Determination of nutrients and heavy metal species in samples from Lake Maracaibo

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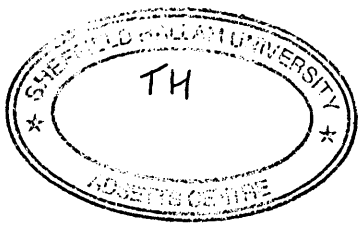
TH

Dedicated to my loved children Sabrina, Jose Alejandro, Samantha and Stefany and their future generations.....

I live with the hope that these lessons of the past and the present ones can avoid future environmental catastrophes.

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Abstract

In this study, a new analytical method for the simultaneous determination of total N, P and S using hydrogen peroxide oxidation has been developed for the analysis of water and sediment samples. The products of the oxidation reaction (nitrate, phosphate and sulphate) were determined by ion chromatography. A method for the simultaneous chemical speciation of arsenic, selenium and chromium was developed using ion chromatography coupled with ICP-MS. Reversed phase chromatography and ICP-MS was used for the simultaneous determination of mercury and selenium species. For the speciation of vanadium a new method using HPLC with reversed phase and ICP-MS detection was developed. The species arsenite, arsenate, selenite, selenate, chromate, methylmercury, inorganic mercury, selenocystine, selenomethionine, vanadium (IV) and vanadium (V) in samples of water, sediment, fish muscle tissue and mussel, were determined using the developed methods. Simultaneous determination of nutrients and metal species were applied to the study of pollution in Lake Maracaibo, Venezuela. The distribution of As, Se, Pb, Hg, Sn and V in sediment was studied using a sequential extraction scheme and related to the physicochemical parameters and nutrient content. The major concentrations of arsenic and lead found inside the lake were associated with the fraction associated with the Fe/Mn hydroxides phase, however, mercury and selenium were distributed at the main zone of the lake in the organic-sulphide fraction. In the strait and the gulf, metals were distributed mainly in the residual phase with the exception of tin. Conditions which favour mercury methylation in the lake are discussed. In the centre of the lake, with anaerobic conditions, methylmercury was the predominant species for mercury. The results found for vanadium and arsenic speciation showed that the predominant species in all the samples of Lake Maracaibo was vanadium (IV) and arsenite, respectively. Results were compared with those from lakes with similar pollution problems.

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CHAPTER I

Introduction

1.-INTRODUCTION

The large lakes in the world have been for many years prime resources. Industrial societies are very heavily water-dependent, and thus population densities and industralization are increasing rapidly on the shores of all large lakes of different latitudes. Effort at removing man-made pollution from this natural environment has not kept pace with the increasing amount of waste materials generated. As a result, man-made pollution has disrupted the natural biological balance in lakes. Two groups of substances in particular have lasting effects on the natural balance in aquatic systems: nutrients, which promote unrestricted biological growth and, in turn, lead to oxygen depletion, and synthetic chemicals and metals that are not eliminated from aquatic ecosystems by natural processes and in the most cases are concentrated through the food chain.

1.1.- Lakes and estuaries

Lakes are masses of waters situated in a depression of the ground without direct communication with the sea (1); these reservoirs are distinguished by water currents typically driven by the wind rather than by gravity. These water currents provide advective transport , generally turbulent, and chemical transport by turbulent diffusion. The pattern of water movement in a lake is also affected by the shape of the lake basin, by variations in water density, by inflow streams and by the Coriolis effect(2). Stratification divides lakes into different layers by inhibiting vertical mixing between the layers. Stratification occurs when the water at the bottom of a lake is denser than the surface water, and water currents are not strong enough to penetrate the boundary between the water layers. Such a density difference is usually due to temperature differences between upper and lower water masses;

the lake is then called thermally stratified. The upper layer, which is typically well mixed, is named epilimnion and the lower layer hypolimnion; the region which separates them is the thermocline. The thermal stratification is common in lakes located in climates with seasonal variations (temperate lakes).

Estuaries are mixing zones between freshwaters and seawaters (3). Water flow in estuaries is more complicated than in rivers and lakes; it is influenced by the inflow of fresh waters from rivers and streams, by tides of the sea, and by the large salinity, and hence density, difference between fresh and seawaters. The density difference tends to create a strong stratification, while the back and forth movement of water driven by tides enhances dispersion and mixing. Stratification in estuaries in some aspects is similar to stratification in lakes; the density difference in estuaries is due to the difference in salinity between fresh water and seawaters rather than temperature differences (2).

In the past decade, there has been a resurgence in the study of pollution in lakes, estuaries and wetlands which cover millions of km^2 of continental area. Lakes are now seen as major regulators in the carbon, nitrogen and phosphorus global geochemical cycles through various processes: sedimentation of detrital organic matter, production of autochtnonous organic matter, precipitation of carbonates, and precipitation of evaporates (4). From 1968 until now, the study of the Great Lakes (USA) has shown how pollution can affect large water bodies, and the steps to eventual restoration (5-8). Fresh water lakes such as Lake Alexandria (9), in the South of Australia, have provided historical information on changes in the N and C cycles. Other studies in the Szczecin Lagoon in the Southern Baltics (10) and Oder Estuary in Poland (11) have yielded valuable information about eutrophication. Eutrophication and subsequent lake-quality deterioration is already visible in many

countries, Lake Zurich in Switzerland which has been under scientific observation for over half a century, is the best example (12); eutrophication can damage many aspects of life including water supply, fisheries, bird life and public health.

The abundance of studies of temperate lakes throughout different seasons including summer, has produced results that have been compared to tropical lakes., however tropical lakes are very different during all parts of the year. Information about nutrients in temperate lakes can not be associated or extrapolated to tropical lakes, because of the fundamental differences in the physical and the biological dynamics of the two types of systems.

These fundamental differences between tropical and temperate Great Lakes have been reviewed recently by Hecky (13) with the following conclusions : tropical lakes have continuously high temperatures throughout the water column and high rates of annual photosynthesis under continuously high solar irradiance. These aspects not only lead to permanent stratification and hypolimnetic anoxia in the deepest tropical lakes, but also they have consequences for oxygen concentrations throughout the water column and can dramatically affect the biogeochemical cycles of carbon, nitrogen and phosphorus. Denitrification and enhanced regeneration of phosphorus from metal oxides cause low nitrogen:phosphorus ratios in the deep waters and create a nitrogen deficit when deep waters mix with surface waters, which is met through N-fixation. In Lake Malawi (a large African tropical lake), nitrogen has a residence time of 2 years while in dimictic Lake Superior, the nitrogen residence time is over 50 years. This disparity in the residence time indicates that nitrogen is poorly recycled to the mixed layer of Lake Malawi. The chronic anoxia of tropical lakes promotes the release of phosphorus bound to metal oxides and allows soil erosion to increase eutrophication .

1.2.-Lake Maracaibo

Lake Maracaibo, Venezuela is a tropical lake (mean temperature 30 °C); it is the largest lake in South America (8^{th} in the World), and covers an area of 13,010 Km^2 . Lake Maracaibo has been classified as of miscellaneous tectonic origin (>36 Ma) with a very ancient structure (14 -15); in this lake the sedimentation rate, of the order of 0.1-1 mm/year, is compensated by subsidence rate; otherwise it would have filled up over 0.1-1 Ma (4, 16). The Lake Maracaibo basin involves six states of the Republic of Venezuela with an area of 82,035 Km^2 (without taking into account the lake itself) and the Republic of Colombia with an area of 16,130 km^2. These basin waters flow into the Gulf of Venezuela. (17) Figure 1.3 shows the location of Lake Maracaibo in South America (Latitude 9°0'-11°0' North, Longitude 71°0'-72.0' West). Lake Maracaibo System which is loaded for 135 rivers with fresh water of 1,900,000 $L.sec^{-1}$ is formed by: the Venezuelan Gulf, the Tablazo Bay, the Strait and the Lake itself. The total extension of the system is 121,422 Km^2 of which 104,900 Km^2 belongs to Venezuela and 16,432 Km^2 belongs to Colombia because it is the born from Catatumbo River, the main water source of the lake (18). The area of the water mirror is 12,780 Km^2 with a volume of 280 Km^3. The lake is 152 Km long and 70 Km wide The mean slope is 0.8 %(19). The Strait covers an area of 479 Km^2, with 40 Km long and 14 Km wide; this Strait (and The Tablazo Bay) is an estuarine zone where there is a mix of fresh water from the lake and seawater from the Caribbean Sea through the Venezuelan Gulf.

Figure 1.1.: Location of Lake Maracaibo

The proximity of Venezuela to the Equator results in minimal annual temperature differences. The climate is predominantly tropical, with a warm zone extending along the coast. The climatic zones are defined by the rainfall rather than by differences in temperature. The dry season extends from December to April, and the wet season covers the remainder of the year. The mean temperature in Lake Maracaibo varies between 29-32 ^{0}C. Figure 1.2 shows a map of the currents inside the lake and the strait. During the dry

season the salinity of the lake increases significantly, but during the wet season (April to December) the water flows from the rivers to the cone (centre of the lake) and maintains an anticlockwise circulation. At the centre, the salinity varies between 4.2 $g.L^{-1}$ and 5.2 $g.L^{-1}$ (17). The concentrations of total phosphorus during the dry season varies between less than 0.05 mgL^{-1} to more than 0.12 mgL^{-1} and in the wet season from less than 0.06 mgL^{-1} to more than 0.12 mgL^{-1}; the concentration of total nitrogen varies during the dry and wet seasons, (between 0.5 and 1.21 mgL^{-1}) but in different sites of the lake(17).

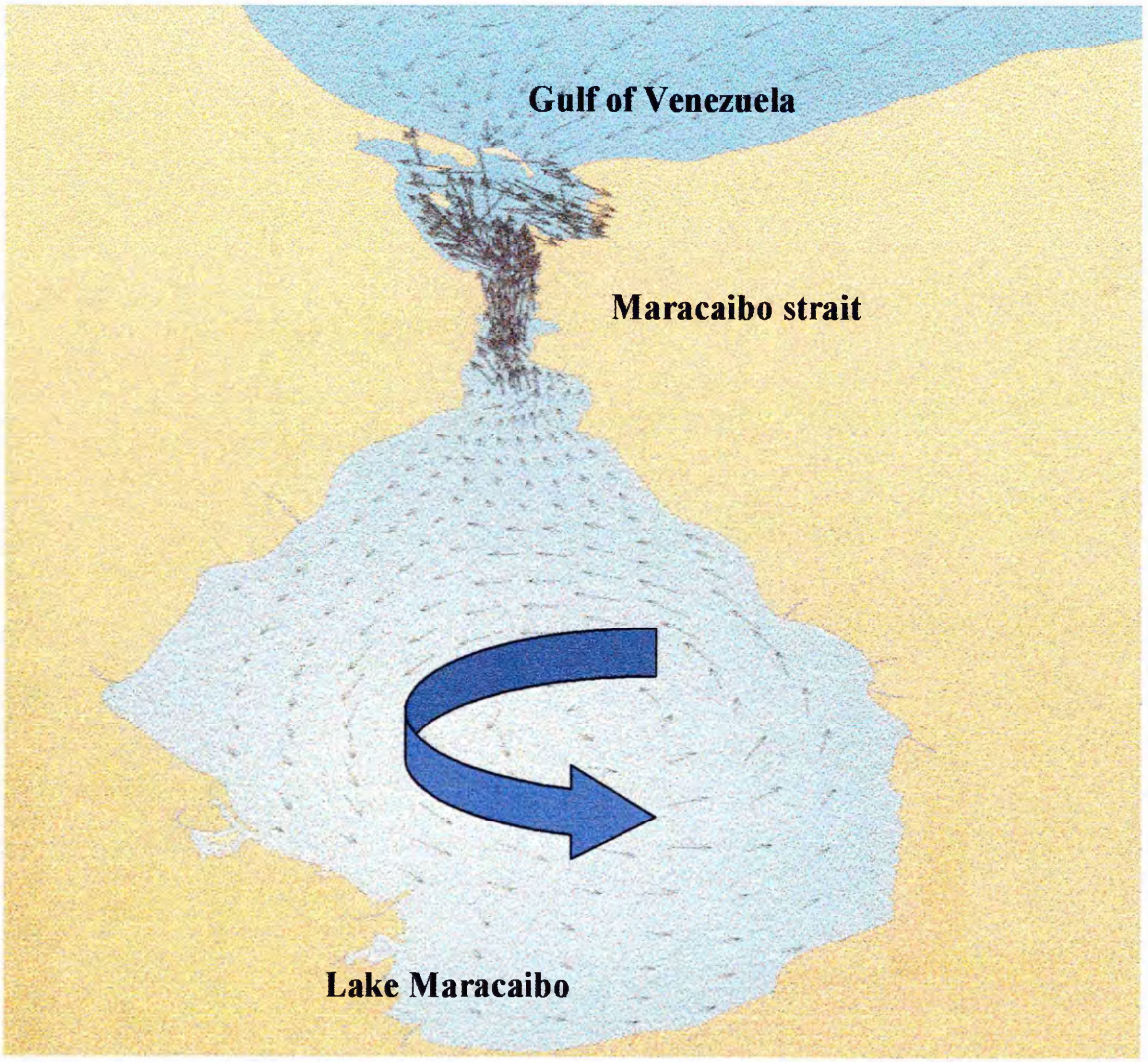


Figure 1.2. The map of water currents inside Lake Maracaibo

70% of the Gross National Product of Venezuela comes from the petroleum extracted from zones lying under Lake Maracaibo, the Misoa Formation sands, which produce basically light crude oils (24-40 ^{0} API)(20), thereby creating a heavy traffic in tankers in and out of the lake. In spite of this, an annual maintenance dredging in Lake Maracaibo has been carried out by the INC (National Institute of Channels) since 1938. This institute was formed to manage a dredging program to maintain a navigable channel in Lake Maracaibo of an average depth of 11m. The maximum normal depth is 32 m and the mean depth is 20 m. Over the last 40 years, the salinity of the water has increased by about 300 % as a result of dredging, which has altered the nature of interaction between the lake and the Caribbean Sea. The lake is surrounded by about 2,000 industries sites and 10,000 oil drilling platforms are situated near to its centre. A growing open coal industry near to the Guasare River (an inflow) and the transport through the lake (non-point contamination source) could also affect the concentrations of As, Hg, V and Pb . In addition Lake Maracaibo receives loads of nutrients from tributaries, sewage discharges, and agricultural sources. Phosphorus mining in the mountains around the lake contributes to the tributary phosphorus loading to the lake. The city of Maracaibo, with population of 3 million people discharges raw sewage directly into the Strait of Maracaibo located on the northern part of the lake. Moreover, the constant dredging of the lake maintain the sediments in a constant re-suspension and open to different reactions with the nutrients that can increase eutrophication and the availability of heavy metals which can increase the concentrations of the most dangerous toxic species.

1.3.- Eutrophication

The term eutrophication describes the condition of lakes or reservoirs involving excess algal growth caused by high nutrient content. The main cause of eutrophication in a water body is the presence of nutrient excess from watershed runoff or sewage. As a result, the dead biomass is accumulated at the bottom of the water body, recycling nutrient carbon dioxide, phosphorus, nitrogen, and potassium. The growth of plants is accelerated, leading to solid material (21). Where river loads of nitrogen (N) and phosphorus (P) have increased (22), eutrophication poses a threat to the quality of reservoir water used for potable supply, especially during the summer months (23)

A number of lakes and lagoons world-wide have been studied, for example the Szczecin Lagoon (Poland), Bodensee (Germany), Zurich (Switzerland) , Laurentian Great Lakes in North America (24) and Lake Biwa in Japan (25). Sediment investigations in Szczecin Lagoon have revealed that 100,000 tons of nitrogen and about 30,000 tons of phosphorus have been retained in the lagoon during the last 100 years (26), The Saginaw Bay in Lake Huron (Michigan), now being remediated , has a loading of phosphorus of 1,544 metric tons per year (27).

Table 1.1 (28) shows the chemical elements needed for plant growth. The eutrophication or enrichment process has been described as a natural process of ageing of a lake (21); the activity of man in the catchment area of lake waters gives rise to domestic, agricultural and industrial wastes and as a consequence the relatively slow process of natural eutrophication is greatly accelerated; thus what might have occurred in a period of thousand years can happen in a few decades.

Table 1.1.: Essential plant nutrient sources and functions(28)

Nutrient	Source	Function and/or constituent
Macronutrients		
Carbon (CO_2)	Atmosphere, decay	Biomass constituent
Hydrogen	Water	Biomass constituent
Oxygen	Water	respiration
Nitrogen (NO_3^-)	Decay, atmosphere (from nitrogen-fixing organisms), pollutants	Protein constituent
Phosphorus	Decay, minerals, pollutants	DNA/RNA constituent
Potassium	Minerals, pollutants	Metabolic function
Sulphur (sulphate)	Minerals	Proteins, enzymes
Magnesium	Minerals	Metabolic function
Calcium	Minerals	Metabolic function
Micronutrients		
B, Cl, Co, Cu, Fe, Mo, Mn, Na, Si, V, Zn	Minerals	Metabolic function and/ or constituent of enzymes

There is a basic relationship between the tropic or nutrient state of a lake and its biological productivity, the increase of which is a function of the nutrients available, and is evidenced by a change in composition and an increase in amount of plankton, benthic fauna and fish production (21). The nutrients which play the predominant role in the phenomena are nitrogen and phosphorus.. Many other substances including potassium, magnesium, sulphates and trace elements (Co, Mo, Cu, Zn, B, Fe, Mn, etc), together with organic growth factors, are also of importance.

The cycling of nutrients has been entensively studied in large temperate lakes, but this is not the case for the large tropical lakes, except for studies by Kilham and Kilham (29) and Lewis (30), the study of Lake Calado, Brazil by Fisher et al (31) and a study from the 80's by Parra-Pardi (32) of Lake Maracaibo, Venezuela. There is a recent paper about nitrogen cycling rates in this lake by Gardner et al (33).

1.3.- Heavy metals

It is well known that the major ions such as sodium, potassium, magnesium and calcium are essential for biological life. For several decades, it has been known that trace quantities of certain elements exert a positive influence on plant, animal and human life. There are at least six transition (Co, Mo, Cu, Zn, Fe, Mn, V) metals that are essential to the growth, development and reproduction of humans. The other elements that do not have a identifiable beneficial biological function are referred to as non essential.

The biogeochemical cycle of a metal is used to understand the possible short and long term problems associated with the release of heavy metals in to the environment . In particular the following problems need to be understood : (a) the physico-chemical forms in which heavy metals can exist in the environment , (b) the processes responsible for transporting the metals through the system, (c) the processes by which the metals are transformed from one compound to another , and (d) the most important pathways by which the trace metals interact with the biota. A conceptual model for an aquatic system has been developed by Hart (34), consisting of a number of compartments or reservoirs coupled by transfer pathways. A heavy metal tends to accumulate in the bottom and surface sediments from which it is released by various physical processes, Figure 1.3. shows a biogeochemical cycle for a lake (34). The system consists of four compartments: (a) the dissolved compartment containing free metal ions, complexed and colloidally- bound metal species; (b) the (abiotic) particulate compartment consisting of both inorganic and organic particulates; (c) the (biotic) particulate compartment consisting mainly of phytoplankton (and bacteria) in lakes and the deep ocean, littoral areas in

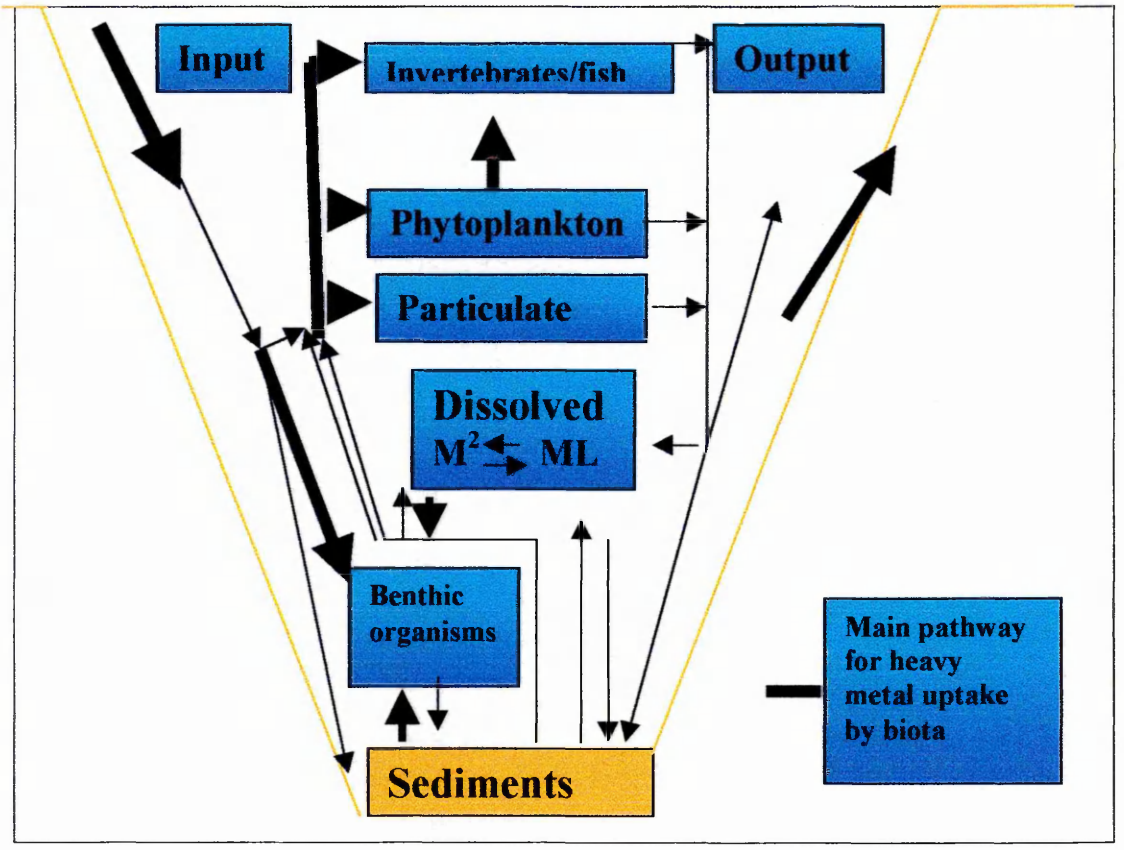


Figure 1.3.: A simplified biogeochemical cycle for a heavy metals in an aquatic system (34).

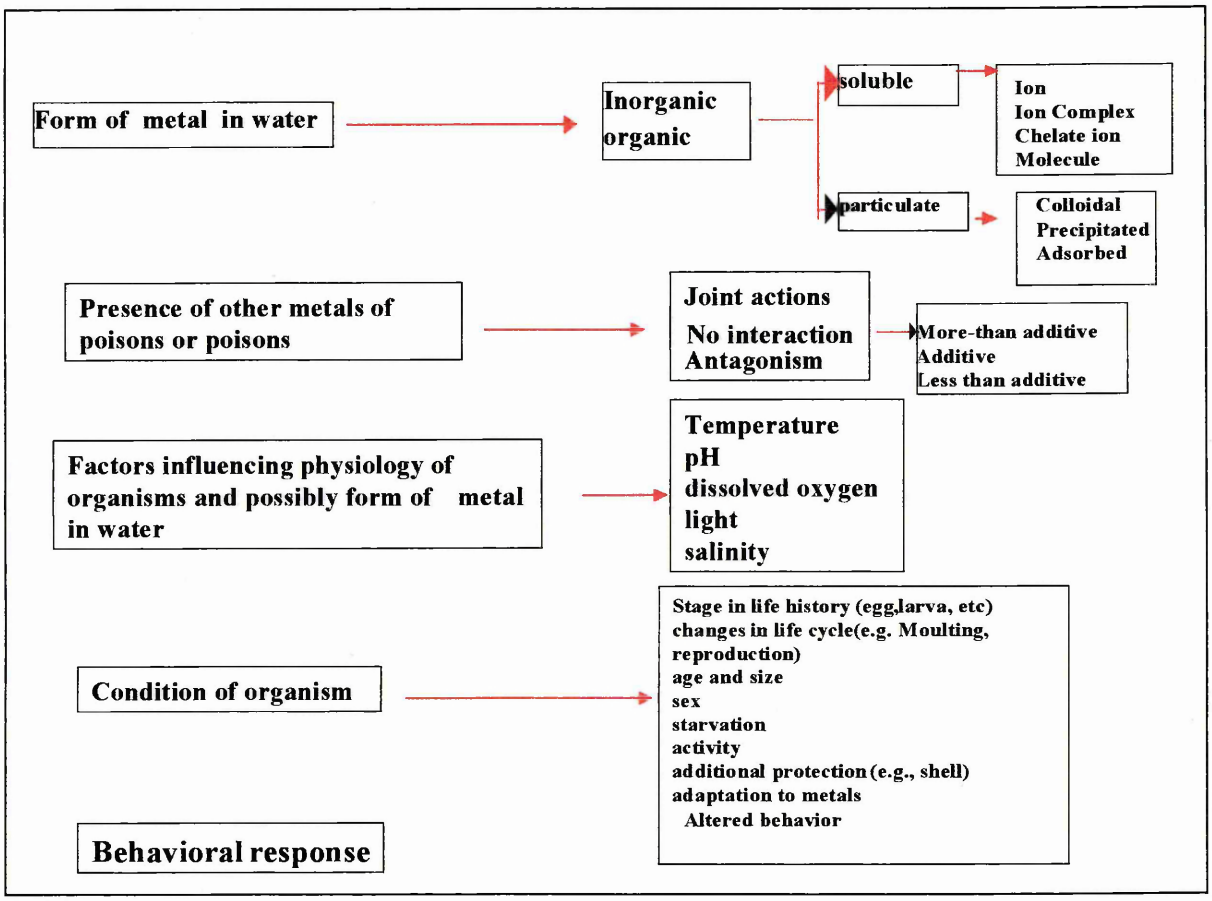
estuaries and attached plants in streams; and d) bottom sediments, the largest reservoir of heavy metals in most aquatic systems.

