



X-ray emission spectrum of typical colloidal material (Amicon PM-10 dialyzate) from Tamar River water (Launceston 21/8/74)



X-ray emission spectrum of typical colloidal material (Amicon PM-10 dialyzate) from Tamar River water (Gunnislake.23/7/74)