The transfer processes of heavy metals between compartments have been discussed in detail by Hart (1982)(34), Salomons and Förstner (1984)(35), Tessier (1992) (36) and others (37-38).

Metals can occurs in various chemical forms resulting from a series of natural bio-(geo)chemical processes. These chemical forms have distinct biological, physical, and chemical properties.

The factors that influence the toxicity of heavy metals in aquatic organisms have been compiled by Bryan (39), as is summarised in Table 1.2. Factors such as temperature, pH and salinity have an influence on the metal toxicity.

Table 1.2: Factors influencing the toxicity of heavy metals in solution (39).



Usually organometallic compounds are much more toxic than ions of the corresponding inorganic compounds. Mercury, lead and tin follow this general rule, whereas arsenic and selenium represent exceptions because most organo-arsenicals are less toxic than inorganic arsenic species, and organic forms of Se are ordinarily less toxic than Se(VI)(40). The toxicity of compounds varies in relation to the compound, e.g., for tin mono- and dialkylated species are less toxic than trialkylated ones. The toxicity of the organometallic

species also varies with the organism monitored. For example, trimethyltin is more toxic for insects, triethyltin for mammals, and tributyltin for fish, fungi and bivalves (41).

The rate of absorption of a metal from solution, or indeed from food, is governed by its chemical form.. Studies of toxicity (24h., LC_{50}), made by O'Hara (42) in crabs, demonstrated that the toxicity of Cd is least at low temperature coupled with high salinity, and greatest at high temperature coupled with low salinity .

D. Boening (43) reported that in aquatic matrices the toxicity of mercury in marine invertebrates, fish and marine mammals is affected by temperature, salinity, dissolved oxygen and water hardness.

1.3.1.Temperature

Most sorption processes of inorganic elements possess negative enthalpy (e.g. are endothermic) (44). Temperature control should always be exercised and reported and may be systematically varied to assess certain thermodynamic properties of sorption reactions.

The observation that heavy metal toxicity increases with higher water temperatures (44) can be explained by elevated respiratory activity. Moreover , the metal solution itself causes increased respiratory activity. The absorption and the release of metals can also depend on temperature. This has been established for mercury, methylmercury, and phenylmercury acetate using rainbow trout (45-46).

Temperature differences influence the mixing of water masses in estuaries. Resistance to mixing in an even partially stratified estuary is proportional to density differences between the water masses; under isothermal conditions, these differences are usually produced by salinity differences, the lower mass being denser and more saline. Differential warming of the upper layer or chilling of the lower can lead to increased stratification and mixing

resistance (3). Although, estuarine dissolved oxygen levels are normally near to saturation, the occasional occurrence of a high summer temperature can result in low oxygen levels producing anaerobic conditions. The reduction of bicarbonate to methane and sulphate to sulphite mark the limit of pε (the hypothetical electron activity) attainable by microbial redox processes. It can be sufficiently low to reduce As(V) to As(III) or Hg(II) to Hg(0), for example, which are species which have differences in toxicity.

1.3.2.- Dissolved Oxygen

The most important oxidizing agent in natural waters is dissolved molecular oxygen, O_2, Upon reaction, each of its oxygen atoms is reduced from the zero oxidation state to the –2 state in H_2O or OH^-. The half reaction that occurs in acidic solution is:

$$O_2 + 4\,H^+ + 4\,e^- \longrightarrow 2\,H_2O$$

in basic aqueous solution it is

$$O_2 + 2\,H_2O + 4\,e^- \longrightarrow 4\,OH^-$$

Because the solubilities of gases increase with decreasing temperature, the amount of O_2 that dissolves at 0°C (14.7 mg/L) is greater than the amount that dissolves at 35 °C (7.0 mg/L)(47). The mean concentration of oxygen in unpolluted waters is about 10 mg/L. The most common substance oxidized by dissolved oxygen in water is organic matter. Similarly, dissolved oxygen in water is consumed by oxidation of dissolved ammonia and ammonium ion. Water that is aerated is constantly replenished with oxygen; however, stagnant water or that near the bottom of a deep lake is usually almost completely depleted of oxygen because of its reaction with organic matter. Since anaerobic conditions are reducing in the chemical sense, insoluble Fe^{3+} compounds that are present in sediments at

the bottom of lakes are converted into soluble Fe^{2+} compounds which then dissolve into lake water:

$Fe^{3+} + e^- \longrightarrow Fe^{2+}$

It is not uncommon to find aerobic and anaerobic conditions in different parts of the same lake at the same time, particularly in the summertime, when a stable stratification of distinct layers often occurs. Water at the top of the lake is warmed by absorption of sunshine by biological materials, while below the level of penetration of sunlight remains cold. Thus conditions in the top layer are aerobic, and consequently elements exist in their most oxidized forms: carbon as CO_2 or H_2CO_3 or HCO_3^-, sulphur as SO_4^{2-}, nitrogen as NO_3^-, and iron as insoluble $Fe(OH)_3$. In the bottom, under anaerobic conditions elements exist in their most reduced forms: carbon as CH_4, sulphur as H_2S, nitrogen as NH_3 and NH_4^+, and iron as soluble Fe^{2+}.

As a result of physiological changes in the organism, these two parameters can, due to chemical processes in water and sediment (e.g., oxidizing-reducing environment) decisively influence heavy metal availability. Thus, the concentration of heavy metals in interstitial waters with anaerobic sediments can be up to 10 times higher than in supernatant water (48).

1.3.3.- pH

pH values play an important role in the interactions between heavy metals and species such as organic compounds. For surface coordination reactions of metallic cations and oxyanions on hydroxylated mineral surfaces, pH is the master variable. The strong pH dependency of adsorption reflects solution hydrolysis or protonation of adsorbing ions, and more importantly, the surface properties of the adsorbent (49).

Metal pollution introduces excessive quantities of certain Lewis acidic metal ions to ecosystems. The biological consequences of metal pollution strongly depend on the resulting chemical species, which is a function of the kind and amounts of Lewis bases, the redox potential and the acidity-alkalinity (pH) characteristics of particular environments. In the biota, the Lewis bases include ligands (in solution as well as on particle surfaces) containing oxygen donors (e.g., -OH; RCOOH); sulphur donors (e.g., -SH), or nitrogen donors (e.g., -NH; $-NH_2$) which can be coordinated to trace metals (e.g., Mg, Mn, Fe, Co, Zn) (50). It is very important to recognize and make use of the general pattern of chemical affinities between Lewis acids and bases, i.e., the position of equilibria for all reactions involving metal coordination.

$$M_i + L_j \rightleftharpoons M_iL_j$$

The redox state of the electron acceptors, M_i, and of the donors, L_j, strongly affects the affinities (e.g., Fe^{+3} vs Fe^{+2} and SO_4^{2-} vs S^{2-})(4). The proportion of free metal ions increases with lower pH, because H^+ ions compete with metal ions for the available ligands:

$$CO_3^{2-} + H^+ = HCO_3^-$$

$$RCOO^- + H^+ = RCOOH$$

$$MeO^- + H^+ = MeOH$$

$$MeOPb^+ + H^+ = MeOH + Pb^{2+}$$

The heavy metal concentrations in acidic lakes, even those with significant smaller pollutional metal input, are much larger than those measured in eutrophic lakes, because metal binding by sorption to particle surfaces decreases with decreasing pH (51).

Also, the toxicity of some heavy metals can increase at basic pH values, for example lead was found to be more toxic at a pH value of 8.5 than at pH 6.5 (52). With respect to organic

substances, changes in the pH values in the water can strongly influence the adsorption or desorption of cations. For example, amino acids, in both water and sediments , can adsorb or desorb cations due to the pH-dependent amphoteric character of the acids.

1.3.4.- Salinity

Salinity is a conservative variable. It offers a convenient index of strength of the buffer systems (carbonate and borate) in seawater. Also, salinity is an indication of the osmotic environment for living organims. In general, salinity in the marine environment is relatively constant and has little influence on heavy metal concentrations. In estuaries, where fresh- and salt-water mix, salinity, however, plays a dominant role in influencing metal concentrations in water.

The salinity gradient in an estuary has several effects on chemical fate and transport. As salinity increases in the region where fresh and salt waters meet, particles brought in by fresh waters tend to stick together (coagulate) and thus settle to the bottom more rapidly. Rising salinity also affects the activity of dissolved ionic chemical species due to increasing ionic strength, thereby changing the position of chemical equillibria in the water. Oxidation/ reduction reactions are affected because oxygen is less soluble in saline waters (1).

In sea water, the concentrations of dissolved heavy metals are generally much lower than in fresh water. Moreover, the high salt content alters the pH- and consequently the metal solubility (fresh water environment pH 7–7.5, marine environment approx. 8.0)(53).

1.4.-Chemical speciation

Chemical speciation as defined by Caroli (54) is "the process yielding evidence of the atomic or molecular form of an analyte". This statement can accommodate both organic

and inorganic substances. The International Union of Pure and Applied Chemistry (IUPAC)(55) defined speciation analysis as "the analytical activity of identifying and measuring species". It is also part of this term to indicate the distribution of species in a particular sample or matrix. The chemical speciation determines the environmental mobility of an element, especially with respect to partitioning between the water and sediment reservoir.

Historically, a general scheme of metal speciation-mainly based on the particle size fractions was introduced by Stumm and Bilinski (1972) (56). A method involving the separation of particulate from soluble metals using filtration through a 0.45 μm pore size membrane filter was developed by Guy and Chakrabarti, (1975)(57).

Following his observations on particulate substances from the Amazon and Yukon Rivers, Gibbs (1973)(58) suggested four categories of heavy metal retention in aquatic solid substances. They can be characterized by the following processes: adsorptive bonding (2), coprecipitation by hydrous iron and manganese oxides, (3) complexation by organic molecules, and (4) incorporation in crystalline minerals.

The concentration of an element in the aqueous phase associated with a sediment is controlled by the formation of well-defined, poorly soluble compounds of the element but this is dependant on the interaction of the dissolved species with the solid/water soluble sediment and particulate phases by adsorption or coprecipitation (59). Metals can be adsorbed at particulate surfaces, occluded in amorphous material, or be present in the lattices of minerals, with each form exhibiting different chemical properties (60).

Based on these mechanisms, **operationally defined speciation** that involve the use of single or sequential extractants to separate species associated with particular sediment

phases has been developed since 1973. The nature of the fraction is largely determined by operational conditions. Ure et al (59) compiled in a table (Table 1.3) the single extractants designed to isolate a particular species , these extractants can, to some extent extract other species.

Table 1.3: Different extracting reagents or procedures and the soil/sediment phase isolated(59).

Phase extracted	Reagent
Water-soluble Soil solution Sediment pore water	Water Centrifugation Displacement Dialysis
Exchangeable	1 mol.L^{-1} $MgCl_2$ 1 mol.L^{-1} NH_4Oac 0.05 mol.$L^{-1}$$CaCl_2$ 1 mol.L^{-1} KNO_3
Organically bound	0.1 mol.L^{-1} $Na_4P_2O_7$ 0.7 mol.L^{-1} NaOCl 0.05 mol.L^{-1}EDTA H_2O_2/ HNO_3/NaOAc
Carbonate	HOAc NaOAc pH=5 EDTA
Mn oxide bound	0.1 mol.L^{-1} NH_2OH. HCl
Fe oxide bound	Dithionite/citrate
Mineral lattice	HF

An improvement of the single extraction scheme was developed by combination of single extractants into a sequential extraction scheme in which the residue of one is extracted by the next extractant in sequence to specifically dissolve different sediment phases or fractions. The use of sequential extraction procedures is justified for its ability to extract metal species from particular soil or sediment phases. The single step methods are usually used to determine mobility of metals in soils and sequential methods are more commonly used for sediments.

Förtsner et al (61) were one of the first groups to propose a 5 -step procedure to isolate the individual fractions. The five fractions were identified as cation exchange, the easily reducible, the moderately easily reducible, the organic fraction and the detrital fraction respectively. Tessier (62) developed a procedure in which the first fraction is represented by the exchangeable metals, and those nominally associated with carbonate, Fe-Mn oxides, organic material and silicate residues were extracted with magnesium chloride, sodium acetate-acetic acid, hydroxylammonium chloride, hydrogen peroxide, and hydrofluoric – perchloric acid, respectively. A number of sequential extraction procedures based on the Tessier method (63-68) have since been developed . Table 1.4 (59) show the methods commonly used for the study of sediments.

It is generally recognised that most extraction schemes are less than perfect, i.e. few extractants can be relied on to release elements solely from a particular phase. In addition, redistribution between phases can occur during the sequential procedure (69). Despite these limitations, these methods provide useful diagnostic information on which environmental impact decisions can be based. Furthermore, they provide a reference method which laboratories world-wide can use for comparisons.

As part of a recent attempt to harmonize methodology for leaching/extraction tests throughout the European Community, the BCR has developed a three-step extraction protocol (73) in which metals are divided into acid soluble/exchangeable, reducible and oxidisable fractions. The method is reproducible and gives good recoveries with respect to acid dissolution (70). However, it lacks specificity and in this respect it is similar to other older schemes. The uncertainty of this method has been studied by Sahuquillo et al (74)

Table1.4: Some examples of sequential extraction schemes(59).

Ref. (70) (Miller et all)		**Ref (71) Shuman**	
Extractant	Metal phase	Extractant	Metal phase
H_2O	Soluble		
1 mol.L^{-1} KNO_3	Exchangeable	1 mol.L^{-1} $MgNO_3$	Exchangeable
0.05 mol.L^{-1} NH_4F	Adsorbed		
0.1mol.L^{-1} $Na_4P_2O_7$	Organic	0.7 mol.L^{-1} NaOCl	Organic
0.01mol.L^{-1} $NH_2OH.HCl$	Mn Oxide		
Citrate/dithionite/bicarbonate	Fe Oxide	0.2 mol.L^{-1} NH_4Ox, pH =3	Amorphous Fe oxides
		0.2 mol.L^{-1} NH_4Ox + 0.1 mol.L^{-1} ascorbic acid	Crystalline Fe oxides
1 mol.L^{-1} HNO_3	Precipitated		
Conc. HNO_3	Residual	0.11mol.L^{-1} $Na_4P_2O_7$.10 H_2O	Sand, Silt, Clay
Ref(63)(Tessier et al)		**Ref (72) (Salomons and Forstner)**	
1 mol.L^{-1} $MgCl_2$	Exchangeable	1 mol.L^{-1} NH_4Oac	Exchangeable
1 mol.L^{-1} NaOAc/HOAc pH=5	Carbonate	0.1 mol.L^{-1} NaOAc/HOAc pH=5	Carbonate
0.04 mol.L^{-1} $NH_2OH.HCl$/ 25 % HOAC	Fe/Mn Oxides	$NH_2OH.HCl$/0.01 mol.L^{-1} HNO_3	Easily reducible Mn Oxide/ Amorphous Fe Oxides
		0.2 mol.L^{-1} NH_4Ox pH=3	Moderately reducible Amorphous Fe oxides
30 % H_2O_2/HNO_3 pH=2 then 3.2 mol.L^{-1} NH_4Ac/ 20 % HNO_3	Organic + Sulfide	30 % H_2O_2/HNO_3 pH=2 then 3.2 mol.L^{-1} NH_4Ac/ 20 % HNO_3	Organic + Sulfide
Conc HF/$HClO_4$	Residual	HF +$HClO_4$	Residual

showing that modification of certain variables is necessary. The pH, type of acid used for adjustment of pH, the temperature and duration of the extraction can influence the metal extractibility.

Chemical speciation could also be based on the identification of **well-defined molecular or atomic structure.** This is achieved by means of advanced chromatographic or electrophoretic techniques which are usually coupled to element specific detectors. The successful approach depends on two factors: selectivity (to determine the proper species

required) and sensitivity (to determine the species at the detection range of the sample). Advanced hyphenated techniques have recently been subject of a book and several reviews (55, 75-81) with emphasis on the interface and the detection (42, 82-83). The element-selective detectors are normally atomic absorption (84-86), atomic emission (87-89), atomic fluorescence (90), mass spectrometry (91-93), inductively coupled plasma (ICP) (94-100), microwave plasma (101), glow discharge (GD) (102). Neutron Activation Analysis (103) or coupled techniques such as ICP-MS (104-112), hydride generation(HG) with fluorescence (113), HG and ICP-MS (114), GC-ICP-MS (115), etc. have also been used. ICP-MS has proved to be a convenient technique for this task and has qualities such as capacity for simultaneous, rapid and precise determination with wide analytical range and low detection limits which allow it to compete successfully with other techniques (116-118). Chromatograpic techniques are used to separate the species according to the nature of the mobile phase in gas, supercritical fluid and conventional liquid chromatography (119-122). The separation mechanism (adsorption, reversed phase, partition, ion-exchange, size exclusion)(123-128), the column used (open, tubular or packed), operational mode (elution, displacement, counter-current) and filling (free, flow or gel) have been studied and used, depending on species to be determined.

1.5.- Aims

New analytical methods that can be used to determine simultaneously various elements are preferred because of reduced cost and analysis time. Most of the techniques used for determination of nutrients such as nitrogen, phosphorus and sulphur are time-consuming and use a lot of reagents, sometimes the use of quite toxic chemicals is also necessary. In this study, a new analytical technique for the simultaneous determination of total nitrogen, phosphorus and sulphur using hydrogen peroxide and ion chromatography has been developed to analyse environmental samples of water and sediment. The products of the oxidation reaction with hydrogen peroxide (nitrate, phosphate and sulphate) have been determined by ion chromatography. However, these products of oxidation can be determined by other detection techniques such as capillary electrophoresis, and traditional wet determination methods available in many laboratories.

In addition, methods for simultaneous determination of metal species have been developed:

1) A method for the simultaneous chemical speciation of arsenic, selenium and chromium has been developed using ion chromatography coupled with ICP-MS. The species arsenite, arsenate, selenite, selenate and chromate can be determined with the developed method in samples of water, sediment, fish muscle tissue and mussels.

2)A method for the simultaneous determination of mercury and selenium species has developed using reversed phase chromatography and ICP-MS. Methylmercury, inorganic mercury, selenocystine and selenomethionine can be determined by this method in samples of sediment, fish muscle tissue and mussel.

3) A new method for the speciation of vanadium has been developed with using HPLC with a reversed phase and ICP-MS detection. Vanadium (IV) and Vanadium (V) can be determined in samples of sediment, fish muscle tissue and mussel using this method.

Only a few papers have been published about nutrients in Lake Maracaibo, these being mainly the work of Parra-Pardi in 1983 (32) and a more recent paper published in 1998 (33). Other publications have been concerned with the geological origin of Lake Maracaibo (14-16, 20). Two papers by Colina and Romero (129-130) about the total mercury concentration in sediments and fish muscle tissue and organs of biological indicators from Lake Maracaibo. Information on the nature of anions in waters and air also has been published (131-132). In this thesis, two sampling campaings were done in Lake Maracaibo and physicochemical parameters measured *in situ* during these samplings. Background concentrations of metals were determined using X-ray fluorescense spectrometry and the total content of the major cations and trace elements by standard methods; all the methodologies presented for simultaneous determination of different nutrient and metal species were applied to the study of this lake. The results have been discussed in order to determine the effects of the excess of nutrients, the aging state of the lake and the metal pollution The distribution of arsenic, selenium, lead, mercury, tin and vanadium in sediment was studied by a sequential extraction scheme and related to the physicochemical parameters and nutrients. Conditions which favour mercury methylation in the lake are discussed. All the results were compared with other lakes that have similar problems of pollution.

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CHAPTER II

Sample collection and pre-treatment

2.1.- INTRODUCTION

2.1.1. Sampling strategy:

Sampling is defined as the total activity which end with the acquisition of the test portion (actual subject of analysis)(1). Sampling starts by taking an increment (an individual portion of material collected by a single operation of a sampling device). The collection of one or more increments or units initially taken for a population represents a primary sample which on division and reduction gives rise to a reduced sample of a mass approximating that of the final laboratory sample. The final step of sampling consists of the selection, removal and preparation of analytical portions from the laboratory sample.

An important requisite for a test sample is to be homogeneous. The homogeneity denotes the degree to which a constituent is uniformly distributed throughout a quantity of material. The degree of heterogeneity is the determining factor of sampling error. In addition, the test sample should be representative, i.e., adequately represents the population of material from which it was taken. The sampling strategy should include a predetermined procedure for the selection, withdrawal, preservation, transportation and preparation of the portions to be removed from a population as samples. This strategy is usually done using a statistical sampling plan to minimize the difference between the properties from the original sampling site and the actual properties of a sample portion.

During sampling, precautions to avoid the change of the characteristics of the sample (contamination, moisture loss or gain) should be considered, particularly during collection and storage.

In this study, two sampling campaigns were made during November 1998 to March 1999 on the sampling points that have been previously established and used for the

ICLAM (The Institute for the Conservation of Lake Maracaibo) during 10 years. These sampling sites have been chosen using a statistical plan following the COVENIN norms (Government of Venezuela accreditation scheme for analytical procedures).

2.2.- SAMPLING

The study area is located between Latitude $9^0$32' and $11^0$0' North and Longitude $71^0$01' – $72^0$01' West (Figure 2.1.). The area was represented by 17 sampling points where the samples were taken during November 1998 and March 1999. Three samples of 1 L of water (at one meter depth) were taken at 15 sites and 1 Kg of sediment sample was taken at 13 sampling sites.

All the *in situ* parameters were measured on the R/V Bergantin , the research vessel of ICLAM. Physicochemical parameters including temperature, pH, conductivity, salinity, dissolved oxygen and redox potential were measured with a Hydrolab Surveyor II at different depths of the lake. Only the parameters at 1.0 m depth and those near to the sediment surface were to considered in this study (Tables 2.1 and 2.2.).

Before sampling all the plastic bottles were carefully acid washed and then rinsed with deionized water. The samples of water were taken using a diaphragm pump (JABSCO, PAR-MAX4, model 30620-00-12), the plastic bottles were rinsed with this water before the sample was taken. The samples were homogenized and kept at –4 °C after sampling and during transportation to England.

The samples of sediment were taken using an Eckman dredge,. The sediments were homogenized and kept in plastic bags at -4°C during sampling, after these were translated to a 50 mL plastic bottle and frozen to -20^0C. The sediments were covered and mantained in the dark until analysis. Before lyophilization sediments were mantained frozen to avoid losses during defrosting.

The samples of lyophilized mussels (*Polymesoda solida*) and fish muscle tissue of Curvina (*Cysnocion acoupa Maracaiboensis*) were supplied by ICLAM.

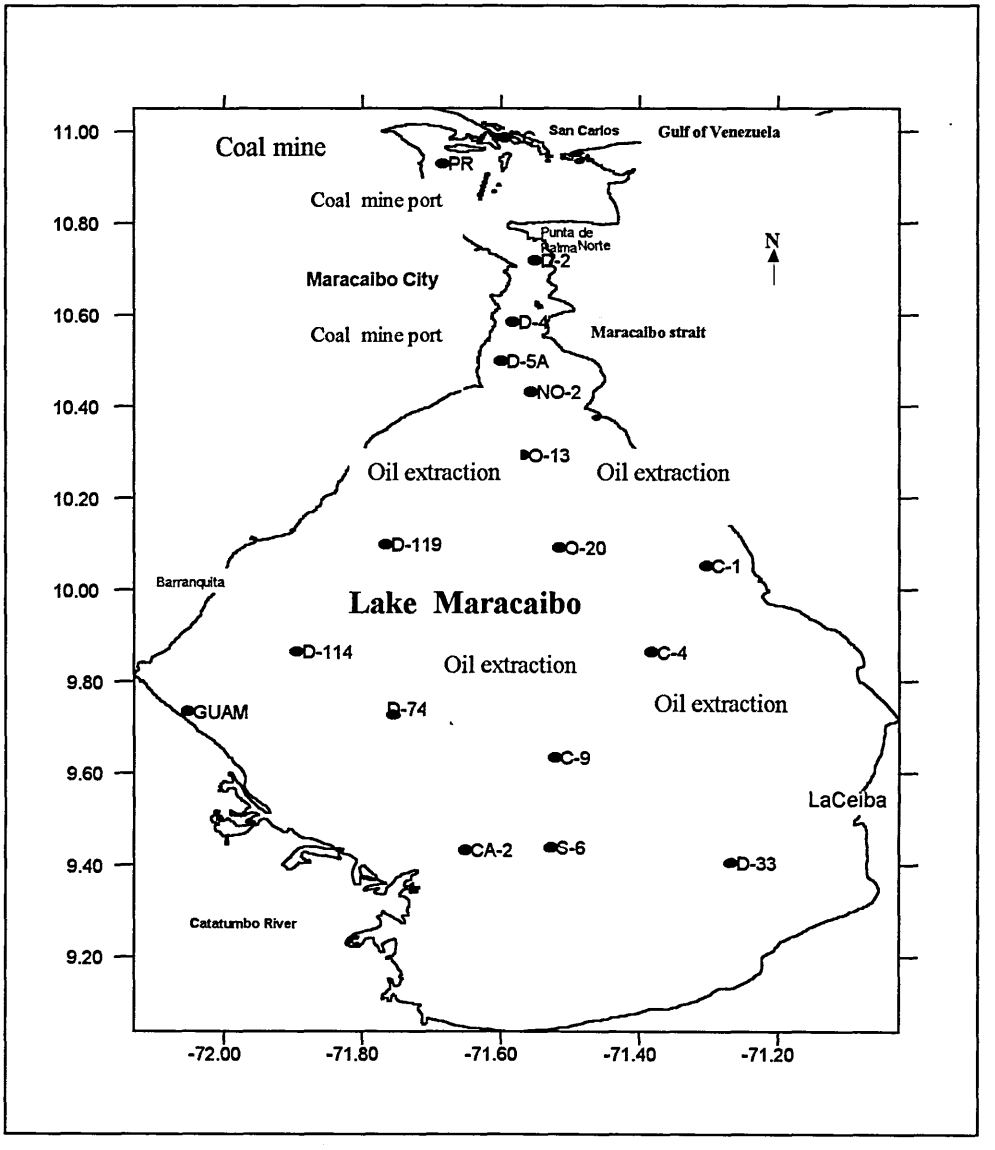


Figure 2.1: Lake Maracaibo and the sampling points

The samples of sediment were lyophilised in a Hereaus Lyophilizer at –44 ^{0}C for 12 h. After lyophilization, the sediments were homogenized with a mortar.

Table 2.1.: Parameters measured in situ for samples of water taken at 1 m of depth.

Site	Temperature (°C)	pH	Conductivity (µS)	Dissolved Oxygen(mgL^{-1})	Salinity ($g\ L^{-1}$)
D-114	29.95	7.54	7.19	6.70	3.9
O-13	30.67	7.78	7.26	7.16	3.9
O-20	30.92	8.01	7.62	8.05	4.1
D-2	29.72	7.97	7.66	6.26	4.2
D-33	29.63	7.91	6.19	7.43	3.3
PR	30.02	8.25	8.02	7.01	4.4
SC	29.53	8.18	8.89	7.18	4.9
D-4	29.73	7.81	7.09	6.03	3.8
D-5a	29.94	7.82	7.03	5.92	3.8
C-9	29.78	7.30	8.18	4.88	4.5
C-1	31.15	8.13	7.09	7.30	3.8
C-11	29.85	7.43	7.73	5.37	4.2
C-1	31.15	8.13	7.09	7.30	3.8
NO-2	7.82	7.82	7.03	5.92	3.8
CA-2	29.28	7.44	6.00	6.10	3.2
S-6	28.89	8.05	7.14	6.56	3.9
D-119	29.36	8.44	6.77	7.52	3.6
D-74	28.45	7.43	7.41	4.90	4.0

Table 2.2.: Parameters measured in situ for the maximum depth, the zone nearest to the surface sediments.

Site	Temperature (°C)	pH	Conductivity (µS)	Dissolved Oxygen(mgL^{-1})	Salinity ($g\ L^{-1}$)	Depth (m)
SC	27.33	8.15	30.9	6.25	19.2	9.9
D-2	28.15	7.73	15.8	3.06	9.2	10.8
D-4	28.54	7.73	7.31	4.9	4.0	14
NO-2	29.21	8.46	6.87	6.28	3.7	14.7
O-13	28.91	7.61	6.56	4.97	3.5	15
O-20	28.46	7.3	7.12	3.49	3.9	27.1
C-9	28.81	7.17	9.74	0.05	5.4	28
CA-2	28.64	7.79	6.72	3.45	3.6	25.2
D-33	28.46	7.08	7.47	1.90	4.1	28.7
C-1	28.68	7.13	8.45	0.79	4.6	28.1
PR	29.74	8.27	8.19	6.80	4.5	3.20
D-5a	29.98	7.84	7.12	5.72	3.8	14.60
C-11	30.06	6.62	14.10	0.01	8.1	27.70

2.3.- Lake Maracaibo System

This lake is connected to the Gulf of Venezuela via the Strait of Maracaibo and the Bay El Tablazo. Sampling points in the three zones are as follows. These zones are:

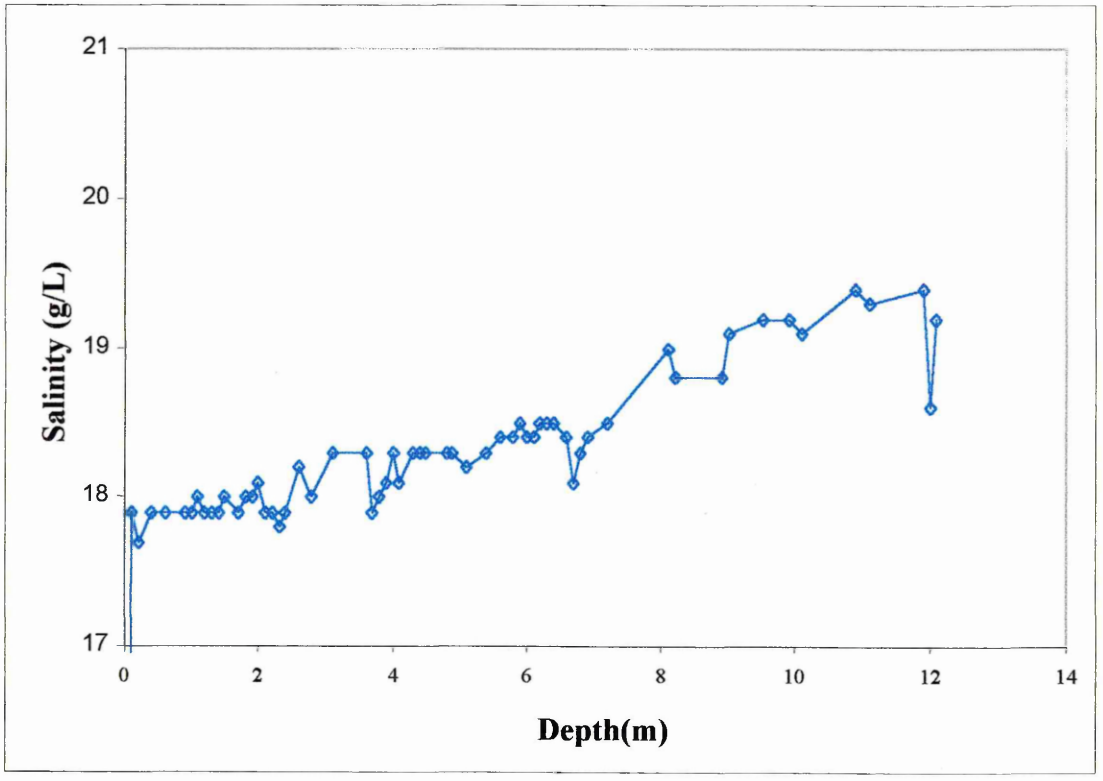
(1)**The Gulf of Venezuela** (mixing zone with the Caribbean Sea) (sampling points SC, PR)

(2) **The Strait of Maracaibo** (intermediate zone)(sampling points (D-2, D-4, D5a, NO-2)

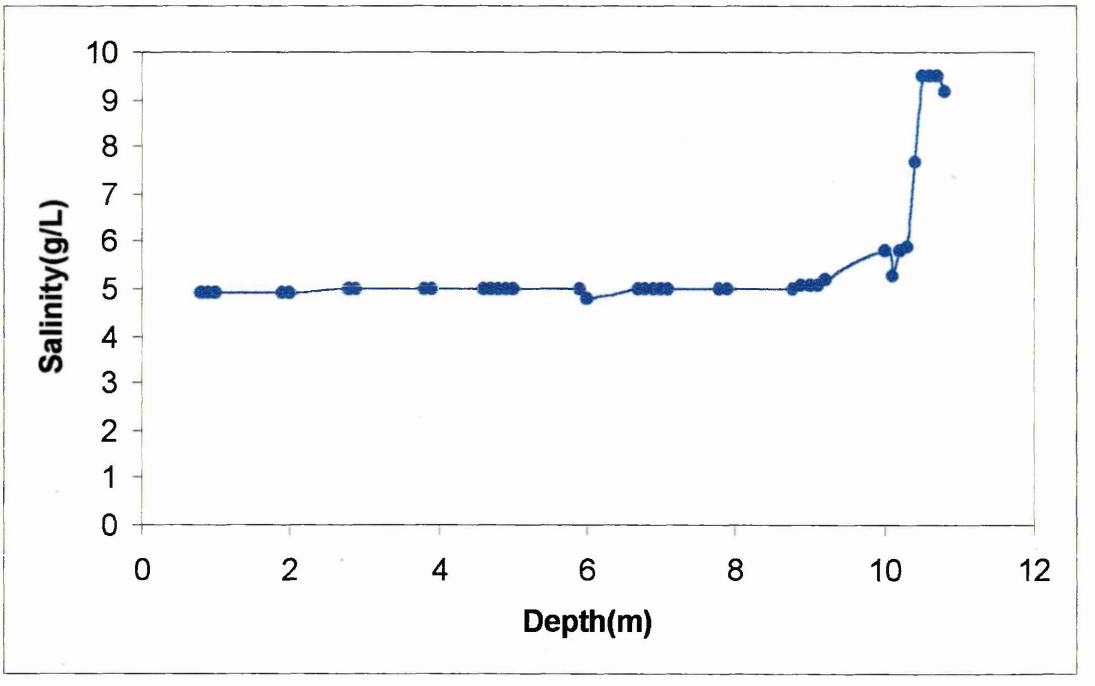
(3)**The lake** (main zone of the lake)(the rest of the sampling points)

Although considerable research has been conducted in describing the salient features of the hydrodynamics of Lake Maracaibo, little has been done in order to quantify the exchange between Lake Maracaibo and the Gulf of Venezuela by characterising the internal processes and their interactions. Furthermore, there have been no attempts to describe the dynamics of the entire system. Figures 2.2. a, b, c show the variation of salinity with depth during 1999 in three different zones of the Lake Maracaibo system , the estuary, the strait and the lake itself (SC, D-2 and C-9)

(a) Point SC



(b) Point D-2



c) Point C-9

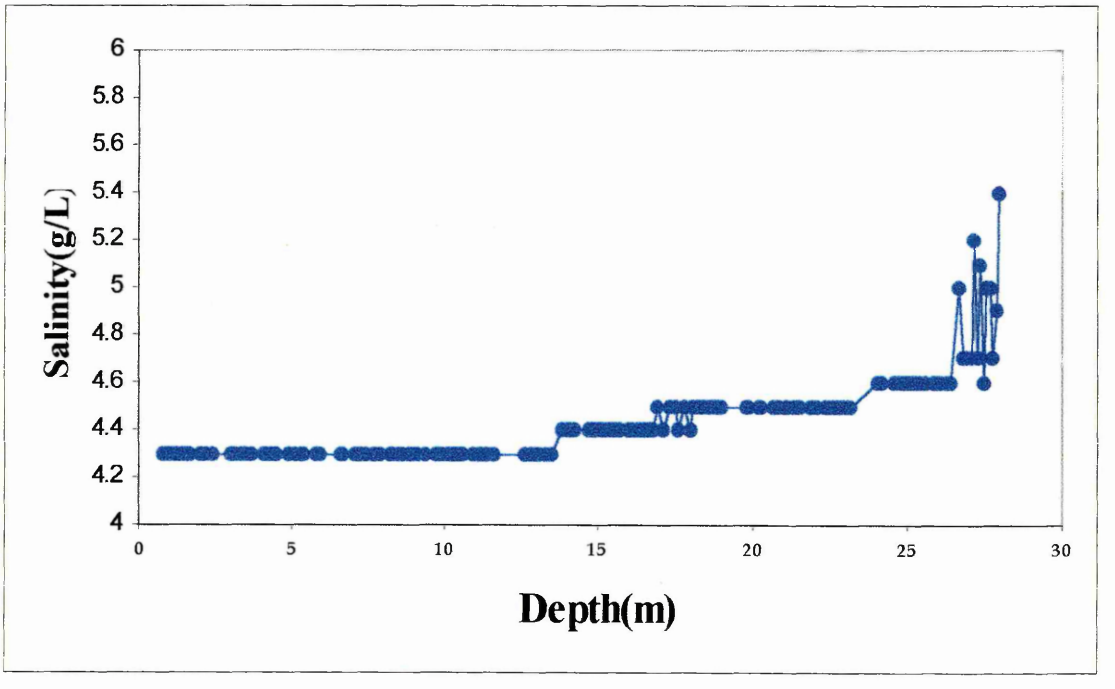


Figure 2.2. (a,b and c): Variations of the salinity with the depth in three zones (estuary, strait and lake itself) of Lake Maracaibo system.

Lake Maracaibo is a stratified lake in which salinity changes with depth. The coned-shaped hypolimnion is defined by higher salinity and lower temperature than the overlying epilimnetic water that circulates at the hypolimnetic layer in a counterclockwise direction (2).

2.4.- REFERENCES

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CHAPTER III

Determination of nitrogen, phosphorus and sulphur.

3.1. INTRODUCTION

3.1.1. Nitrogen

Nitrogen occurs in numerous chemical compounds and in various environmental compartments (1). ^{14}N and ^{15}N of relative natural abundance 99.63 % and 0.37 % respectively, are the two stable isotopes . The breakdown of the stable N_2 molecule is the limiting step in the incorporation of nitrogen into its inorganic and organic chemical forms (2). Elemental nitrogen is also incorporated into chemically bound forms, or fixed by biochemical processes mediated by microorganisms (see Figure 3.1). Biological nitrogen fixation is a key biochemical process in the environment and is essential for plant growth in absence of synthetic fertilizers (3).

Nitrification, the conversion of N(-III) to N(V), is a very common and extremely important process in water and soil. Aquatic nitrogen in thermodynamic equilibrium with air is in the +5 oxidation state as nitrate, whereas in most biological compounds, nitrogen is present as N(-III), such as $-NH_2$ in amino acids.

$$2\,O_2 + \ NH_4^+ \rightarrow NO_3^- + 2\,H^+ + H_2O$$

Nitrate reduction is a microbial process by which nitrogen in chemical compounds is reduced to lower oxidation states. In the absence of free oxygen, nitrate may be used by some bacteria as an alternative electron receptor.

Generally, when nitrate ion functions as an electron receptor, the product is NO_2^-:

$2\ NO_3^- + CH_2O \rightarrow 2\ NO_2^- + H_2O + CO_2$

An important case of nitrate reduction is denitrification, in which the reduced nitrogen product is a nitrogen-containing gas, usually N_2:

$4\ NO_3^- + 5\ CH_2O + 4\ H^+ \rightarrow 2\ N_2 + 7\ H_2O + 5\ CO_2$

Denitrification is the process by which nitrogen is returned to the atmosphere.

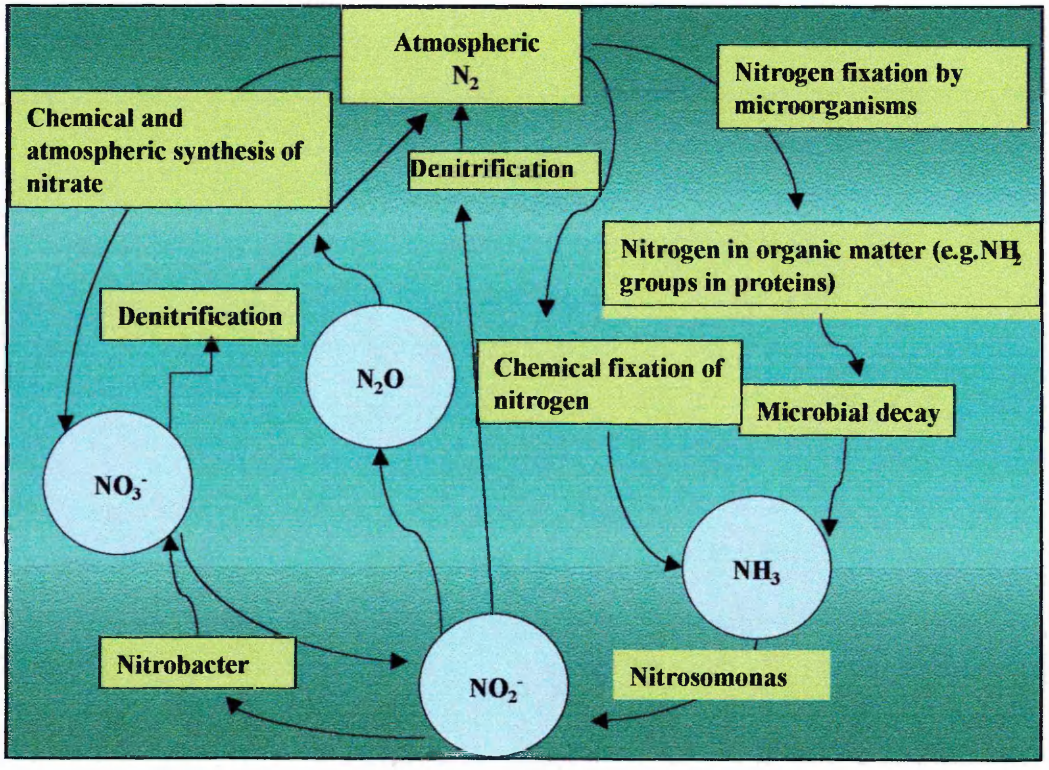


Figure 3.1.: The nitrogen cycle which describes the dynamic processes through which nitrogen is interchanged among the atmosphere, organic matter, and inorganic compounds (3).

Nitrogen is present as free nitrogen (N_2) and as salts (NH_4^+, NO_3^-, NO_2^-) in soil and water (2). As an essential element for plant growth, it is a constituent of proteins, amino acids, vitamins, chlorophyll, enzymes, etc. Consequently, nitrogen availability may often be the limiting factor in plant growth and yield of agricultural crops.

3.1.2. Phosphorus

Phosphorus is a highly reactive element and forms compounds with various elements by direct bonding with or through oxygen. Phosphorus exhibits nine formal oxidation states from +5 to –3. Typical oxoacids such as orthophosphate (+5), hypophosphate(or di-phosphate) (+4); and hypophosphite, (+1) and their derivatives are well known. Sodium diphosphate is familiar as the phosphorylation agent for biological substances. Phosphorus oxo acids, phosphate and its polymers are important in nature and in industry orthophosphoric acid is used as raw material in the manufacture of fertilizers, detergents, surfactants and flame retardants (4).

Although cyclopolymers such as trimetaphosphate exist in nature, linear polymers of phosphate such as di-(*pyro*), tri- (tripoly) and polyphosphate are the most abundant.

The phosphorus cycle is shown in Figure 3.2.. There are no common stable gaseous forms of phosphorus. In the geosphere, phosphorus is held largely in poorly soluble minerals, such as hydroxyapatite, a calcium salt, deposits of which constitute the major reservoir of environmental phosphate. Soluble phosphorus from phosphate minerals and others sources, such as fertilizer, is taken up by plants and incorporated into nucleic acids, which make up

the genetic material of organisms. Mineralization of biomass by microbial decay returns phosphorus to the salt form from which it may precipitate as mineral matter (4).

Biodegradation of phosphorus compounds is important in the environment for two reasons. The first one is that it provides a source of algae nutrient orthophosphate from the hydrolysis of polyphosphates. Secondly, biodegradation deactivates highly toxic orthophosphates compounds, such as the orthophosphate insecticides

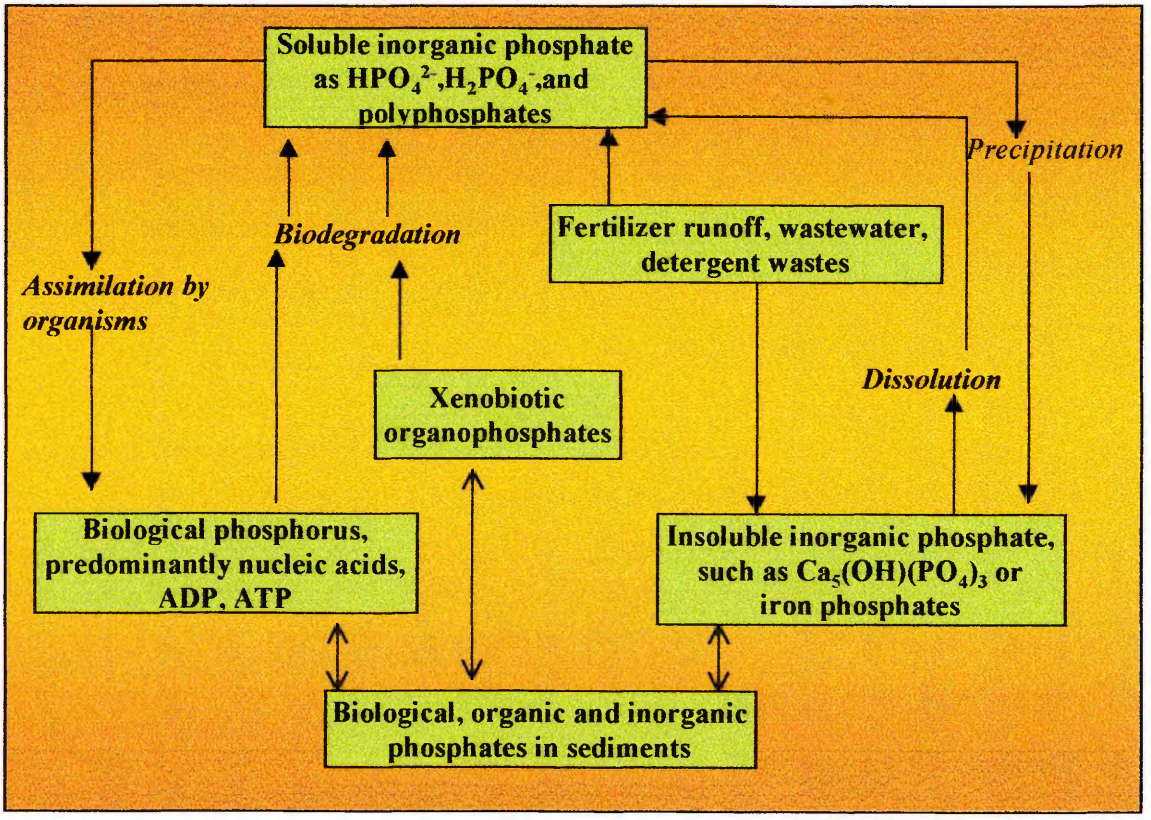


Figure 3.2.: The phosphorus cycle (4) showing phosphorus-containing species found in the environment

Phosphorus occurs in waters, either in dissolved or particulate forms and, as inorganic or organically bound species (5). Total phosphorus concentrations in water can vary from less

than 0.01 mgL^{-1} in small, near pristine, mountain streams to over 1 mgL^{-1} in heavily polluted rivers (5).

Phosphorus entering a wetland or stream is typically present in both organic and inorganic forms. These forms are dissolved inorganic phosphorus, dissolved organic phosphorus, particulate inorganic and particulate organic phosphorus. Dissolved inorganic phosphorus is considered bioavailable, whereas organic and particulate phosphorus forms generally undergo transformations to inorganic forms before being considered bioavailable (6). Seawater contains various organic esters of phosphorus as well as orthophosphate.

Due to the pre-eminence of phosphorus in primary production in all kinds of aquatic environments, research has focused on the origin and fate of phosphorus in lakes and seas (7-10). In sediment from lakes, it has been demonstrated that there are many cases in which phosphorus has been the limiting nutrient (7). The classification of the trophic status of standing water bodies is still based on the total phosphorus concentrations suggested by Vollenweider and modified by Wetzel in 1983 (11).

Some lakes in Central Europe have been regularly analysed during the last few years. Lakes such as Bodensee, Zurichsee and Greifensee have different degrees of eutrophication as a result of increases in phosphate concentration (12); for example, the phosphate loads in Lake Bodensee (Germany)have increased from 3 to 6 mg per m^3 per year (12).

3.1.3. Sulphur

Sulphur is the tenth most abundant element in the earth's crust (0.03-0.1 % w/w) and it is found in both the elemental form and in metal sulphide ores The four naturally occurring

stable sulphur isotopes are ^{32}S (95%), ^{33}S (0.76%), ^{34}S (4.22%) and ^{36}S (0.014%). The figures in brackets denote their natural abundance (13).

The cycling of sulphur on the Earth's surface has been greatly increased since the start of the Industrial Revolution by the demand for fuel, metals and fertilizers. Despite a great deal of study that the sulphur cycle has received in the past few years, there is still some uncertainity about many of the sources of the element. The sulphur and nitrogen cycles have a number of similarities, but one of the most important differences is that the major reservoir for nitrogen is the atmosphere, whereas the major available reservoir for sulphur is the earth's crust.

The sulphur cycle, illustrated in Figure 3.3., is relatively complex in that it involves several gaseous species, poorly soluble minerals, and several species in solution. It interacts with the oxygen cycle to form sulphur dioxide SO_2, an atmospheric pollutant, and soluble sulphate ion, SO_4^{2-}. Among the significant species involved in the sulphur cycle are gaseous hydrogen sulphide, H_2S, mineral sulphides, such as PbS; sulphuric acid, H_2SO_4, the main constituent of acid rain; and biologically bound sulphur in sulphur-containing proteins.

Insofar as pollution is concerned, the most significant part of the sulphur cycle is the presence of pollutant SO_2 gas and H_2SO_4 in the atmosphere. The former is a toxic gaseous air pollutant evolved in the combustion of sulphur containing fossil fuels.

Microorganisms and the sulphur cycle:

There is a strong analogy between sulphur and nitrogen in the way that microorganisms influence their biogeochemical cycling. Each element tends to be present in living

organisms in its most reduced form, i.e. nitrogen(-3) as amino groups, $-NH_2$, and sulphur (-2) as hydrosulphide groups, -SH. Sulphur is an important secondary constituent of amino acids and proteins. The ability of this sulphur to form sulphur-sulphur bonds allows cross-linking in proteins by so-called disulphide linkages. When organic sulphur compounds are decomposed by bacteria, the initial excreted sulphur product is often hydrogen sulphide, H_2S,

$$R\text{-}SH \longrightarrow H_2S + RH$$

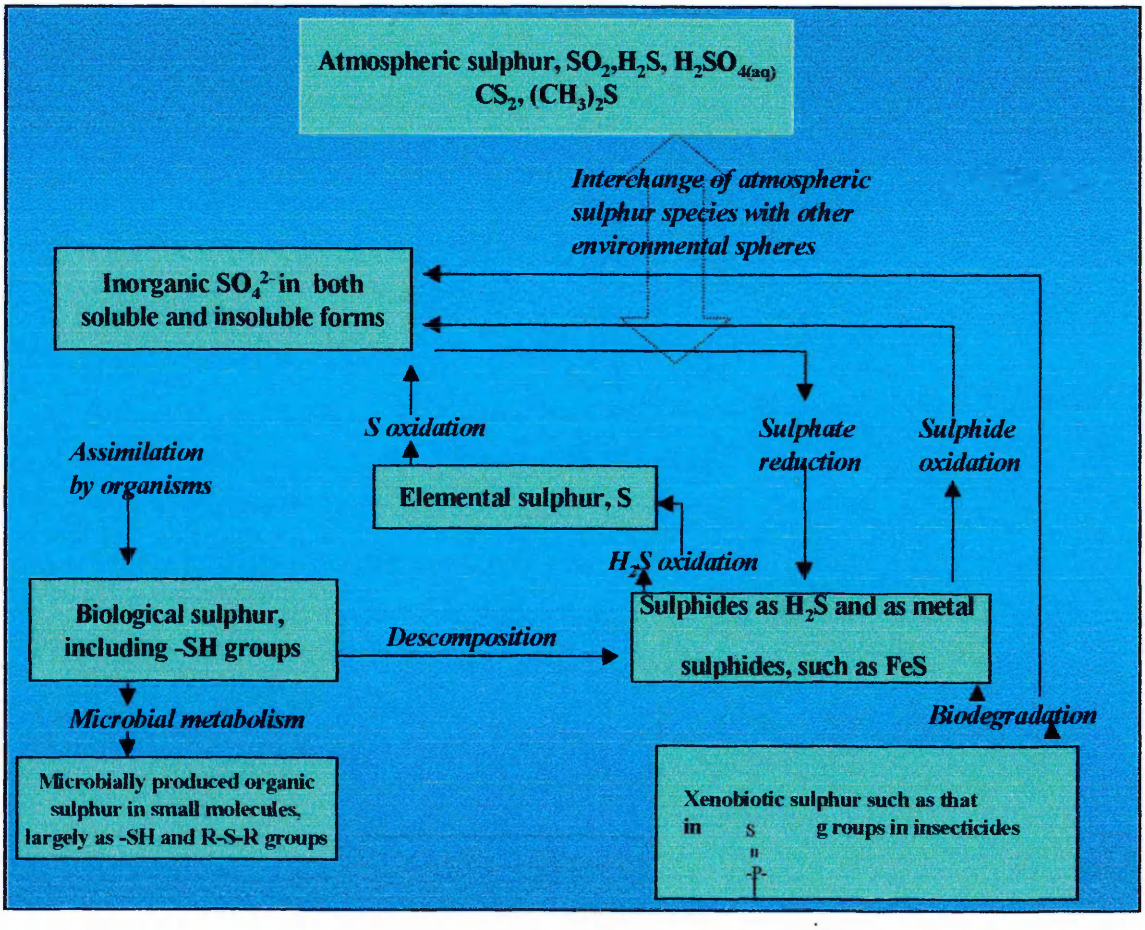


Figure 3.3.: The sulphur cycle(13). Sulphate ion, is found in varying concentrations in practically all natural waters. Organic sulphur compounds are common in natural aquatic systems and the degradation of these compounds is an important microbial process.

in the same way that organic nitrogen compounds yield the hydrogen containing products ammonia, NH_3, or ammonium ions, NH_4^+. Many marine phytoplankton produce compounds that break down to produce dimethylsulphide, $(CH_3)_2S$.

Dimethyl sulphide is a major biogenically produced sulphur compound, releasing about 20-40$TgSa^{-1}$from the oceans .The formation of hydrogen sulphide (H_2S) is a characteristic feature of anaerobic marine sediments due to the high levels of sulphate, as compared with nitrate, in the sea. The hydrogen sulphide that is produced may be released as a gas to the atmosphere, where it is oxidised or may undergo reaction with metal ions in the sediments or water column to form insoluble sulphides. The later transition metals and those metals which come after the transition metals in the periodic table are especially likely to form insoluble sulphides (14). Iron , because it is present in relatively large quantities, forms the major sulphides mineral reservoir such as triolite, FeS, and as iron pyrites FeS_2. The black colour of many sediments is partially due to the presence of iron sulphides as well as organic matter.

3.1.4.-Analytical determination of nutrients

The nitrogen, phosphorus and sulphur cycles are of particular significance in a number of biological and non-biological processes in the environment (15). Natural and anthropogenic effects can cause localised inter-related changes to the cycles. In order to assess the impact and extent of the changes, it is essential to develop analytical methods which allow the simultaneous determination of two or all three constituents in a wide variety of environmental samples.

Phosphorus determination involves two general steps, conversion of the phosphorus species to dissolved orthophosphate followed by determination of dissolved orthophosphate. Three digestion methods involving either perchloric acid, nitric-sulphuric acid mixture or persulphate solution are usually used. The phosphate generated is determined colorimetrically (16-18). Determination of phosphorus by inductively coupled argon plasma spectrometry is possible but requires that the instrument is adapted to work in the low ultraviolet region. (19)

Several methods have been described in the literature for the determination of sulphur which include gravimetric, turbidimetric, ion selective methods, chemiluminescence and capillary gas chromatography (20-22). These methods are both time- and reactant-consuming. Recently ICP-AES has been used to determine sulphur with the disadvantage that the recoveries and interference show dependence on the wavelength used. Calcium and boron are considered spectral interferences, and potassium, magnesium and phosphorus cause inter-element interferences (23).

The total dissolved nitrogen content of natural and marine waters and sediments are important quality parameters, given that nitrogen is an essential nutrient for primary production, and in some cases may be the limiting factor. Dissolved nitrogen in natural waters include inorganic species (nitrate, nitrite, ammonia) and organic matter such as amino acids, enzymes, nucleic acids, vitamins and alkaloids . Historically, analysis of total nitrogen is by the Kjeldahl method (24). The sample is digested with a mixture of concentrated sulphuric acid and potassium sulphate and selenium or mercury is added as a catalyst. Using this method dissolved organic nitrogen and ammonia are measured, but the

oxidized forms, nitrate and nitrite, must be determined separately. Another limiting factor in the nitrogen determination using the Kjeldahl method is the time required (ca. 12 hours). Other methods that have been used include photooxidation (25-26), generic combustion (27), pyrochemiluminescence (28) and peroxidisulphate oxidation (29-30).

A modified alkaline persulphate procedure has been developed for the simultaneous determination of nitrogen and phosphorus after oxidation to nitrate and phosphate, respectively (31). This digestion method has also been used, followed by ion chromatography to determine the anions, nitrate and phosphate, but the method is subject to interference in the determination of phosphate due to a large sulphate peak (32-34) .

In one of the first attempts at simultaneous determination, Ebina *et al* (16) developed a method of oxidizing nitrogen and phosphorus to nitrate and phosphate, respectively using alkaline potassium peroxodisulphate. The composition of the oxidizing solution was carefully chosen so that its pH changed from basic to acidic during the oxidation step. The change in pH was necessary because oxidation with potassium peroxodisulphate of nitrogen and phosphorus occurs under basic and acidic conditions, respectively. The nitrate and phosphate ions were then determined colorimetrically.

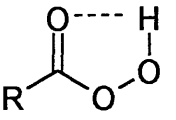
In a different approach, Collins at al (35) developed a method for the combined analysis of total phosphorus and Kjeldahl nitrogen in complex matrices using a pressure microwave digestion and final colorimetric determination of phosphorus. More recently, Matilainen and Tummavuori (23) investigated the application of ICP-AES to the determination of water soluble sulphur in fertilizers and reported on spectral and interelement effects.

To be able to analyse both bound and water soluble fractions, samples have to be digested. However, existing digestion methods are not easily adapted to simultaneous determinations because the use of oxidants such as nitric and sulphuric acids and potassium peroxodisulphate precludes the determination of one or more of the analytes. The use of hydrogen peroxide as an oxidant has a number of benefits compared with some of the more traditional oxidants (36). These include long term storage stability, and when the oxidising power of the peroxide is spent, only water is left as the by-product, thus eliminating the need for expensive effluent disposal treatments. In addition, it is a relatively inexpensive reagent, etc (37). Furthermore, samples digested with hydrogen peroxide can be used in analyses involving ion chromatography (38), potentiometry (39), colorimetry (40), UV-induced photoxidation (41), and other traditional techniques such as the cadmium reduction method (N)(42) and the ascorbic acid method (P) (43).

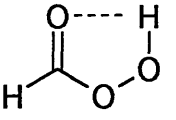
The oxidation strength of hydrogen peroxide is much enhanced when it is activated by the presence of an alkali, acid, metal ions or UV light. Activation via peroxyacid formation is the most common industrial use of H_2O_2 (36, 44-45).

Organic peroxyacids take part in a wide range of oxidation reactions often resulting in high product yields (46). Organic peroxyacids , or peroxyacids, are derivatives of hydrogen peroxide in which one of the hydrogen atoms is replaced by one acyl or aroyl group.

Monoperoxyacids contain one peroxycarboxyl (-CO_3H) group: diperoxyacids contain two.



For example: Peroxyformic acid:



Pure peroxyformic acid has probably never been prepared, but concentrated solutions are known; e.g. a mixture of 98 % hydrogen peroxide and 20 g of formic acid can contain up to 48% of peroxyformic acid under vacuum. Peroxyformic acid solutions of lower concentrations are obtained when less concentrated hydrogen peroxide (30%v/v) is used, with a resulting increase in safety (46).

About 25 % of active oxygen is lost within 24 h even at 0^{o} in a 90 % v/v solution of peroxyformic acid. The decomposition products are reported to be carbon dioxide and water or formic acid and oxygen (46):

$HCO_3H \rightarrow H_2O + CO_2$

$2HCO_3H \rightarrow HCO_2H + O_2$

In the presence of UV light H_2O_2 and ROOH can be degraded as follows:(47)

$H_2O_2 + h\nu \rightarrow 2OH^{\cdot}$

$RO_2^{\cdot} + HO_2^{\cdot} \rightarrow ROOH + O_2$

$ROOH + h\nu \rightarrow RO^{\cdot} + OH^{\cdot}$

$RO_2^{\cdot} + HO_2^{\cdot} + h\nu \rightarrow RO^{\cdot} + OH^{\cdot} + O_2$ (Net equation)

In aqueous phase,

$H_2O_{2\,(aq)} + h\nu \rightarrow 2{\cdot}OH_{(aq)}$

Hydrogen peroxide can oxidise transition metals such as Fe (II),

$Fe(II) + H_2O_{2(aq)} \rightarrow Fe(III) + {\cdot}OH_{(aq)} + OH^-$ in acidic medium (48)

and oxidise gaseous sulphur dioxide as well

$SO_2 \rightarrow H_2O \rightarrow SO_{2(aq)}$

$SO_2^-(aq) \rightarrow H^+ + HSO_3^-$

$HSO_3^- \rightarrow H^+ + SO_3^{2-}$

Bisulphate anions (HSO_3^-) can reacts with hydrogen peroxide over a pH range 3 to 6 producing sulfuric acid (47):

$HSO_3^- + H_2O_{2(aq)} + H^+ \rightarrow SO_4^{2-} + 2H^+ + H_2O$

Examples of the applications of hydrogen peroxide include the following: the degradation of the nitrogen containing organic compounds (49), degradation of PCB (50), degradation of humic acids (51), use in a tertiary treatment scheme with activated carbon (52), destruction of chemical pollutants (53), degradation of organophosphorus pesticides (54), monitoring oxygen demand in polluted waters (55), determination of sodium hydrosulphite in waste liquid by ion chromatography (56) and many other applications (57).

Speciation of nitrogen, phosphorus and sulphur in aquatic systems has become increasingly important due to the realisation that the environmental behaviour of these elements (i.e., bioavailability, bioaccumulation and biogeochemical transport) is often critically dependent on its physco-chemical form (58). There are many methods for the determination of nitrogen, phosphorus and sulphur species, some of them using techniques such as separation by precipitation (59), colorimetry (60), titrimetry (61), ion selective electrodes

(62), anion exchange chromatography (63), photo-oxidation (64), capillary electrophoresis (65), electron microscopy (66), ion chromatography (67), integrated pervaporation (68), liquid chromatography-mass spectrometry (69) and others. There is a simultaneous method for the speciation of sulphur and nitrogen in humic substances that uses x-ray as a detection technique (70).

3.1.5.- Microwave digestion

Microwave heating involves direct absorption of energy by the sample material being digested. Microwaves are electromagnetic energy, which is non-ionising radiation that causes molecular motion by the migration of ions and rotation of dipoles, but does not causes changes in molecular structure (71). Microwave energy has a frequency range from 300 to 300 000 MHz.

A microwave unit used in a laboratory consists of six major components: the microwave generator (the magnetron), the wave cavity, the wave guide, the mode stirrer, a circulator and a turntable.

The magnetron produces microwaves that are radiated from its antenna into the wave guide. The microwave guide is a reflective metal that directs the waves into the microwave cavity. As the microwaves enter the cavity, they are reflected by the mode stirrer to assist in homogeneity the microwave field inside the cavity. To improve the homogeneity of the microwave field, the samples are rotated through the variable field. A laboratory microwave unit has vessels in a turntable which contain the samples to digest. The closed-vessel systems have a number of advantages over open vessel systems. The pressure raises the boiling point of the acids, achieving higher temperatures, which reduce the time

required for digestion (72) , losses of volatile elements are eliminated by closed system, less acid is required because little evaporation of the sample digest occurs and the hazardous fumes are contained within the vessel.

In this study, a simultaneous procedure to determine nitrogen, phosphorus and sulphur is described. Hydrogen peroxide, formic acid and a microwave digestion system were used to oxidise nitrogen to nitrate, phosphorus to phosphate and sulphur to sulphate which were determined by ion chromatography. The methodology was validated using a number of inorganic and organic N,P and S containing compounds and reference materials.

3.2. MATERIALS AND METHODS

3.2.1. Apparatus

A Dionex QIC analyzer ion chromatograph equipped with a Dionex AG4A guard column, a Dionex AS4A anion separation column, and a Dionex AMMS-II suppressor and conductivity detector was used. The sample was injected into the chromatograph via a 100 μL sample loop, and eluted with a solution of 1.8 mM sodium carbonate /1.7 mM sodium bicarbonate at a flow rate of 1 mL min^{-1}. A chart speed of 0.5 cm s^{-1} , conductivity range setting of 30 μS, and conductivity suppressor solution of 12.5 mM H_2SO_4 were used throughout.

A Milestone model MLS-1200 Mega microwave system (24010 Sorisole, Italy) was used for the digestion of the samples.

The digestion programme was as follows:

STEP	POWER (W)	TIME (min)
1	250	5
2	0	15
3	600	10
4	Ventilation	10

3.2.2. Reagents

The column eluent was prepared from reagent grade sodium carbonate and bicarbonate, and distilled deionized water (18 MΩ -cm, nanopure, Millipore Corporation, Massachusetts 01730, USA) The suppressor solution was prepared from 1.4 mL Aristar grade sulphuric acid (Merck, Poole, Dorset, UK) and made up to 2 L with distilled deionised water. The following analytical grade compounds were subjected to the digestion treatment: sodium nitrite, urea, L-cysteine and ammonium nitrate (all supplied by Merck, Poole, Dorset, UK), L-lysine and sodium pyrophosphate (both supplied by Aldrich, Gillingham, Dorset, UK), sodium sulphite (East Anglia Chemicals, UK). A 22% $^{v}/_{v}$ solution was prepared from Aristar grade 30% $^{v}/_{v}$ hydrogen peroxide. A reference material rain water LGC 6018 was used to test the ion chromatograph response.

3.2.3.- Sample preparation

To test the efficiency of the oxidation procedure, solutions containing 50 μL of formic acid and 40-100 mg L^{-1} in nitrogen , phosphorus or sulphur were prepared.

Standard reference materials oyster tissue (NIST, SRM 1566a) and Buffalo River sediment (NIST SRM 2704) were used to validate the digestion procedure.

3.2.4. Stock standard solutions

Individual 1000 mg L^{-1} stock standard solutions of nitrate-N, phosphate-P, sulphate -S and nitrite-N were prepared from Aristar grade reagents (supplied by Merck) by dissolving 6.0679 g $NaNO_3$, 4.3937 g KH_2PO_4, 1.8145 g K_2SO_4 and 0.2020 g of $NaNO_2$, respectively, in one litre of distilled deionised water.

Mixed anion standard solutions of 1.0, 2.5, 5.0 and 10.0 mg L^{-1}, respectively, were used to calibrate the ion chromatograph.

3.2.5. Sample digestion

Ten mL of hydrogen peroxide solution were added to 5 mL of sample or 0.2 g of a reference material and 50 μL of formic acid added to the microwave sample vessel. The mixture was capped and the microwave programme initiated. At the end of the first run, the sample was allowed to cool to room temperature, a further 10 mL of the same strength hydrogen peroxide solution was added and then the same programme was repeated. After oxidation, the digest was cooled to room temperature, made up to 100 mL with distilled deionised water, and analysed on the ion chromatograph. Each compound was digested and analysed at least five times.

3.3. RESULTS AND DISCUSSION

3.3.1.- Concentration of the oxidising solution

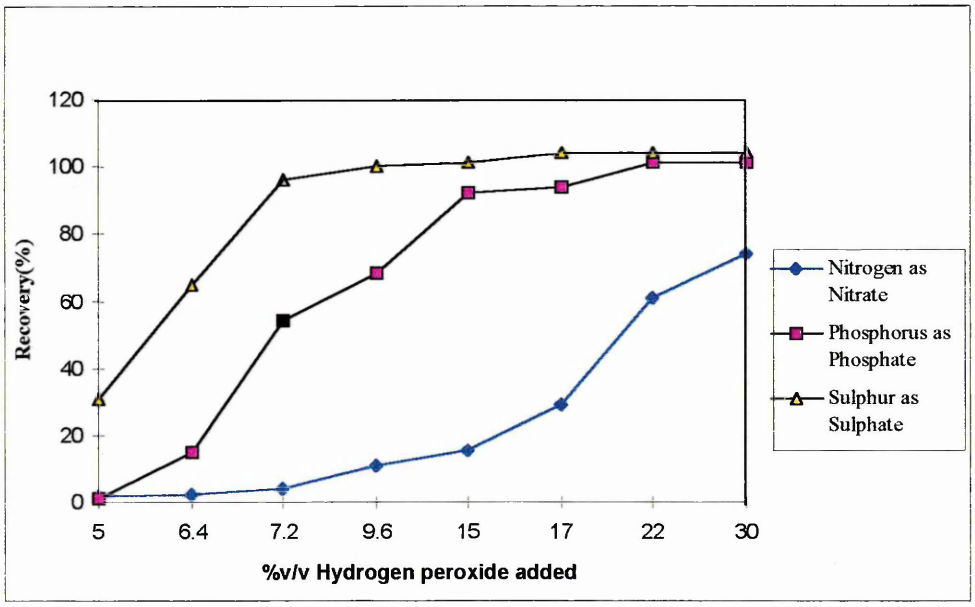


Figure 3.4: Effect of varying hydrogen peroxide concentration on the recovery of nitrogen, phosphorus and sulphur from urea, sodium pyrophosphate, and L-cysteine, respectively after the first digestion.

Figure 3.4 shows the effect in percent recovery of varying hydrogen peroxide concentrations on the conversion of urea, sodium pyrophosphate and L-cysteine to nitrate, phosphate and sulphate, respectively.

It has been suggested that the oxidising power of hydrogen peroxide is enhanced when it is activated by either acid, metal ions or is exposed to UV light (20).

The extent of conversion of urea to nitrate was much improved (Figure 3.5) when a second 10 ml aliquot of the same concentration hydrogen peroxide solution was added and the sample subjected to the microwave programme for a second time. In subsequent

experiments, 22 % $^{v}/_{v}$ hydrogen peroxide and the two stage digestion procedure were used to test the efficiency of the oxidation process on a variety of compounds

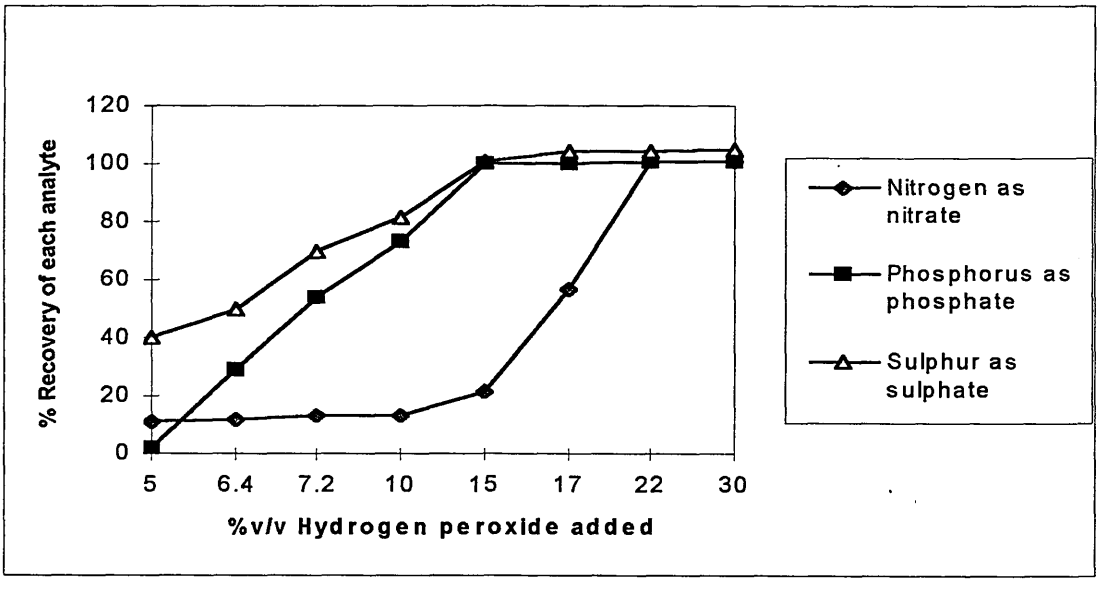


Figure 3.5.: Effect of varying hydrogen peroxide concentration on the recovery of nitrogen, phosphorus and sulphur from urea, sodium pyrophosphate, and l-cysteine, respectively after the second digestion.

Tables 3.1 and 3.2 summarise the extent of oxidation expressed as recoveries of total nitrogen, phosphorus and sulphur. Varying the amounts of urea, L-cysteine and sodium pyrophosphate did not affect the extent of oxidation (see Table 3.2). The very good recovery values indicate that the oxidation process is efficient at converting N, P and S from the form in which they occur in these compounds. The efficiency of the procedure in oxidizing compounds containing nitrogen-nitrogen bonds or amide groups, and condensed polyphosphates is currently being assessed. A comparison of the expected and found values for N, P, S (Table 3.1) using a paired-t test was found not to be statistically significant at the 95% confidence limits except for the L-cysteine for which high recoveries were

obtained. The difference in the results could be due to the poorer sensitivity for the determination of sulphate ions at low concentrations.

Table 3.1: Recoveries of nitrogen, phosphorus and sulphur as nitrate, phosphate and sulphate ions from different concentrations (mgL^{-1}) of pure compounds after digestion with 22% $^{v}/_{v}$ hydrogen peroxide (n=5).

Compound	$N\text{-}NO_3^-$ Expected	$N\text{-}NO_3^-$ Found	$P\text{-}PO_4^{3-}$ expected	$P\text{-}PO_4^{3-}$ found	$S\text{-}SO_4^{2-}$ Expected	$S\text{-}SO_4^{2-}$ found
Urea	9.93	9.96±0.62				
L-Lysine	4.00	4.01±0.04				
Ammoniun nitrate	6.49	6.68±0.06				
Sodium nitrite	10.0	10.02±0.08				
L-Cysteine	2.26	2.12±0.01			5.17	6.10 ± 0.01
Sodium Pyrophosphate			9.78	9.80±0.13		
Sodium sulphite					5.38	5.35±0.04
Mix l-Cysteine and sodium pyrophosphate	2.26	2.12±0.01	9.78	9.65±0.26	5.17	6.11±0.02

Table 3.2: Recoveries of nitrogen, phosphorus and sulphur using different concentrations of analyte and 22% $^{v}/_{v}$ hydrogen peroxide.

Compounds	Concentration expected (mgL^{-1})	Concentration found(mgL^{-1})	% Recovery
Urea ($N\text{-}NO_3^-$)	5.00	4.85	97.0
	9.93	10.40	104.7
	6.00	5.42	90.3
	6.24	5.50	88.1
	8.00	7.45	93.1
L-Cysteine($S\text{-}SO_4^{2-}$)-	23.10	22.59	97.7
	11.48	12.11	105.4
	15.11	14.44	95.5
	10.00	10.50	105.0
	5.17	6.10	117.0
Sodium Pyrophosphate ($P\text{-}PO_4^{3-}$)	10.00	9.56	95.6
	20.50	22.19	108.2
	6.49	6.72	103.5
	31.8	30.13	94.7
	9.78	9.70	99.2

3.3.2.- Analytical performance

A chromatogram of a mixture of L-cysteine and sodium pyrophosphate after oxidation is shown in Figure 3.6. The mean ± sd retention times for nitrate, phosphate and sulphate ions were: 4.11 ± 0.14 , 6.60 ± 0.05 and 8.65 ± 0.24 min, respectively. The three peaks are very well resolved and as a result samples containing widely different proportions of the analytes can be analyzed without interferences.

Calibration graphs obtained from mixed anion standards gave the following highly linear best-fit equations:

Nitrate : $y = 1.18 \times 10^7 x - 7.18 \times 10^6$ ($r^2 = 0.9970$)

Phosphate: $y = 4.71 \times 10^7 x - 3.78 \times 10^6$ ($r^2 = 0.9886$)

Sulphate: $y = 4.37 \times 10^6 x - 3.76 \times 10^6$ ($r^2 = 0.9865$)

y = Peak area (arbitrary units)

x= Anion concentration (mgL^{-1})

Detection limits were calculated from the calibration graphs using the method of Miller and Miller (7). The results were 0.123 mg/L N-nitrate, 0.251 mg/L P- phosphate and 0.850 mg/L S-sulphate. The detection limits based on 0.2 g of sediment were 0.006% w/w N, 0.012 % w/w P and 0.042 % w/w S .

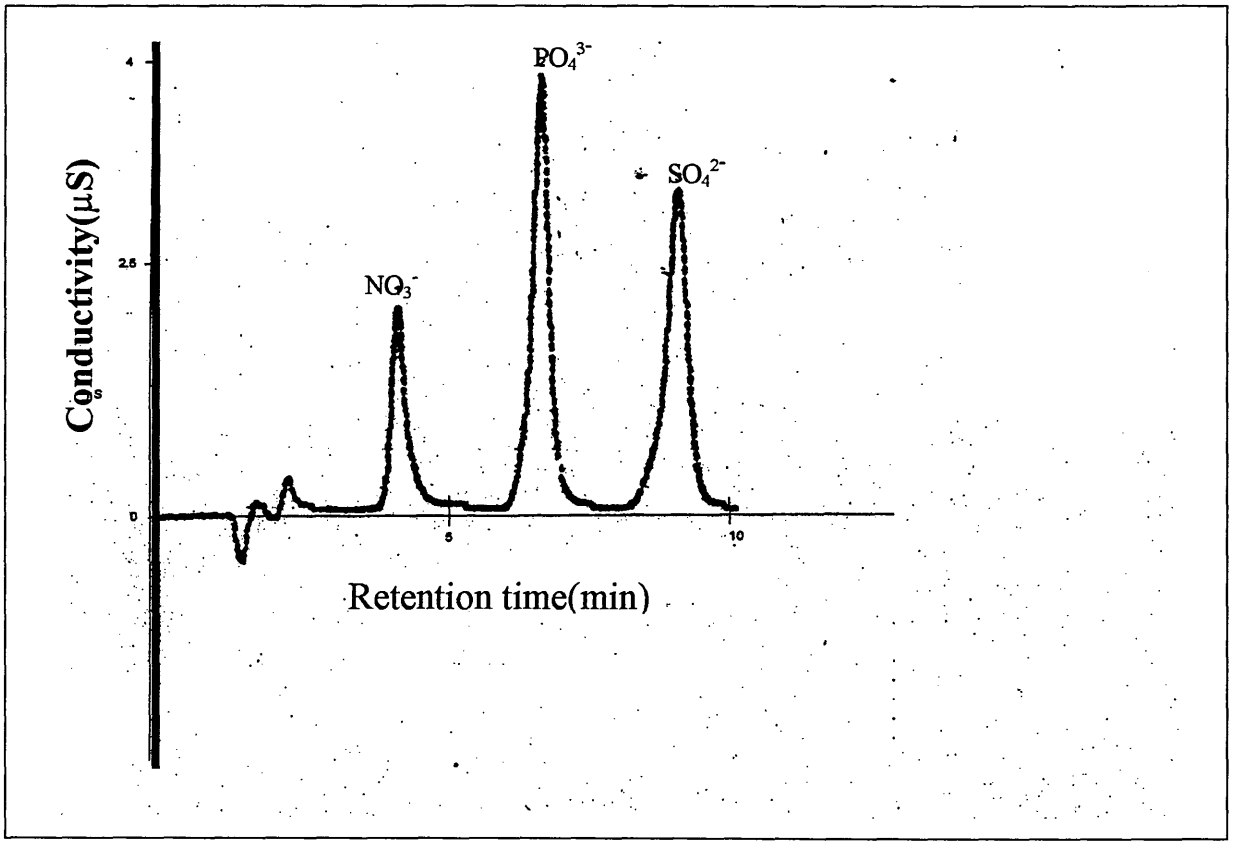


Figure 3.6: Chromatogram of a sample containing L-cysteine and sodium pyrophosphate after oxidation to nitrate (1), phosphate (2) and sulphate(3).

3.3.3. -Method validation

The N, P and S contents for NIST SRM 1566a oyster tissue and NIST SRM 2704 Buffalo river sediment samples digested with 22% $^{v}/_{v}$ hydrogen peroxide are given in Table 3.3. Satisfactory agreement with the certified values was obtained. The presence of a sample matrix did not have an adverse effect on the recoveries.

Table 3.3 : Comparison of the quantities of nitrogen phosphorus and sulphur found using the proposed method and the reported values for the standard reference materials (n=3).

Element		Oyster Tissue (%w/w± 95% confidence limit)	Buffalo River (%w/w± 95% confidence limit)
N	Found	6.62 ± 0.28	
	Reference value	6.81	
P	Found	0.62±0.02	0.09±0.01
	Certified	0.62±0.02	0.10±0.01
S	Found	0.87±0.01	0.43±0.05
	Certified	0.86±0.02	0.40±0.01

The proposed method for the oxidation of N, P and S followed by the determination of the nitrate, phosphate and sulphate ion by ion chromatography gave satisfactory results for the compounds tested. The effectiveness of this procedure is demonstrated by the good recoveries obtained for the two SRMs, oyster tissue and Buffalo river sediment. However, this work was focused on the application of the method to more recalcitrant compounds where the N, P and S atoms are in ring systems.

3.3.4- Chemical speciation of nitrogen, phosphorus and sulphur.

This previously reported method (38) was modified in order to extend the range of compounds that can be analysed for total nitrogen, phosphorus and sulphur. Parameters affecting the extent of oxidation such as microwave power, hydrogen peroxide

concentration and microwave program sequence were optimised. By altering the amount of hydrogen peroxide added to the sample, and the stepwise use of the microwave programme, it was possible, depending on the nature of the compound, to control the extent of the oxidation. Anions formed after oxidation of the samples were separated and determined by ion chromatography with conductivity detection. The developed procedures were validated using pure compounds: sodium nitrite, sodium sulphite, L- cysteine, lysine, phosphonitrile chloride, saccharin, urea, and reference material prawn GBWO8572.

3.3.4.1. Modified Method:

The instrumental settings for the microwave digestion were modified so that stepwise oxidation of the following compounds could be achieved. Parameters such as hydrogen peroxide added to an organic acid, the power of the microwave, and time of digestion were studied in order to control the oxidation to nitrate, phosphate and sulphate respectively.

The modified microwave programme was as follows (Table 3.4):

Table 3.4: Microwave conditions in each step used with the modified method.

STEP	POWER (W)	TIME (min)
1	250	5
2	0	15
3	450	10
4	0	10
5	650	10
6	Ventilation	15

3.3.4.2. Sample digestion:

Ten mL 30 % v/v hydrogen peroxide were added to 1 mL of sample or 0.2 g of a reference material followed by 50 μL of formic acid in the microwave vessel. The mixture was capped and the microwave programme initiated. For organic nitrogen and sulphur compounds, at the end of the first run, the sample was allowed to cool to room temperature, a further 5 mL of the same strength of hydrogen peroxide solution was added and then the same programme was repeated. For cyclic compounds and the reference material, an additional step was included after addition of 5 mL hydrogen peroxide. After oxidation, the digest was cooled to room temperature, made up to 25 mL with distilled deionised water, and analysed on the ion chromatograph.

3.3.4.3.- Inorganic and linear organic compounds

The Figure 3.7 (a,and b) shows variation of the conversion of urea to nitrate when different powers of the microwave, and one or two oxidation steps were used. An appreciable increase in the recovery is seen with the additional step. The nitrite (Figure 3.8a and b) and ammonium ions (Figure 3.9 , a and b) are converted to nitrate in only one step.

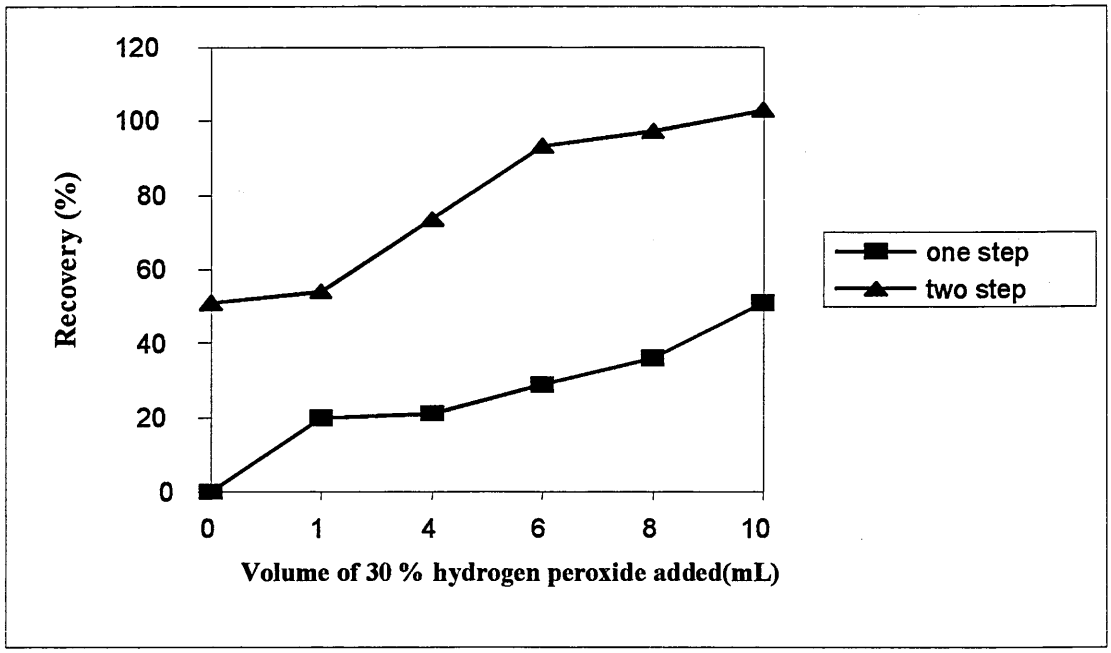


Figure 3.7.(a): Recoveries of nitrogen from a solution of 9.72 mg.L^{-1} of urea when different microwave program steps are used.

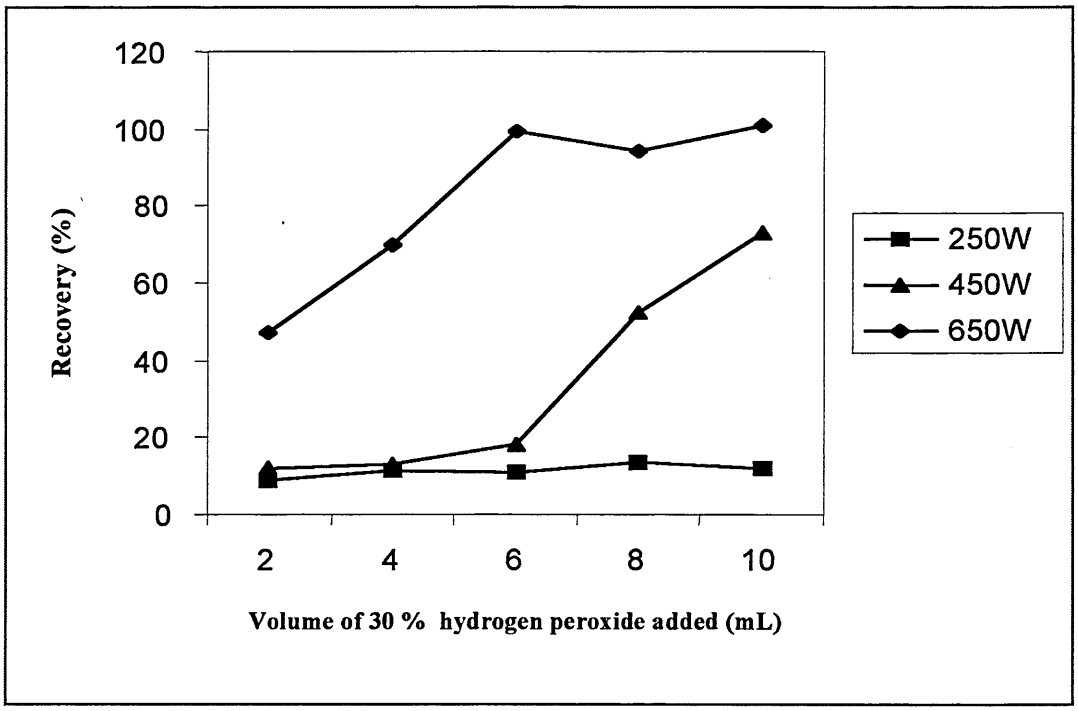


Figure 3.7(b): Recoveries of nitrogen using different power.

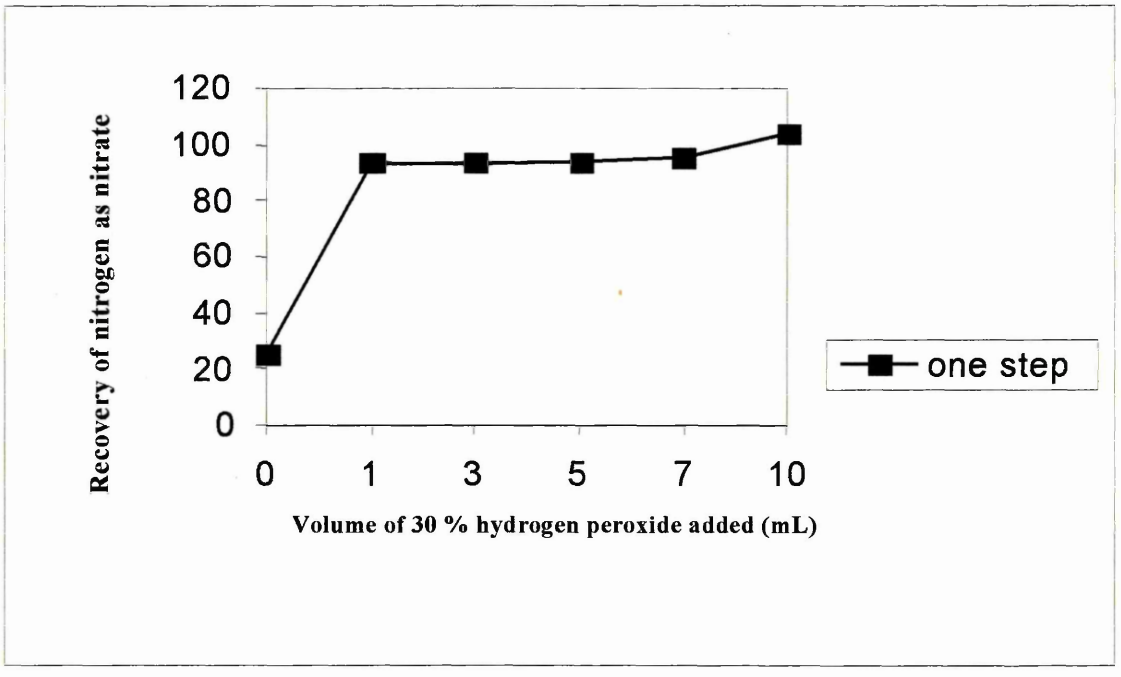


Figure 3.8(a).: Recoveries of nitrogen from a solution of 40 mg.L^{-1} of sodium nitrite when a one step program is applied.

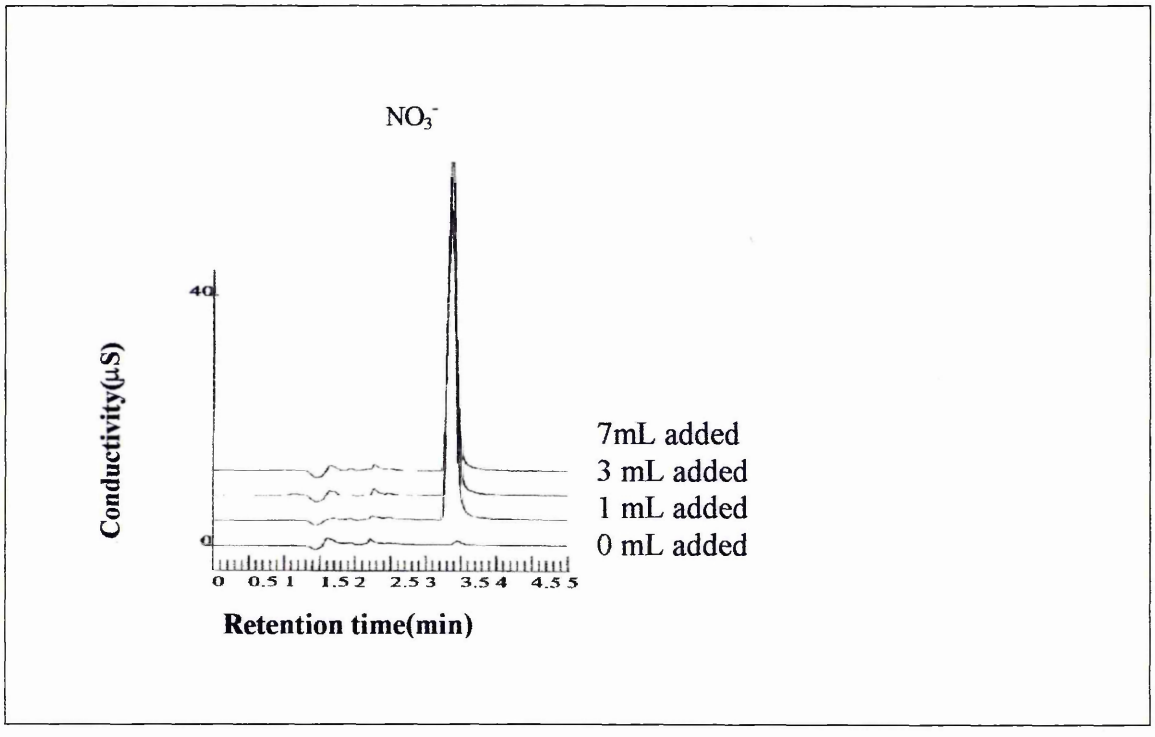


Figure 3.8.(b): Chromatogram of the 40 mg.L^{-1} solution of nitrite after oxidation with hydrogen peroxide.

The Figure 3.8(b) shows the chromatogram following oxidation of the nitrite solution with different concentrations of hydrogen peroxide; the nitrate peak increases in size with the addition of hydrogen peroxide until total conversion to nitrate is achieved.

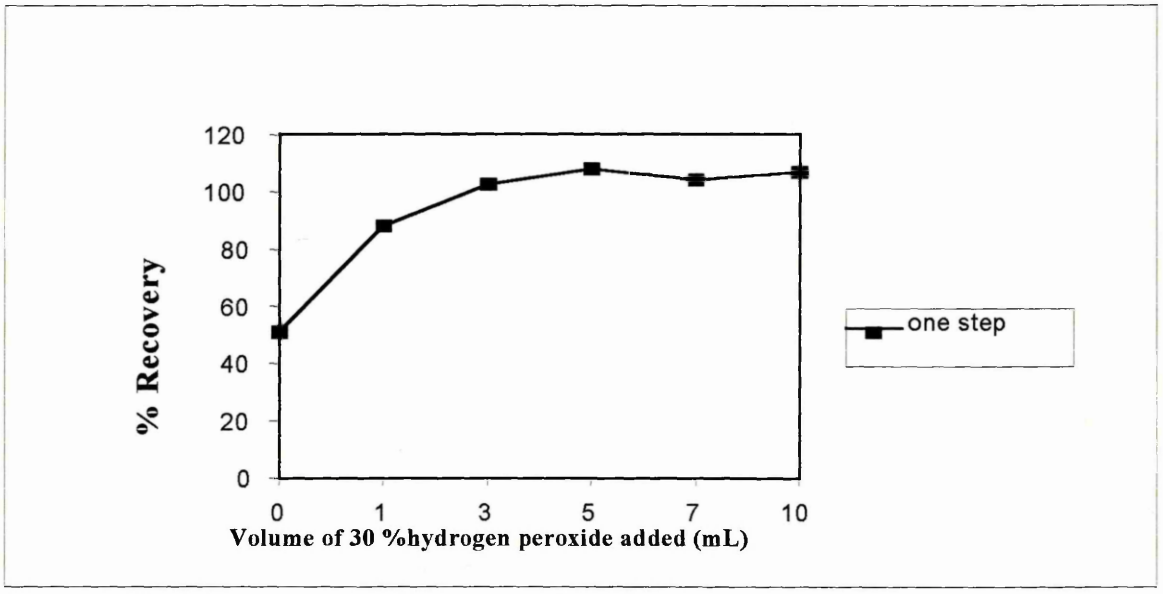


Figure 3.9.(a): Recoveries of nitrogen from ammonium chloride when a one step program is applied to the sample

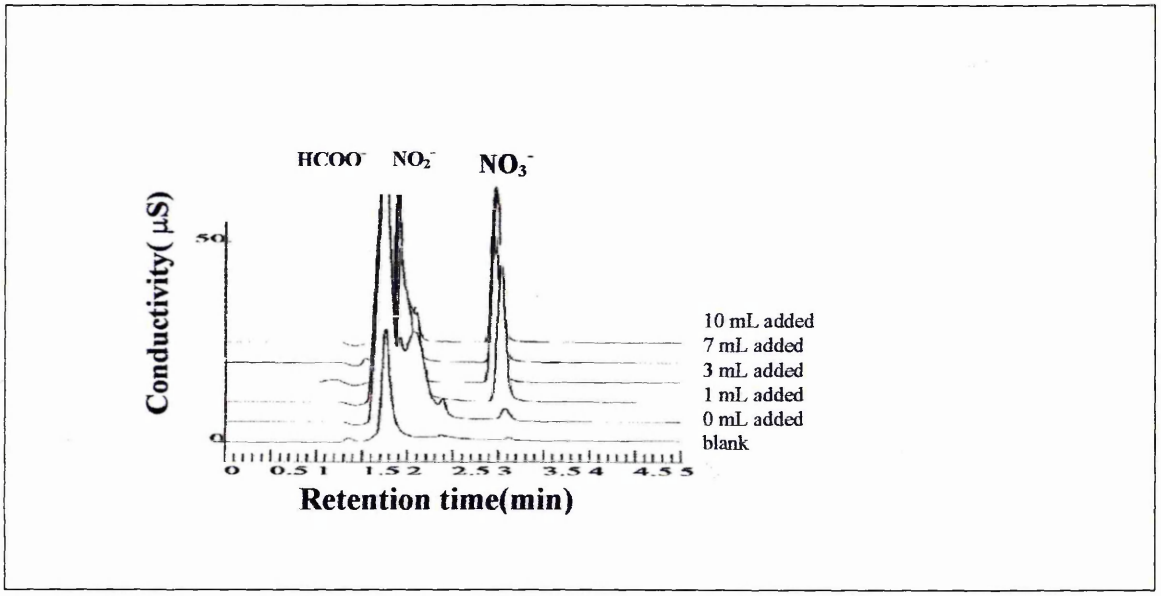


Figure 3.9 (b): Chromatogram showing the variation of the nitrate peak from ammonium chloride when different volumes of hydrogen peroxide are added to the sample.

Organic compounds such as urea, L-Lysine and L-cysteine require a two step programme in order to convert to them to nitrate and sulphate, respectively (Table 3.5).

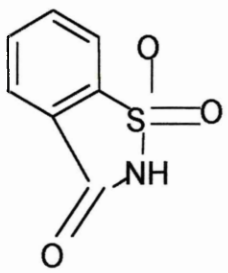
Table 3.5: Recoveries of nitrogen, phosphorus and sulphur obtained using different compounds and the modified programme.

Compound	Added (mg/l)	Found (mg/l)	Recovery (%)
Sodium sulphite(S)	25.8	26.0 ± 0.2	100.7
Sodium pyrophosphate (P)	30.9	30.9 ± 0.1	100.1
Sodium nitrite (N)	8.1	8.6 ± 0.6	106.0
Ammonium chloride(N)	40.0	42.5 ± 2.1	106.0
L-Lysine (N)	7.1	7.2 ± 0.2	101.4
Urea (N)	17.7	17.0 ± 1.2	96.0
Saccharin (N)	2.3	2.2 ± 0.1	95.6

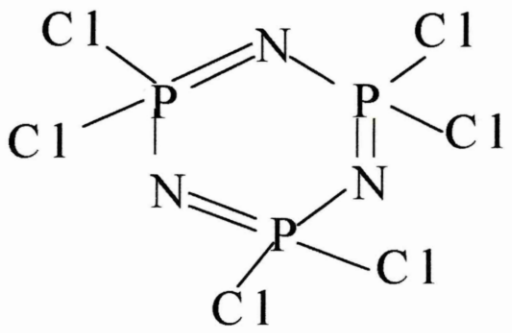
3.3.4.4.- Cyclic organic compounds:

Experiments were performed with the cyclic compounds saccharin (FW= 183.19) and phosphonitrile chloride (FW= 347.66).

Saccharin



Phosphosnitrile Chloride



In Figure 3.10 (a and b), the recoveries of saccharin in response to varying amounts of hydrogen peroxide added and the number of steps used, and the resulting chromatograms, are shown. All of the nitrogen is converted to nitrate in the third step. Sulphate is also formed in this step.

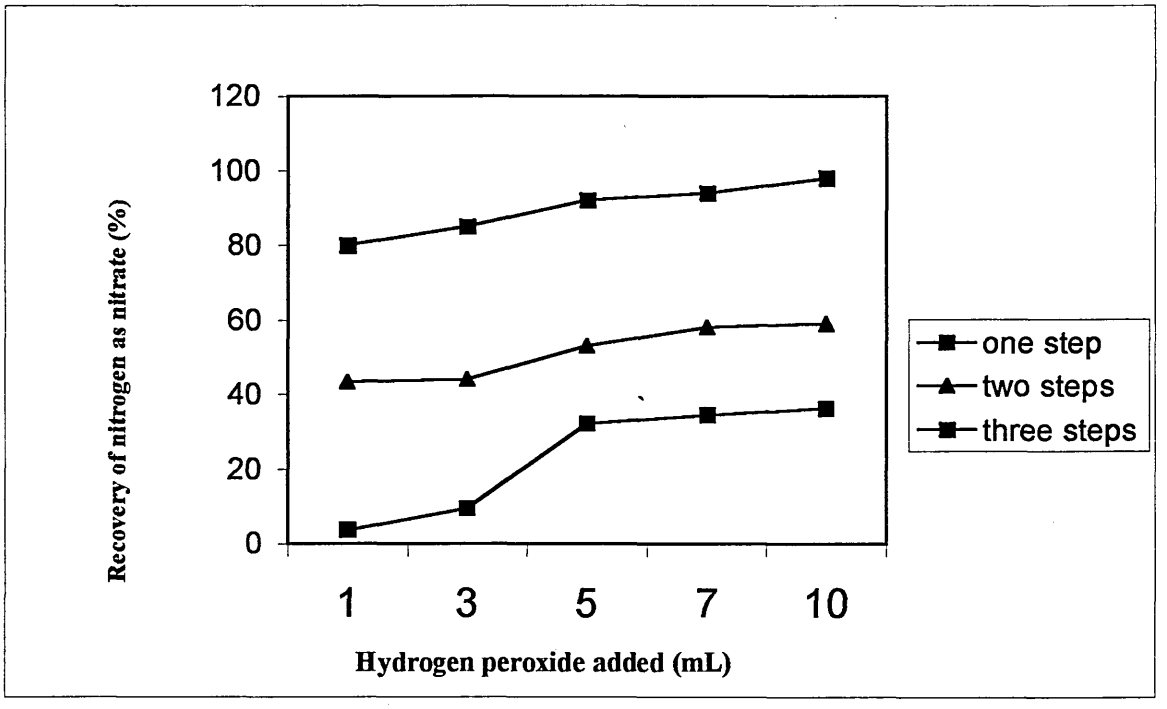


Figure 3.10 (a): Variation of the recoveries of nitrogen from saccharin when a different step program is used.

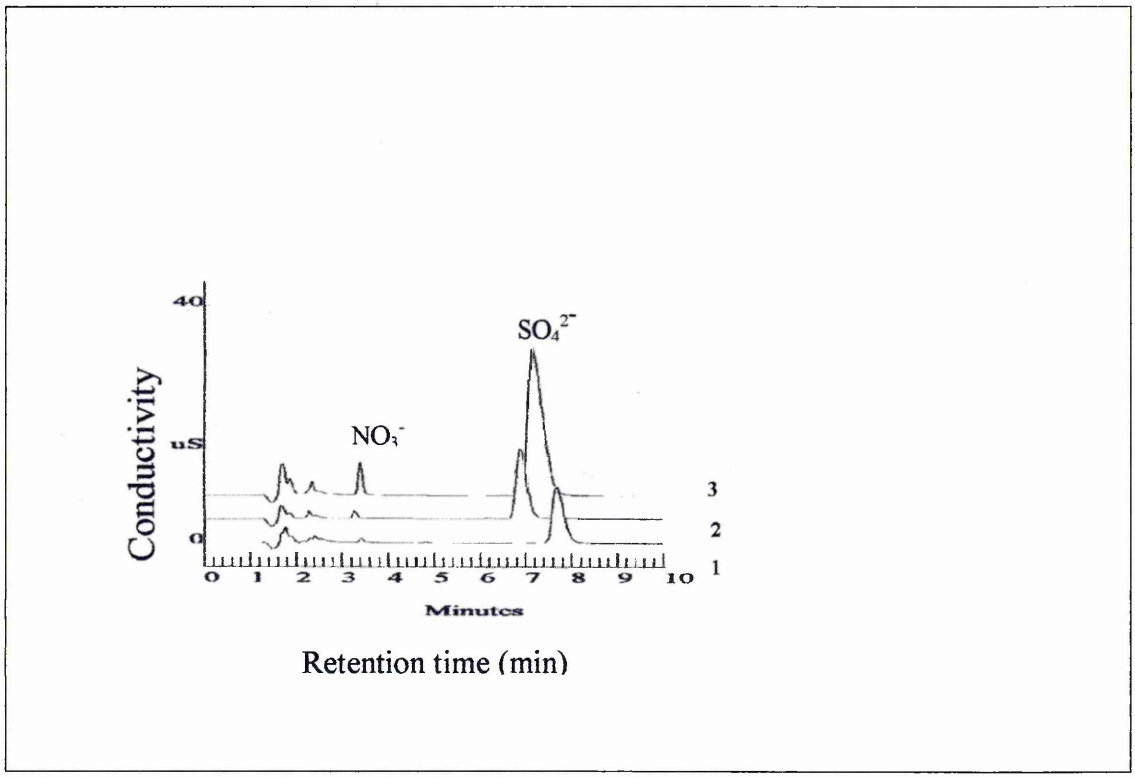


Figure 3.10(b) Chromatogram of the saccharin solution after oxidation.

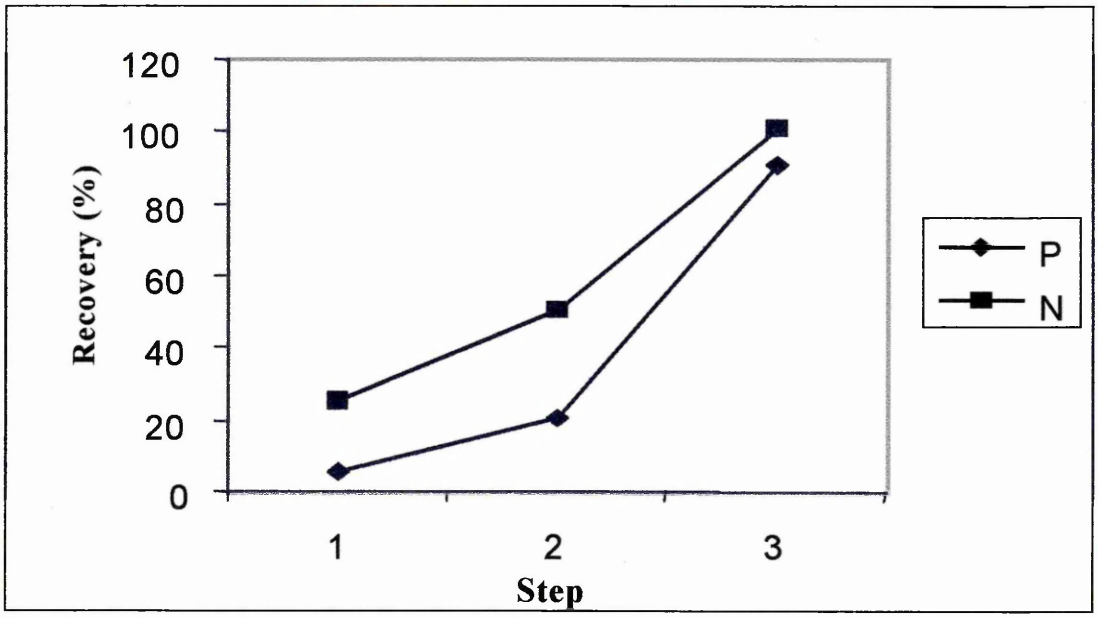


Figure 3.11: Recoveries of phosphorus and nitrogen from a solution of phosphonitrile chloride using a three step program method.

Figure 3.11 shows a graph of percentage of conversion of N and P from a solution of 51.4 mg L^{-1} digested in one, two and three steps. Three steps are required to convert all the phosphorus in phosphonitrile chloride to phosphate.

The Table 3.6. shows the recovery of nitrogen and sulphur from a mixture of nitrite, urea and saccharin and a mixture of L-lysine, saccharin and cysteine.

Table 3.6 : Recoveries of nitrogen obtained from two mixtures : Mixture 1 : containing 12.71 mg-N.L^{-1} as nitrite, 9.72 mg-N.L^{-1} urea and 2.96 mg-N.L^{-1} saccharin; Mixture 2: 4.05 mg-N.L^{-1} as nitrite, 7.12 mg-N.L^{-1} L-lysine and 2.96 mg-N.L^{-1} saccharin respectively.

Element	One step	Two steps	Three steps	Added (mg/l)	Found (mg/l)
Mix 1 (N) (mg-N L^{-1})	18.5	22.8	25.0	25.4	25.6
Recovery(%)	72.8	89.6	100.7		
Mix 2 (N) (mg-N.L^{-1})	7.7	11.4	15.0	14.3	15.0
Recovery(%)	54.1	79.8	104.8		

The Figure 3.12 shows the presence of a sulphite peak in the first and the second steps, before the sulphate peak.

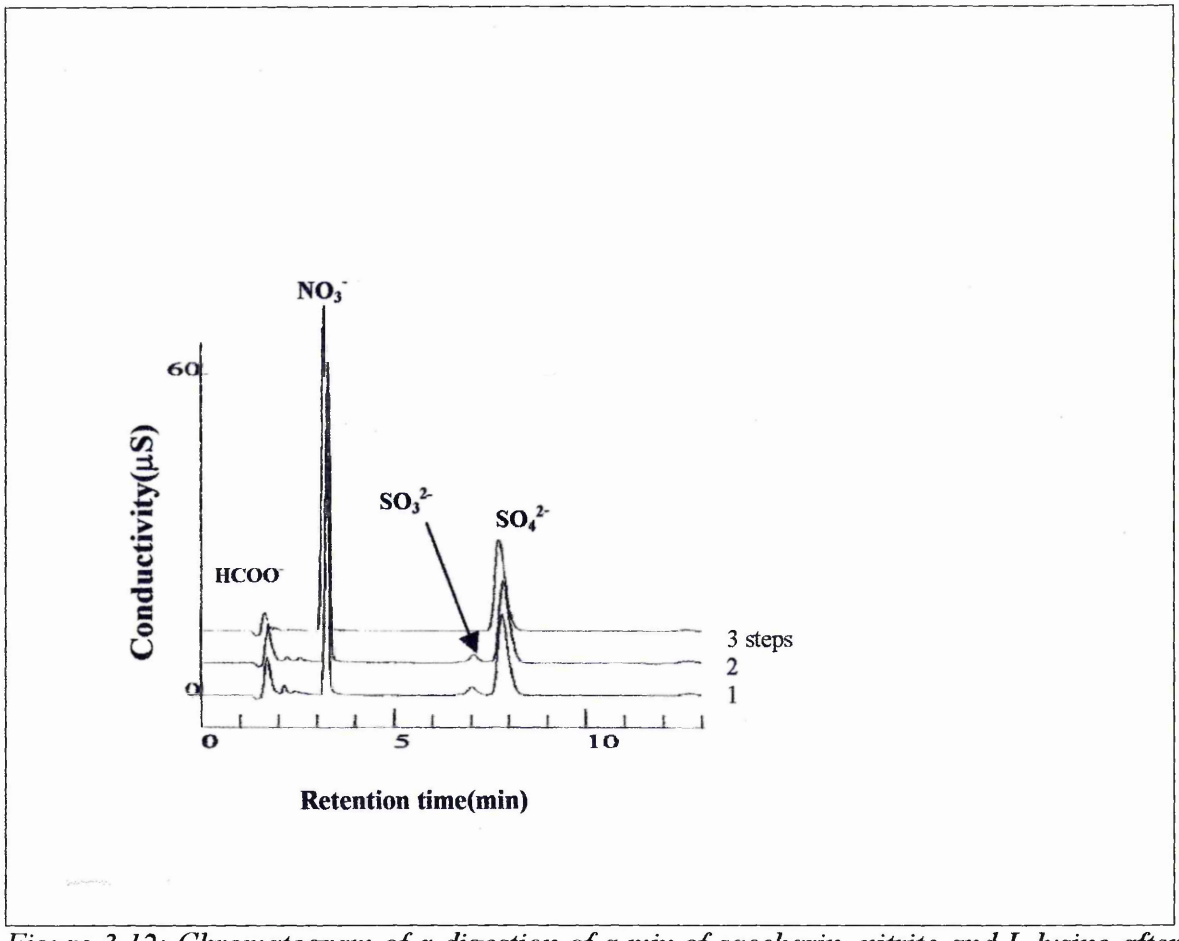


Figure 3.12: Chromatogram of a digestion of a mix of saccharin, nitrite and L-lysine after the three steps runs method.

As shown in Table 3.7 most of the nitrogen is converted to nitrate in the third step from the prawn reference material (Prawn GBW08572). An increased recovery of 70 % from the second to the third steps indicates that the nitrogen is found probably in a cyclic compounds, whereas most of the phosphorus is present as phosphate or related inorganic form.

Table 3.7: Recoveries of nitrogen and phosphorus obtained from a reference material (Prawn GBW08572) using the modified programme.

Element	One step(%)	Two steps(%)	Three steps(%)	Added (%w/w)	Found (%w/w)
N	21.5	25.0	95.9	14.1	13.6
P	34.8	93.6	108	0.85	0.91

In order to ensure that there are not cyclic compounds present, if is advisable to carry out the three step digestion programme so that all nitrogen is converted to nitrate. The varying amount of recovery after each step is maybe indicative of the nature of the compounds present in the sample. On the basis of this difference in behaviour it maybe possible to obtain speciation information from the samples.

3.3.4.5.- Environmental results.

The method for nitrogen, phosphorus and sulphur total content was applied to the samples of water and lyophilised sediment from Lake Maracaibo, and the ion chromatographic determinations were validated.

Table 3.8: Results of the reference materials (Rain water LGC 6018) for ion chromatography.

Element	Rain Water (found) ($mg.L^{-1}$)	Rain Water (RM) ($mg.L^{-1}$)
N	1.1 ± 0.1	1.0 ± 0.3
S	4.8 ± 0.4	5.3 ± 0.2

**SD: Standard deviation*

Table 3.9: Results of the total ($mg.L^{-1}$) nitrogen, phosphorus and sulphur in water samples from Lake Maracaibo determined by the three steps program.

Sampling points	N (mg/L)	SD	P (mg/L)	SD	S(mg/L)	SD
PR	2.2	0.2	<1.0		24.7	0.8
SC	1.6	0.0	<1.0		22.6	1.4
D-2	9.5	0.4	<1.0		18.4	0.7
D-4	76.8	0.9	4.7	0.1	54.3	0.5
D5a	11.6	0.6	6.3	5.9	53.0	1.4
NO2	135.0	7.8	1.2	0.1	270.3	9.6
O-13	75.7	1.1	<1.0		76.3	3.1
O-20	101.4	0.4	<1.0		733.0	122.3
C-1	19.8	1.3	<1.0		187.6	15.5
C-11	60.5	6.0	10.8	1.7	250.5	22.1
C-9	63.7	4.4	6.5	0.6	942.4	37.7
CA-2	109.0	2.9	8.1	2.2	239.0	19.4
D-33	64.2	0.8	10.6	1.4	140.0	1.3
D-119	126.1	1.9	<1.0		575.7	99.7
D114	97.4	0.1	<1.0		1009.0	10.9
S-6	124.0	1.9	<1.0		128.1	12.5
Guam	75.6	0.5	20.3	0.1	1227.6	121.9
D-74	5.40	0.5	1.2	0.0	1.06	0.0

with two reference materials for nitrate, phosphate and sulphate in rain water and in river water, giving a good agreement. The results are shown in Tables 3.8 and 3.9.

The forms of greatest interest in waters are nitrate, nitrite, ammonia and organic nitrogen, and all can be oxidised by the hydrogen peroxide in the proposed method for total nitrogen.

Nitrogen levels in aquatic system, as with phosphorus, are intimately linked with excessive algal growth, as are seen in Lake Maracaibo. Total nitrogen levels in waters can vary from as low as 0.1 mg L^{-1} to in excess of 10 mgL^{-1} in heavily polluted aquatic systems (58). These total nitrogen levels are exceeded in most of the sampling points in Lake Maracaibo, for which total nitrogen varies in the range 1.6 – 135.0 mg/L.

Apart from the natural input of nitrogen from rainfall, the main inputs of nitrogenous matter into freshwater is from agricultural land (58), via wastewater point discharges or diffuse runoff. Lake Maracaibo also receives wastewater discharges from the cities of Maracaibo, Cabimas and Santa Rita, without any pre-treatment. Most of the area around the Lake is covered by farms that also can contribute to the nitrogen input to the lake.

Total phosphorus in waters can vary from less than 0.01 mgL^{-1} in uncontaminated waters to over 1 mgL^{-1} in heavily polluted rivers (73). Nitrogen may be a limiting nutrient in some situations (74) but phosphorus is generally regarded as the limiting nutrient for primary production (75), as shown by the results determined in Lake Maracaibo. Excessive loading of phosphorus in its various physico-chemical forms is known to be causal factor in the eutrophication of waters . Furthermore, classification of the trophic status of standing water bodies is still largely based on the total phosphorus concentration (76). The maximum levels of phosphorus encountered in Lake Maracaibo (ca. 20.3 mg L^{-1}) correspond to a

general level of productivity of a hyper-eutrophic lake, in terms of the Vollenweider classification, modified by Wetzel (77), as shown in study of this lake during 1998 (78).

Table 3.10: Total nitrogen, phosphorus and sulphur ($\mu molg^{-1}$) found in sediments during the sampling of Lake Maracaibo and determined by the three step program.

Sampling points	N	SD	P	SD	S	SD
PR	3.9	0.3	<0.03		320.5	12.0
SC	0.9	0.4	<0.03		33.0	3.0
D-2	3.2	0.3	<0.03		287.8	39.1
D-4	4.8	0.3	<0.03		770.3	29.0
D5a	1.7	0.1	1.2	0.1	11.7	1.20
NO2	5.2	0.3	1.3	0.1	470.9	11.6
O-13	2.6	0.1	9.5	0.1	1719.2	35.4
O-20	10.6	1.1	40.6	3.9	1947.3	40.5
C-1	4.2	0.4	31.1	0.3	132.8	13.4
C-11	9.6	1.9	12.9	1.2	1248.0	27.6
C-9	8.1	1.5	15.0	1.0	1176.9	69.5
CA2	0.1	0.1	27.1	1.0	1412.3	30.6
D-33	1.0	0.1	10.4	1.0	1975.6	91.1

The values for total nitrogen content in water and sediments, are very high if they are compared with a subtropical bay in Oahu, Hawaii, for example, where Stimson and Larned determined the nitrogen efflux from the sediments (79). The maximum concentrations of the dissolved nitrogen in positions close to the sediments were in the range of 0.38 – 0.72 μM. The concentration in water one meter in depth in Lake Maracaibo exceeds this range, but the concentration of total phosphorus was lower (except O-20) than those found in

other lakes such as Lannngjon, Flaten and Gommaren in Sweeden where the range of total phosphorus is [36.2 – 62.6 $\mu mol.g^{-1}$] (80).

Sediments can accumulate sulphur in the range of 132.8 ($\mu mol.g^{-1}$) where pyrite is the most common mineral form of sulphur (81). In Lake Maracaibo, the concentration of sulphur is in the range 11.6 – 1975 $\mu mol.g^{-1}$ and these concentrations could be associated with the intrusion of salt waters from the Caribbean Sea to the lake. It is also characteristic of depletion of dissolved oxygen, with concentrations of oxygen around zero $mg.L^{-1}$ in the centre. In this zone, sulphur occurs as a reducible form of mostly HS^-, and it can result in the precipitation of metals such as Hg, Pb and Se.

3.4.- CONCLUSIONS

The proposed method for the microwave oxidation with hydrogen peroxide of nitrogen, phosphorus and sulphur followed by the determination of the nitrate, phosphate and sulphate ion by ion chromatography gave satisfactory results for the compounds tested, however, this method was modified in order to applied the methodology in more recalcitrant compounds where nitrogen, phosphorus and sulphur atoms were in ring system. The amount of recovery after each step in the modified method could be indicative of the nature of the compounds present in the sample. The results of Lake Maracaibo showed high concentrations of the three elements (N,P and S) in the samples of water and sediment. Lake Maracaibo can be classified as a hyper-eutrophic lake because the high concentrations of phosphorus.

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CHAPTER IV

Distribution of metals in samples from Lake Maracaibo

4.1.- INTRODUCTION

4.1.1.- Distribution of metals in sediments

The sediments represent the major sink for material in the aquatic environment. The main pathway to the sediments is the deposition of suspended particles. Such particles may only be in transit though the ocean from continental origin or be formed in situ by chemical and biological processes. Sinking particles can scavenge material from solution. The formation of marine sediments depends upon chemical, biological, geological, and physical influences. There are four distinct processes that are important in the formation of sediments: a) the source of the material b) the material and its distribution which is influenced by the transportation history, c) the deposition process that must include particle formation and alteration in the water column and d) the diagenesis which is a process that occurs after deposition.

The components of sediments are classified according to origin : Lithogenous materials which are those that come from the continents as a result of weathering processes. The most important components in the lithogenous fraction being quartz and the clay minerals (kaolinite, illite, montmorillonite and chlorite) (1). Kaolinite typifies intense weathering observed in tropical and desert conditions. Therefore, it is relatively enriched in equatorial regions. Hydrogenous components are those produced abiotally within the water column. Biogenous material is produced by the fixation of mineral phases by marine organisms. There are two further minor components which are cosmogenous material, derived from extraterrestrial sources, and anthropogenic components, notably heavy metals which can have a significant influence on sediments in coastal environments (1).

4.1.1.1.- Chemical and physical process in sediments

4.1.1.1.1.-Chemistry of particles:

Surface charge:

Particles in seawater tend to exhibit a negative surface charge. There are several mechanisms by which particles can develop this charge. It can be produced by crystal defects (i.e. vacant cation positions) or cation substitution. For example, clay minerals are layered structures of octahedral AlO_6 and tetrahedral SiO_4, substitution of Mg^{II} and Fe^{II} for the Al^{III} in octahedral sites or replacement of Si^{IV} can cause net negative charge. Also a charge can result from differential dissolution of an electrolytic salt as barite ($BaSO_4$) and finally, organic material can be negatively charged because they possess acidic functional groups (2).

Adsorption processes can also lead to the development of a negatively charged particle surface. One example is the specific adsorption of anionic organic compounds onto surfaces of particles. Another mechanism relates to the acid base behaviour of oxides in suspension. Metal oxides (most commonly Fe, Mn) and clay minerals have frayed edges resulting from broken metal-oxygen bonds; the surfaces can be hydrolysed and exhibit amphoteric behaviour:

$$X\text{---}O^-_{(s)} + H^+_{(aq)} \rightleftharpoons X\text{----}OH_{(s)}$$

$$2X\text{---}OH^-_{(s)} + 2\,H^+_{(aq} \rightleftharpoons 2\,X\text{--}\,OH_2{}^+_{(s)}$$

The hydroxide surface exhibits a different charge depending on the pH.

Adsorption process:

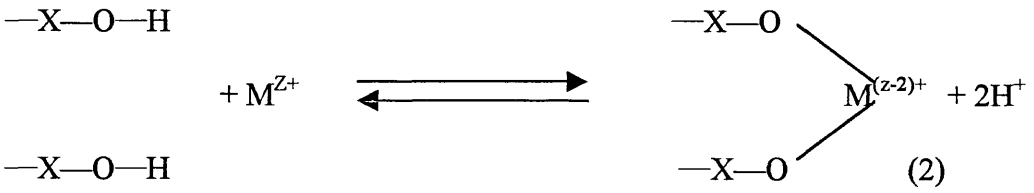
Physical or non-specific adsorption involves relatively weak attractive forces, such as electrostatic attraction and van der Waals forces. Adsorbed species retain their

coordinated sphere of water and, hence, cannot approach the surface closer than the radius of the hydrated ion. Adsorption is favoured by ions having a high charge density, i.e., trivalent ions in preference to univalent ones. Additionally, an entropy effect promotes the physical adsorption of polymeric species, such as Al and Fe oxides, because a large number of water molecules and monomeric species is displaced. Chemisorption or specific adsorption involves greater forces of attraction than physical adsorption. As hydrogen bonding or π orbital interactions are utilised, the adsorbed species lose their hydrated spheres and can approach the surface as close as the ionic radius.

Hydrated oxide surfaces have sites that are either negatively charged or readily deprotonated . The oxygen atoms tend to be available for bond formation, a favourable process for transition metals; an incoming metal ion, M^{Z+}, may eliminate an H^+ ion as:

$$—X—O—H + M^{Z+} \rightleftharpoons —X—O—M^{(z-1)+} + H^+ \quad (1)$$

Alternatively , two or more H+ ions may be displaced forming a chelate as shown below:



(2)

A metal complex, ML_n^{z+}, may be coordinated instead of a free ion by displacement of one or more H^+ ions in a manner analogous to the above reaction. In addition, the metal complex might eliminate a hydroxide group, giving rise to a metal-metal bond as:

$$—X—O—H + ML_n^{z+} \rightleftharpoons —X— ML_n^{(z-1)+} + OH^- \quad (3)$$

Ion exchange reactions:

Both mineral particles and particulate organic material can take up cations and release an equivalent amount of another cation into solution; this process is termed cation exchange. There are factors that influence the affinity of cations towards a given surface; the surface coverage increases as a function of cation concentration. The affinity for the exchange site is enhanced as the oxidation state and the charge density of the hydrated cation increase. In order of increasing charge density, the group I and II cations are: Ba<Sr<Ca<Mg<Cs<Rb<K<Na<Li

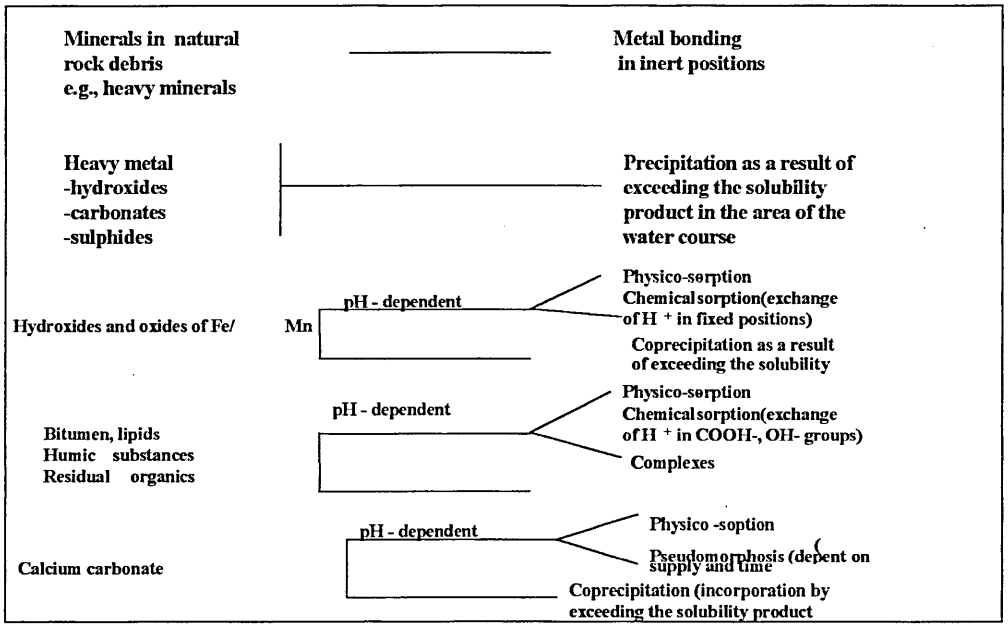
4.1.1.2. Association in sediments

There are four types of association in the bonding process of sediment formation (3):

1.- Adsorptive bonding ; 2.- Coprecipitation by hydrated iron and manganese oxides; 3.-Complexation by organic molecules and 4.-Incorporation in crystalline minerals.

These four types of association have been expanded by Forstner and Pachineelam (4) as in Table 1., which includes all of main types of association both in natural and polluted water systems.

Table 4.1: Carrier substances and mechanisms of heavy metal bonding (3)



Incorporation in mineral crystals: Heavy metals, as major, minor or trace components, can be transported and deposited in the mineral substances of natural rock debris. The silicate minerals feldspar and quartz usually have very low heavy metal contents. The distribution of elements in minerals is determined by the physico-chemistry of the source medium (magma, lava, aqueous solution) and by crystal-chemical factors, i.e., ionic radius, valence and electron configuration (4)

Precipitation and co-precipitation: The heavy metals can be precipitated as hydroxides, carbonates and sulphides when their solubility product is exceeded. The process of precipitation of metal hydroxides results in different forms that can also coprecipitate or redissolve. The heavy metal sulphides are practically insoluble at neutral pH; in addition, the solubility of carbonates in aqueous solution is dependent on the CO_2 partial pressure.

Cation exchange and adsorption: A number of sediment-forming materials with a large surface area are known, particularly clay minerals, freshly precipitated iron hydroxides, amorphous silica acids, and also organic substances. All these are capable of sorbing cations from solution and releasing equivalent amounts of other cations into the solution by ion exchange. In addition, all fine-grained materials with a large surface area are capable of accumulating heavy metals ions at the solid liquid interface as a result of intermolecular forces by adsorption phenomena.

4.1.3. Heavy metals in lakes and estuaries

4.1.3.1.- Heavy metals in lakes:

There are a number of studies of heavy metal pollution in lakes carried out in North America and Europe (5). There are three main biogeochemical processes that affect the cycling of trace elements in lakes: the algal production and degradation, Fe and Mn redox cycling, and sulphide precipitation. Other important factors include trace metal

complexation with ligands (e.g. carbonate and humic substances) and the effects of competition for surface exchange sites arising out of the increased cation (e.g. Ca^{2+}. NH_4^+) concentrations that occur in anoxic water lakes (6). In well-oxygenated waters, the stable forms of Fe and Mn are their higher oxidation states [Fe(III) and Mn(III/IV)], which are completely hydrolyzed oxides present as colloids or particles. Where oxygen is absent the lower redox states, Fe(II) and Mn(II), are favoured. These divalent ions are very soluble in the pH range of 4-8. In seasonal or permanently stratified lakes, where the bottom is anoxic, Fe(II) and Mn(II) can accumulate.

The removal of trace elements as sulphides in anoxic lakes has been widely recognized. Elements for which sulphide precipitation is an important factor in their accumulation in sediments include As, Cd, Cu, Co, Ni, Pb and Zn (7)

The Great Lakes region represents one of the most important reservoirs in the world and includes nine states of the USA; there are studies on trace element pollution and its impact in this aquatic system (5); for example, studies of Batterson and McNabb (8) in Lake Lansing, Michigan showed that arsenic as arsenite becomes oxidized to arsenate, As(V), in aerobic epilimnetic water; in this portion of the lake, arsenate exists as the anion $HAsO_4^{2-}$ and this, like phosphate, can be adsorbed, occluded or precipitated with hydrous ferric oxides; thus ferric iron controls arsenate solubility in oxic portions of a lake basin. The turbulent dispersion and convection of the lake can transport some of the arsenate and metal complexes into the oxygen-depleted hypolimnion; once there, reduction of these species is likely to take place in the water or on the surface of anaerobic sediments. Depending on the pH, Eh, iron and sulphur concentrations, arsenite, insoluble arsenic sulphides or ferrous arsenic sulphides could result. Ferguson and Anderson (9) report that at low Eh in the presence of S^{2-}, As(III) should be effectively removed from the water column as insoluble sulphides. This trace element

isomorphous replacement into the crystalline lattice of Fe sulphides can influence its concentrations in anoxic waters (10). Trace elements associated with Fe monosulphides can be released and redistributed during their transformation to pyrite (11); naturally occuring Fe sulphides can adsorb Au, Cd, Cu, Pb and Zn and can play an important role in metal deposition and formation of deposits (12). However, Agget and O'Brien (13), in their study of Lake Ohakuri showed that the most important mechanism for the adsorption of arsenic at the sediment-water interface was adsorption onto hydroxyiron (III) species, and thus there is no evidence for the precipitation of arsenious sulphide as was suggested by Ferguson and Anderson (9).

Studies by J. Hlavay and K. Polyák (14) of Lake Balaton, Hungary, the largest lake in Central Europe, showed that significant amounts of Pb were found mainly in the acid soluble- fraction, bound to organic matter-, and sulphide- fractions. Similar results were found in studies by Stalikas *et al* (15) on soils irrigated by lake waters; lead was mainly associated with the carbonate, organic and residual phases, vanadium linked to the residual phase, and arsenic associated with carbonate and residual phases.

4.1.3.2.- Heavy metals in estuaries:

Estuaries and the associated offshore areas are the sites of most of the great fisheries of the world. The estuaries also, being inshore, are more sensitive than any other marine environment to the influence of pollution from man. Estuarine sediments consist of several geochemical phases such as carbonates, iron and manganese oxides, organic matter and clays. These diverse components that constitute the sediment matrix do not usually exist as separate particles but rather as aggregates and act as reservoirs of trace metals in the environment (16).

The oxides of Fe Mn, Al and Si, together with the reactive particulate organic matter and clays, provide the sorption sites for dissolved metals. Under oxic conditions, Fe and

Mn oxides, together with organic matter, are generally regarded as the most important scavenging or carrier phases for the labile trace element fraction in this aquatic environment. Competition among metals for the available ligand, can also substantially influence the sorption of a metal.

Ngiam and Lim (17) have reported the determination of Cu, Pb, Zn, Cd, Fe and Mn in tropical estuarine anoxic and oxidized sediments by a sequential extraction scheme. It was concluded that these metals (except Cu) existed mostly as sulphides in the organic/sulphide fraction in anoxic sediments. Balkis and Gagatay (18) studied the Erdek Bay (Turkey), an estuary with oxic surface sediments; the results of the sequential scheme indicated that the metals investigated (Pb,Cu, Zn, Ni and Cr) were mainly associated with the residual aluminosilicate-mineral phases. Lead was also associated with the Fe/Mn oxyhydroxide phase. The percentage of Pb associated with the different fractions in the Pearl River Estuary (China) (19) was in the following order: residual > Fe-Mn oxide> organic>carbonate>exchangeable, using the sequential extraction procedure of Tessier referred to in Chapter I. Arsenic in sediments from the Humber Estuary (UK)(20) was associated with the iron and clay fraction.

4.1.4.- Analytical techniques used for the determination of metals

4.1.4.1.- Inductively coupled plasma atomic emission spectrometry

An ICP (Inductively coupled plasma) is a plasma sustained in a quartz torch placed in a radio frequency (27.12 MHz) oscillating magnetic field. Argon is chosen as the plasma gas for its inertness, optical transparence in the UV-VIS part of the spectrum, high first ionization energy and moderate low thermal conductivity, so that heat is retained within the plasma fireball, sustaining stable operation of moderate power inputs (20). The

temperature in the plasma reaches up to 8000 °C. The vortex flow of coolant gas prevents the torch from melting.

4.1.4.2.- Inductively coupled plasma mass spectrometry

An ICP argon plasma is used in this technique as the ion source. Plasma gases are extracted through an orifice into a chamber held at 1 torr and then passed into a mass analyzer for dispersion and measurement.

An ICP mass spectrometer is composed for (1) a sample introduction system, (2) an argon plasma torch configured at 90 ° with respect to the conventional ICP AES operation, thus allowing plasma gases to be sampled through an orifice, via differential pumping unit into the quadrupole mass filter and (3) a quadrupole mass spectrometer and associated data collection electronics which permit rapid scanning of selected mass ranges between 0 and 300 daltons. Atomization source may be placed in a glove box for a radiactive materials analysis (21). The sample is usually taken up in solution and introduced into the plasma via a pneumatic nebulizer and a conventional spray chamber. The salt load is limited to ca 0.2- 1 % by sampling orifice clogging. Ion which pass through the quadrupole are detected by an electron multiplier. Output pulses are fed to a multi-channel are swept synchronously with the mass scan. Depending on the number of elements with rapid switching between them, or scanning mode over the whole mass range or pre-selected parts of it, can be used. ICP-MS is a versatile, sensitive analytical technique which offers a simple approach to the analysis of a wide variety of metals in a variety of biological materials.

4.1.4.3.- X-ray fluorescence spectrometry (XRF)

Bombardment of an atom with high energy photons, electrons or photons induces removal of inner electrons (from K, L or M shells). The orbital vacancies formed are

filled by outer orbital electrons giving rise to the emission of X-ray photons. The measurement of their energy (wavelength) and intensity forms the basis of XRF techniques. They are usually divided according to the design and principle of operation of the spectrometer into wavelelngth dispersive XRF (WDXRF), energy dispersive XRF(EDXRF) and, a modification of the latter, total reflection XRF(TXRF). Since there is a simple relationship between wavelength and energy the same basic type of information is provided and the same character of interferences encountered. There are significant differences in terms of sensitivity, selectivity, versatility and speed. XRF methods are usually applied to direct analysis, sample pre-treatment is often required to enhance its performance. Solid homogeneous samples as metals, glasses, ceramics or polymers disks can be analyzed directly or after polishing the surface. Signal intensity depends on particle shape and size, particle size distribution and packing density so these must be kept uniform. Inhomogeneity and particle size problems can further overcome by fusion of the material, usually with Li borate fluxes, to give smooth surfaced amorphous glass disks (20,22).

4.1.5.- Principal Component Analysis(PCA)

One consequence of automation is that many variables can be determined simultaneously for the same sample, for example with inductively coupled plasma emission technique, various metals can be measured simultaneously. The set of measurements which is used to characterise the sample is called the pattern. When only two variables are measured for each sample the pattern can be represented graphically by a point where the co-ordinates of the point are the values taken by the two variables (23). This point can also be defined by a vector, drawn to it from the origin and known as a pattern or data vector; the co-ordinate system is known as the pattern space. The basis of all pattern recognition methods is the pattern vector for similar samples lie

close together in the pattern space, forming clusters. However, when more than two variables are measured, graphical representation is no longer possible; if n- variables are measured, each variable will be represented by a point in n-dimensional space and mathematical methods are needed to detect clustering. One such method, known as principal component analysis (PCA), allow the pattern vectors to be projected onto a plane in such a way that as little information as possible is lost. The most important use of the PCA (as factor analysis) is to represent the n-dimensional data structure in a smaller number of dimension usually two or three. This permit one to observe grouping of objects, outliers, etc which define the structure of a data set.

In this work, the distribution of metals in Lake Maracaibo has been studied. The total content of major cations and trace elements was determined after digestion by ICP-AES and ICP-MS, respectively. Oxides were determined by X-Ray Fluorescence Spectrometry. The distribution of six metals (arsenic, selenium, lead, tin, mercury and vanadium), by sequential extraction scheme using the BCR protocol of Davidson et al (24) was determined in the three aquatic systems of the Lake Maracaibo,i.e. the Estuary or Tablazo Bay; the Maracaibo Strait, and the main zone of the lake. Some correlations between the total concentration of metals and the physicochemical parameters or total concentrations of nutrients were found. Principal component analysis was used to determine the most polluted sampling points in the lake.

4.2.- MATERIAL AND METHODS

4.2.1.: Determination of major cations

The total content of the major cations sodium, potassium, calcium, magnesium, iron and manganese in sediments were determined as follows by a method which is based on method ISO 11466, using *aqua regia* as the extraction medium.

4.2.1.1.- Procedure

3 g of sample was introduced into a 250 mL reaction vessel containing roughened glass beads (2-3 mm of diameter). It was moistened with about 2mL of water and to this was added a mixture of 21 mL of hydrochloric acid (12 $molL^{-1}$, density = 1.19 $g.mL^{-1}$) followed by 7 mL of nitric acid (15.8 $mol.L^{-1}$, density = 1.42 $g.L^{-1}$). It was then connected to an absorption vessel and a condenser (lengths of aprox 340 mm) added to the reaction vessel and then allowed to stand for 16 h at room temperature. The reaction mixture was heated under reflux conditions for 2 h,. after which the heating was stopped and the system allowed to cool. The contents of the absorption vessel were added to the reaction vessel via the condenser, rinsing both the absorption and the reaction vessels with 10 mL of nitric acid (0.5 mol L^{-1}). The contents of the reaction vessel were filtered into a 100 mL volumetric flask, using 0.5 mol L^{-1} to wash the reaction vessel. It was diluted to 100 mL with 0.5 mol L^{-1} nitric acid.

4.2.1.2 Reactants

Stock solutions of 1000 mg L^{-1} sodium, potassium, calcium, magnesium, iron and manganese were purchased from Merck . Calibrations curves were prepared from these stock by dilution to 0.5 to 10 mg L^{-1}.

The following wavelength and detection limits were used for the determination of each major cations:

Metal	Wavelength(nm)	Detection limits(mg L^{-1})
Calcium	317.93	0.07
Magnesium	285.21	0.06
Iron	259.94	0.01
Potassium	766.49	0.01
Sodium	589.59	0.07
Manganese	257.61	0.01

4.2.1.3. Instruments

A ICP Spectro model P was used for the determination of the major cations using the following conditions (Table 4.2.)

Table 4.2: Instrumental conditions used during the study for the ICP-AES.

Conditions	
Rf Power/W	1500
Carrier gas flow rate ($L.min^{-1}$)	1.00
Uptake speed ($mL\ min^{-1}$)	1.0
Pump speed rps	0.12
Torch	Fassel torch
Coolant gas flow	17 $Lmin^{-1}$
Number of repetitions	3

4.2.2.- X-ray fluorescence spectrometry analysis of oxides and silicates

The fusion technique was used for sample preparation, and the instrument calibrated using theoretically based correction coefficients (22) to determine oxides and silicates in samples of sediment from Lake Maracaibo. Anaytes determined include the following Na_2O, MgO, SiO_2, P_2O_5, SO_3, K_2O, CaO, TiO_2, V_2O_5, Cr_2O_3, Mn_3O_4, Fe_2O_3, ZnO, SrO, Y_2O_3, ZrO_2, BaO and HfO_2.

4.2.2.1.-Instruments:

The spectrometer used for this study was a Philips PW2400 fitted with a rhodium target end window X-ray tube and Philips X-40 analytical software.

4.2.2.2.-Procedure

Each calibration sample was prepared by weighing, to four decimal places, the required amount of a pure salt of the element with 10.0000 g of $Li_2B_4O_7$, the total weight being sufficient to produce a bead of 40 mm diameter. The weights and suppliers of the chemicals are given in Table 4.3.

The fusion was carried out in a 95% platinum-5% gold crucible at 1,250°C . The fusion time was 12 min, with vigorous swirling after 6 and 9 min. The casting dish, which is of the same alloy as the crucible, was placed in the muffle furnace for 3 min before casting was due to take place. After casting, the bead was cooled, in the dish, over an air jet of about 1.5 L m^{-1} until the bead was seen to have separated from the casting dish. It was then moved over a second air jet of about 4 L m^{-1} and cooled further until the bead was cool enough to handle. Preparation time was approximately 17 min. Analytical lines used for each elements are given in Table 4.4.

Table 4.3.: Chemicals used for calibration samples

Standard	Chemical	Source	Weight(g)
25 % Na_2O	Na_2CO_3	Aldrich(20442-0)	0.4275
100% MgO	MgO	Aldrich(20371-8)	1.0000
100% Al_2O_3	Al_2O_3	Aldrich(20260-6)	1.0000
100% SiO_2	SiO_2	Aldrich(20435-8)	1.0000
25% P_2O_5	$(NH_4)_2HPO_4$	Aldrich(37998-0)	0.3200
5% SO_3	Li_2SO_4	Aldrich(20365-3)	0.0690
50% K_2O	K_2CO_3	Aldrich(20408-0)	0.5300
100% CaO	CaO	Aldrich(22953-9)	0.5000
10% TiO_2	TiO_2	Aldrich(20473-0)	0.1000
50%V_2O_5	V_2O_5	Aldrich(20485-4)	0.3000
25% Cr_2O_3	Cr_2O_3	Aldrich(20306-8)	0.1000
100% Mn_3O_4	MnO_2	Aldrich(20375-0)	0.9000
100% Fe_2O_3	Fe metal	99.999% purity	0.6994
25% ZnO	Zn metal	99.999% purity	0.1400
50% SrO	$Sr(NO_3)_2$	Johson Matthey Specpure	0.8000
100% Y_2O_3	Y_2O_3	Aldrich(20492-7)	1.0000
100% ZrO_2	ZrO_2	Aldrich(20499-0)	1.0000
50% BaO	$BaCO_3$	Aldrich(20271-1)	0.4000
100% HfO_2	HfO_2	Heraeus(004010)	1.0000

Table 4.4.: Measurement parameters for X-ray fluorescence spectrometry analysis

Element	Line	KV	mA	Coll(mm)*
Al	Kα	50	60	0.70
Ba	$L\beta_1$	50	60	0.15
Ca	Kα	50	60	0.15
Cr	Kα	50	60	0.15
Fe	Kα	50	60	0.15
Hf	$L\beta_1$	60	50	0.15
K	Kα	50	60	0.30
Mg	Kα	50	60	0.70
Mn	Kα	50	60	0.15
Na	Kα	50	60	0.70
P	Kα	50	60	0.30
S	Kα	50	60	0.30
Si	Kα	50	60	0.30
Sr**	Kα	60	50	0.15
Ti	Kα	50	60	0.15
V	Kα	50	60	0.15
Y	Lα	50	60	0.30
Zn	Kα	60	50	0.15
Zr	Lα	50	60	0.30

*Collimator spacing in mm

Sr** Kα measured with 0.10 mm brass filter over the x-ray tube window.

4.2.3.- Sequential extraction

4.2.3.1.- Reagents:

All the reagents were Analar grade except were stated. Acetic acid (Aristar), nitric acid (Aristar), hydrogen peroxide 30 % v/v, ammonium acetate (Analar), hydroxylammonium chloride (Analar) and ammonium acetate (Analar) were purcharsed from Merck. All the solutions were prepared using double deionized water grade II (MilliQ). Arsenic, lead, mercury, vanadium, tin and selenium 1000 mg L^{-1} stock solutions were purchased from Merck.

Standard solutions were prepared by dilution of the stock solutions with deionised water (Milli –RO/Milli-Q system from Millipore, 18 mΩ). The standards were stored at 4 ^{0}C in the dark.

The following certified and reference materials were used to validate the methodology during the determination of the metals in the different extractable phases and the total content.

Certified Materials from The National Water Research Institute of Canada (NWRI RM)Lake Ontario water (preserved in 0.2 % nitric acid):

TM 23.5, TMDA 51.4 and TM 52.4 were used

Reference Materials:

Polluted Marine Sediment IAEA 356 Reference Material was used to validate the total metals determination.

4.2.3.2.-Apparatus:

An ICP-MS (Hewlett Packard 4500) was used as detector. The instrumental conditions are summarised in Table 4.45

Table 4.5.: Instrumental conditions used during this study for the ICP-MS.

Conditions	For spectrum analysis
Rf Power/W	1200
Carrier gas flow rate ($L.min^{-1}$)	1.25
Sample Depth/mm	6.0
Pump speed rps	0.12
Uptake speed rps	0.5
Acquisition Mode	Spectrum Analysis
Acquisition Time (sec)	1.23
Torch	Fassel torch
Spray chamber	Cyclonic
Nebulizer	Babington
Coolant gas flow	10 $Lmin^{-1}$
Number of repetition	3

4.2.3.3- Procedure

The method used for the sequential extraction was that developed by Davidson et al (24):

Step one:

The exchangeable, water and acid soluble phases

A 40 mL volume of acetic acid (0.11 mol L^{-1}) was added to 1 g of dry sediment in a 100 mL centrifuge tube. The tube was shaken for 16 h (overnight) at ambient temperature on a mechanical shaker. The extract was separated from the solid residue by centrifugation at 4000 rpm. The liquid was decanted into a clean container and stored

at 4 °C for analysis. The residue was shaken for 15 min, centrifuged and washings discarded.

Step two:

The reducible (e.g. iron/ manganese oxides) phase

A 40 mL volume of hydroxylammonium chloride (0.1 $molL^{-1}$, adjusted to pH 2 with nitric acid) was added to the residue from step one. The extraction procedure was repeated as described above, i.e. the sample was shaken overnight, the extract separated by centrifugation, and the residue washed with deionised water.

Step three :

The oxidisable (e.g. organic matter and sulphides) phase

A 10 mL volume of hydrogen peroxide (8.8 mol L^{-1}) was carefully added, in small aliquots, to the residue from step two. The centrifuge tube was covered with a watch glass and the contents digested at room temperature for one hour with occasional manual shaking. Digestion was continued by heating the tube to 85°C in a water bath for one hour. The watch glass was then removed and the tube contents reduced to a small volume (1-2 mL). A second 10 mL aliquot of H_2O_2 was added and the tube was again covered and heated to 85°C for one hour. The cover was removed and the volume reduced as before. A 50 mL volume of ammonium acetate solution (1 $molL^{-1}$, adjusted to pH 2 with nitric acid) was added to the cool, moist residue. The sample was then shaken, centrifuged and the extract separated as described in step one. The solid residue was retained for microwave digestion.

Residual:

The solid residual was heated until dryness and transferred to a microwave vessel for digestion with 2:1 mixture of concentrated HCl and HNO_3 . The program used was the same for the trace element total content which is described in the following paragraph

The total content of trace elements

0.2 g of sediment was transferred in a microwave vessel, and 4 mL of concentrated HNO_3 and 1 mL of H_2O_2 (30 % v/v) were added. The vessel was transferred into the microwave and the following program was run (Table 4.6):

Table 4.6.: Microwave program used during this study

Step	Time (min)	Power(W)
1	5	250
2	2	0
3	5	400
4	2	0
5	5	500
6	2	0
7	5	600
8	10	Ventilation

After the run, the vessel was cooled and opened, 1 mL of nitric acid and 0.25 mL of hydrogen peroxide were added. The microwave programme was then run again. The vessel was cooled, opened and the contents transferred to a 25 mL volumetric flask and made up to volume with deionised water. The trace elements were determined using a ICP-MS (conditions described before). The following isotopes and calibration curves were used.

Metal	Isotope(m/z)	Calibration curve
Chromium	53	$y = 6.0x10^4X + 8.2x10^3$
Cobalt	59	$y = 5.9x10^4X + 2.4x10^4$
Nickel	60	$y = 1.3x10^4X + 1.0x10^4$
Copper	63	$y = 3.1x10^4X + 4.8x10^4$
Zinc	66	$y = 9.64x10^3X + 9.96x10^3$
Arsenic	75	$y = 7.1x10^3X - 3.4x10^3$
Selenium	82	$y = 7.7x10^2X + 1.6x10^2$
Cadmium	111	$y = 9.5x10^3X + 3.8x10^3$
Tin	118	$y = 1.9x10^4X - 2.0x10^4$
Mercury	202	$y = 8.0x10^3X + 4.3x10^3$
Lead	208	$y = 5.4x10^4X + 3.9x10^3$

Vanadium and titanium were measured by ICP-AES at a wavelength of 311.07 nm and 334.94 nm respectively.

The method was validated with the reference material RM IAEA–356 estuarine sediment.

4.3.- RESULTS AND DISCUSSION

4.3.1.-Major cations and trace elements :

4.3.1.1. Major Cations:

Table 4.7. shows the results for the major cations found in the background analysis of the Lake Maracaibo.

Table 4.7.: Concentrations of the major cations (mg.Kg^{-1}) found in sediments during the sampling of the Lake Maracaibo.

Sampling point	Na	K	Ca	Al	Mg	Mn	Fe
PR	8.48E+02	6.91E+02	9.65E+02	3.24E+03	1.38E+03	7.26E+01	1.00E+04
SC	1.51E+03	6.24E+02	1.93E+04	2.95E+03	1.16E+03	1.38E+02	9.95E+03
D-2	1.46E+03	1.01E+03	1.48E+03	4.47E+03	1.80E+03	1.09E+02	1.09E+04
D-4	1.78E+03	1.11E+03	1.68E+03	4.37E+03	2.16E+03	1.36E+02	1.07E+04
D-5a	2.88E+02	5.58E+01	2.43E+02	1.13E+02	6.76E+01	3.82E+00	3.17E+02
NO2	1.78E+03	1.11E+03	1.68E+03	4.37E+03	2.16E+03	1.36E+02	1.07E+04
0-13	1.66E+03	1.14E+03	8.00E+02	5.49E+03	1.70E+03	1.71E+02	1.14E+04
O-20	1.87E+03	1.27E+03	7.60E+02	6.84E+03	1.89E+03	3.25E+02	1.22E+04
C-1	7.82E+02	4.37E+02	4.67E+02	2.80E+03	1.03E+02	1.15E+02	1.66E+04
C-11	1.62E+03	8.45E+02	5.21E+02	4.25E+03	1.17E+03	5.76E+02	6.59E+03
C-9	1.71E+03	5.04E+02	1.04E+03	2.49E+03	9.14E+02	1.71E+03	5.32E+03
CA-2	9.99E+02	8.32E+02	6.08E+02	4.45E+03	1.18E+03	7.24E+01	1.04E+04
D-33	1.87E+03	1.43E+03	9.20E+02	8.91E+03	2.01E+03	3.69E+02	1.39E+04

Correlations:

The Pearson product moment correlations between each pair of variables (25) were calculated. These correlation coefficients range between -1and +1 and measure the

strength of the linear relationship between the variables. P-values below 0.05 indicate statistically significant non-zero correlations at the 95%confidence level. The following pairs of variables have P-values below 0.05: Na and K, Na and Al; Na and Mg; K and Al; K and Mg; K and Fe; Al and Mg; Al and Fe.

Correlations between Na and K were found; both elements belong to group I of the periodic table. Their compounds have similar solubilities but their geochemical behaviour differs in a number of ways. Sodium and calcium undergo isomorphous replacement in silicates because of their similar ionic radii, whereas potassium is found in separate primary igneous minerals. The weathered potassium silicates release potassium ions but these are even more strongly adsorbed by negatively charged clays and organic colloids than sodium ions (26). Unlike sodium, the potassium is readily reincoporated into silicate structures with the formation of clay minerals and its concentration in biological material is about 15 times greater that of sodium. As a consequence the concentration of potassium in fresh waters is one-third that of sodium.

4.3.1.2.- Trace elements, total content in waters

Data in Tables 4.8, 4.9 and 4.10 represent the checking of the accuracy of the method using three different water reference materials for metal determination. A good agreement were found for all these reference materials ($p<0.05$) when the results were compared with those obtained for the metals in the present study.

Table 4.8: Results for the reference material TM 23.2

Metal	Found ($\mu g.L^{-1}$)	Certified ($\mu g.L^{-1}$)
As	9.4 ± 0.1	8.5±1.6
Cr	5.8±0.1	6.5±1.4
Pb	3.4±0.0	3.8±1.0
Se	5.0±0.1	4.2±1.4
V	2.2±0.1	2.1±0.7

Table 4.9: Results for the reference material TMDA 51.2

Metal	Found ($\mu g.L^{-1}$)	Certified ($\mu g.L^{-1}$)
As	17.5±0.3	15.3±3.4
Cr	58.2±1.1	62.5±6.6
Pb	68.6±1.5	72.9±10.6
Se	11.7±0.3	12.0±3.0
V	48.8±1.1	47.6 3.0

Table 4.10: Results for the reference material TMDA 54.2

Metal	Found ($\mu g.L^{-1}$)	Certified ($\mu g.L^{-1}$)
As	28.0±0.4	25.0±4.2
Cr	395.8±5.0	432.0±32.1
Pb	493.1±9.3	531.0±54.4
Se	15.2±0.2	15.0±3.0
V	342.0±0.2	343.0±26.2

The metal content found in water samples from Lake Maracaibo is shown in Table 4.11.

Table 4.11: Mean concentrations of dissolved arsenic, chromium, lead, tin, vanadium, mercury and selenium in water samples from Lake Maracaibo ($\mu g.L^{-1}$):

Site	As	Cr	Pb	Sn	V	Hg	Se
O-13	30.7	7.8	7..3	2.0	9.0	2.1	7.2
O-20	30.9	8.0	7.6	2.0	11.0	1.9	8.6
D-2	29.7	8.0	7.7	1.7	14.0	1.9	6.3
D-33	29.6	7.9	6.2	1.9	14.0	2.0	7.4
PR	30.0	8.3	8.0	1.8	11.0	3.0	7.0
SC	29.5	8.2	8.9	1.9	11.0	2.2	7.2
D-4	29.7	7.8	7.1	1.9	12.0	2.0	6.0
D-5a	29.9	7.8	7.0	1.0	12.0	2.0	5.9
C-9	29.8	7.3	8.2	1.19	11.0	2.2	4.9
C-1	31.2	8.1	7.1	2.4	15.0	2.5	7.3
C-11	29.9	7.4	7.7	3.3	11.0	4.3	5.4
NO-2	7.8	7.8	7.0	1.9	12.0	2.8	5.9
CA-2	29.	7.4	6.0	2.0	10.0	2.0	6.1
S-6	28.9	8.1	7.1	2.0	12.0	1.9	6.6
D-119	29.4	8.4	6.8	1.9	12.0	1.8	7.5
D-74	28.5	7.4	7.4	1.0	14.0	2.2	4.9

The total concentration of arsenic in waters varies between 7.82 to 31.15 $\mu g\ L^{-1}$. For arsenic these concentrations are high if they are compared with the typical concentrations in open ocean and coastal sea-water which are in the range of 1-2 $\mu g\ L^{-1}$(27). The source of contamination is probably an open-cast coal mine located on the edge of Lake Maracaibo. The total selenium concentration is below the average found in surface ocean and well waters, which usually contain less than 50 $\mu g\ L^{-1}$ (28). The concentration of chromium is below the limit of the EPA which is 50$\mu g\ L^{-1}$(29). The values of Hg in water were higher than the normal estuarine waters (ca. <50 $ng\ L^{-1}$)

and lakes (10-50 ng L^{-1})(30). The limit for lead of US Public Health is 50 μg L^{-1} ; the values of the Lake Maracaibo waters were below this limit(31).

4.3.1.3 Trace elements, total content in sediments

The Table 4.12. (a and b) shows the results for the total content of all trace elements in sediments determined by the developed microwave digestion method.

Table 4.12. (a and b): Mean concentrations of the trace elements (mg Kg^{-1})
(a)

Sampling points	Cu	Ni	Cd	Cr	V	Ti	Pb	Zn
PR	3.7	3.4	3.66	8.3	30.8	47.4	23.6	46.7
SC	3.2	8.9	5.4	11.2	31.6	23.5	43.3	47.7
D-2	6.2	6.1	10	11.6	46.3	88.7	58.5	48.2
D-4	7.4	7.3	4.9	11	72.7	19.3	58.5	53.4
D-5a	1.0	0.7	0.37	0.9	1.77	7.2	10.1	4.7
NO2	7.4	7.3	4.9	11.0	32.3	19.3	6.2	53.4
0-13	10.3	6.1	4.4	11.7	91.8	80.4	24.8	50.7
O-20	12.6	7.2	4.6	13.1	113.5	176.7	86.0	49.6
C-1	3.0	4.2	5.4	6.1	47.5	67.6	4.0	24.7
C-11	11.4	5.9	2.7	6.5	81.2	38.6	64.9	26.2
C-9	15.4	5.4	2.41	7.9	62.3	33.7	69.6	36.3
CA-2	6.7	6.0	3.8	8.6	61.7	89.7	6.2	28.7
D-33	13.3	9.8	5.3	15.0	79	182.6	110.0	48.5

(b)Concentrations in ($\mu g\ Kg^{-1}$)

Sampling points	As	Sn	Hg	Se
PR	6080.9	17.1	404.2	21.2
SC	10099.5	17.2	258.5	94.4
D-2	9313.0	24.8	281.7	159.0
D-4	8000.0	27.1	124.7	160.0
D-5a	40.7	7.2	190.2	ND
NO2	2033.0	0.5	52.7	590.8
0-13	2369.0	0.3	130.3	511.0
O-20	4082.0	0.2	130.3	683.9
C-1	2159.0	0.2	188.7	687.4
C-11	5774.0	0.2	280.5	691.2
C-9	5314.0	0.3	187.4	812.3
CA-2	2153.0	1.1	520.1	260.0
D-33	5057.0	0.8	148.3	631.9

Correlations:

The Pearson product moment correlations between each pair of variables with P-values below 0.05 which indicate statistically significant non-zero correlations have been calculated. At the 95% confidence level, the following pairs of variables have P-values below 0.05: Cu and Se; Cu and Pb; Cu and V; Ni and Cr; Ni and Zn; Ni and Pb; Cd and Cr; Cd and Zn; Cr and Ti; Cr and Zn; Cr and Pb ; Ti and Pb ; Ti and V; As and Sn; Se and Sn; Se and V ; Pb and V .

Similar correlations between Pb and V have been found in the Gulf of Mexico with similar petroleum- related activities (32). Also, correlations have been found between Ni and Pb and V and Ti in sediments from Kuwait which had oil fires during the Gulf

War in 1991(33).

Correlations between total arsenic and the total phosphorus concentration were found ($r=0.6411$, $n=13$, $p<0.018$) and total selenium and total sulphur concentrations ($r=0.5768$, $n=13$, $p<0.039$). These results were expected because the respective pairs of variables (As and P) and (Se and S) belong to the same group of the periodic table.

Correlations were found between Se and Depth ($r= 0.7230$, $n=13$, $p<0.005$); pH ($r= -0.6600$, $n=13$, $p<0.014$) and dissolved oxygen (DO) ($r= -0.5404$, $n=13$, $p<0.05$). No correlations were found between As and the physicochemical parameters.

4.3.2 -X-ray fluorescence spectrometry results

The results of X-Ray fluorescence spectrometry are given in the Table 4.8 (a and b)., concentrations are given in % w/w.

Table 4.13(a and b): X-ray fluorescence spectrometry results(%w/w) for the samples of sediments from Lake Maracaibo

Points	Na_2O	MgO	Al_2O_3	SiO_2	P_2O_5	SO_3	K_2O	CaO	TiO_2	V_2O_5
PR	0.47	0.61	6.08	83.14	0.05	0.12	0.90	0.47	0.51	0.02
SC	0.29	0.30	2.38	71.79	0.06	0.06	1.42	8.79	0.15	0.01
D-2	0.81	1.08	11.85	68.41	0.12	0.01	1.45	0.53	0.73	0.02
D-4	0.95	1.49	16.11	57.23	0.21	0.02	1.89	0.69	0.80	0.03
D5a	0.06	0.01	0.73	97.16	0.01	0.016	0.44	0.18	0.11	<0.014
NO2	0.68	0.76	9.23	72.27	0.12	0.01	1.30	0.38	0.52	0.02
O-13	1.43	1.33	16.50	50.10	0.14	0.12	1.99	0.33	0.65	0.03
O-20	1.01	1.29	17.31	48.99	0.15	0.1	2.11	0.30	0.60	0.03
C-1	0.34	0.51	4.75	78.70	0.25	0.07	0.57	0.16	0.27	0.01
C-11	0.92	1.13	15.01	49.86	0.12	0.1	1.95	0.32	0.47	0.03
C-9	1.02	1.12	14.36	47.58	0.15	0.04	1.80	0.40	0.46	0.02
CA-2	0.78	0.85	10.87	65.66	0.14	0.01	1.42	0.29	0.59	0.02
D-33	1.08	1.15	16.28	50.30	0.15	0.02	1.92	0.29	0.60	0.03

(b)

Sampling points	Cr_2O_3	Mn_3O_4	Fe_2O_3	ZnO	SrO	Y_2O_3	ZrO_2	BaO	HfO_2
PR	0.01	0.02	3.59	0.01	<0.008	0.01	0.06	0.05	0.01
SC	0.06	0.05	4.85	0.01	0.02	0.01	<0.017	0.05	0.01
D-2	0.01	0.05	5.71	0.02	0.01	0.01	0.03	0.06	0.01
D-4	0.01	0.06	6.31	0.02	0.01	0.01	0.02	0.06	0.01
D5a	0.09	0.01	0.17	0.01	0.01	0.01	0.02	0.03	0.01
NO2	0.04	0.04	3.99	0.02	<0.008	0.01	0.04	0.1	0.01
O-13	0.01	0.09	6.69	0.02	0.01	0.01	0.01	0.08	0.01
O-20	0.01	0.16	7.28	0.02	0.01	0.01	0.01	0.13	0.01
C-1	0.04	0.04	7.69	0.01	0.01	0.01	0.03	0.05	0.01
C-11	<0.024	0.4	5.47	0.01	0.01	0.01	<0.017	0.15	0.01
C-9	0.01	0.64	5.65	0.02	0.01	0.01	0.01	0.22	0.01
CA-2	0.01	0.04	6.4	0.01	<0.008	0.01	0.03	0.09	0.01
D-33	0.01	0.11	5.93	0.02	<0.008	0.01	<0.017	0.07	0.01

Limit of determinability(L.O.D.)(%w/w): SO_3 : 0.016 ; Cr_2O_3 ; 0.024; V_2O_5 : 0.014; SrO: 0.008; ZrO_2: 0.017.

Correlations:

The following pairs of variables have correlations with $p<0.05$ (95 % of confidence): Fe_2O_3 and V_2O_5;, Fe_2O_3 and Cr_2O_3; Fe_2O_3 and P_2O_5; Fe_2O_3 and K_2O; Fe_2O_3 and Al_2O_3; Fe_2O_3 and SiO_2; Fe_2O_3 and Na_2O; Fe_2O_3 and MgO. Al_2O_3 and SiO_2; Al_2O_3 and Na_2O; Al_2O_3 and MgO; Al_2O_3 and V total. Similar correlations between % Al_2O_3 and total V have been observed in the Gulf of Mexico (32).

Table 4.14.shows the results of the principal components analysis (PCA) using the Statgraphics program (25) for the different sampling sites.

Table 4.14 : The eigen values for the three principal components.

Sampling site	Component 1	Component 2	Component 3
PR	-2,12534	-1,9331	0,635728
SC	-0,541212	-3,04569	0,891025
D-2	0,344223	-3,11663	0,07483
D-4	0,397966	-1,98982	0,421493
D 5a	-5,74657	1,7443	-0,113156
NO2	0,25357	0,339748	-1,57195
O-13	1,11893	0,609787	-0,920445
O-20	3,17701	0,766038	-0,140658
C-1	-1,13605	0,613193	-2,04273
C-11	-0,19987	1,44116	1,79935
C-9	0,778882	2,51182	3,11197
Ca-2	-0,201003	1,478	-1,6673
D-33	3,87945	0,581203	-0,478158

Three components had eigen values that represent 78 % of the variability of all the data reported in this work. According to this table the sampling points D-33, O-20, O-13 and C-9 have the main values for the principal component 1, and the points C-11, CA-2, C-9 and D5a for the component 2. These results indicate that these sampling points are having a greater influence on the variability of data for total metal concentration either because they are more polluted; because D5a is a low concentration point, which is affecting negatively all the data. D5a is located in the Strait of Maracaibo near to the

shore and it is not so affected by pollution as are the other points, probably because the currents from the lake do not reach it.

4.3.3.- Sequential extraction

Table 4.15 presents the results obtained for the Reference Material IAEA-356 for total content of each metal studied.

Table 4.15: Comparison of the results obtained with the reference material IAEA-356

Metal	Found ($\mu g.g^{-1}$)	Certified($\mu g.g^{-1}$)**
As	23.06±4.61	26.9[22.6-30.0]
Cr	60.5±0.90	69.8 [62.9-74.4]
Pb	351..2±2.9	[301-365]
V	43.1±3.0	55.5[32.8-60.1]
Hg	7.4 ± 0.7	7.62 [6.74-7.98]
Sn	53.7±2.6	[43.6-62.2]
Se*	0.54 ± 0.02	0.76 [0.40-1.58]

***Information value* ***Median*

4.3.3.1 Arsenic:

The results showed that arsenic in Lake Maracaibo is present in large amounts [0.04 – 10.1 $\mu g.g^{-1}$], Table 4.16 The highest concentration of the element is at the point SC (ca. 10.1$\mu g.g^{-1}$) and points D-4, D-2, the source of the contamination could be coal mining ports located near of this sampling area. In the main zone of the Lake, the total concentration of arsenic varies between 0.04 – 5.8$\mu g.g^{-1}$. It is known that environmental conditions in sediment can influence arsenic concentrations and potential mobility. The anaerobic conditions found in the centre of Lake Maracaibo increases the total arsenic concentration (see C-11 and C-9 sites, 5.5-5.8 $\mu g.g^{-1}$). In general, arsenic is well

distributed in the various phases in all of the Lake Maracaibo System as is shown in Figure 4.1. The major concentration of arsenic found inside the lake was associated with the step two, that extractable with hydroxylammonium chloride, the fraction associated with the Fe/Mn hydroxous phase (10-60%). Fe oxides are generally the major carrier phase for arsenic (5).

Table 4.16: Arsenic concentrations ($\mu g\ g^{-1}$) and recoveries in the four sequential extraction steps.

Samples	1	2	3	Residual	Total	Total content	%Recovery
PR	0.57	0.41	2.76	3.20	6.94	6.08	114.1
SC	0.05	0.18	0.11	5.80	6.14	10.09	60.8
D-2	0.23	0.41	2.52	1.80	4.96	6.08	81.6
D-4	0.27	0.46	2.02	0.44	3.19	6.34	50.3
D5a	0.00	0.05	0.02	0.01	0.08	0.04	195.1
NO-2	0.50	0.77	0.34	0.44	2.05	2.03	100.5
O-13	0.39	0.96	0.76	1.46	3.56	2.37	150.3
O-20	0.44	1.66	0.95	1.78	4.83	4.08	118.2
C-1	0.07	0.24	0.20	1.67	2.19	2.16	101.1
C-11	1.39	1.34	1.68	1.04	5.45	5.77	94.3
C-9	0.93	1.75	1.66	1.50	5.84	5.31	109.8
Ca-2	0.38	0.55	0.31	0.51	1.74	2.19	79.5
D-33	0.40	0.82	0.55	0.25	2.03	2.20	92.3

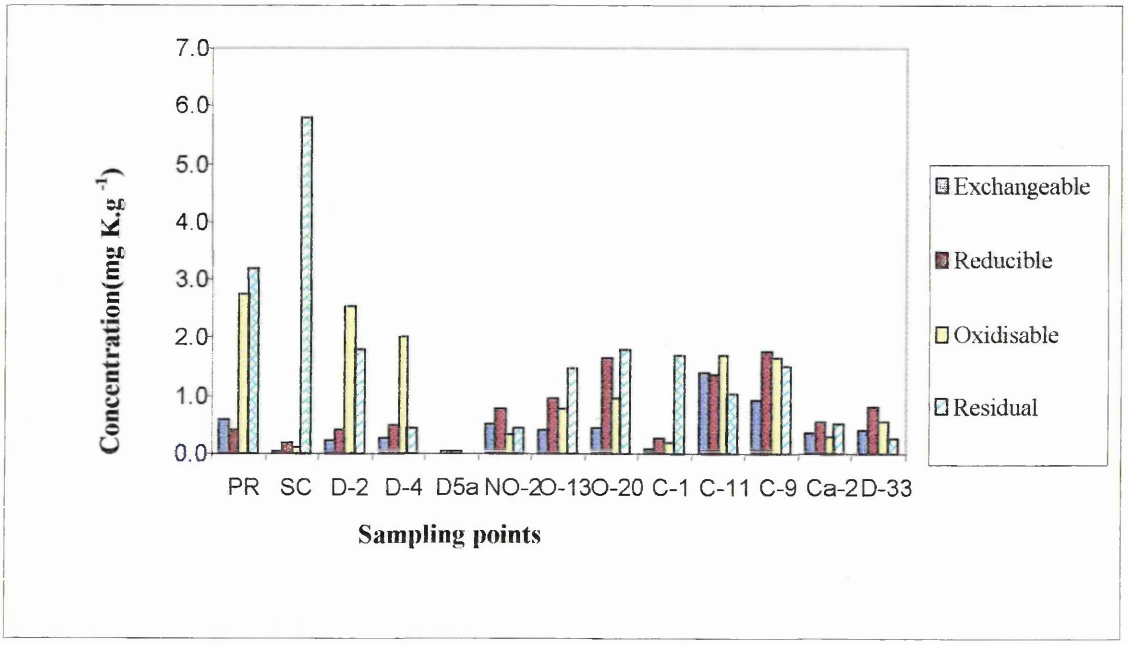


Figure 4.1: Distribution of arsenic in the sediment of Lake Maracaibo.

The distribution of arsenic in the strait of Maracaibo is mainly in the oxidisable and organic matter nominal phase (15-80%); there are raw sewage discharges from the heavy industrialised area of the city of Maracaibo (3 million population), a major source of organic matter, and a nearby source of arsenic pollution of an open-cast coal mine that can produce this effect. The arsenic in the residual phase is mainly present in the sample taken from PR at the entrance to the Gulf of Venezuela. The phase of exchangeable arsenic was present essentially in the centre of the lake (0-25%); under reducing conditions , Mn(II) associated with exchangeable fractions and sulphides can be the primary Mn forms present; half of the reduced Fe(III) is converted to Fe(II) carbonate (34); both Fe(II) and Mn(II) could be the carrier phases for arsenic in sediment in the centre of the lake. Under these conditions, binding to sulphide and insoluble large molecular weight humic acid can control the iron behaviour (35).

The first extraction phase (exchangeable metal, water and acid soluble) and the second extraction of the iron /manganese oxyhydroxides phase were mainly present and higher in the lake zone. The behaviour of arsenic in the gulf and the strait were similar (paired t-test, not statistically significant differences, $p<0.05$) with the exception of the residual phase that was present at a higher level in the zone near to the Gulf of Venezuela and the mixing zone with the Caribbean Sea waters and also to the effect of arsenic contamination from lake-side open-coast coal mine.

A correlation was also found between levels of arsenic in the residual phase and the amount of CaO as determined by X-Ray Fluorescence spectrometry ($r=0.8328$, $n=13$, $p<0.0004$).

4.3.3.2.- Selenium:

The total concentration of selenium in Lake Maracaibo varied from 0.02 to 0.85 $\mu g.g^{-1}$; the results are shown in Table 4.17 and Figure 4.2. The sequential extraction results

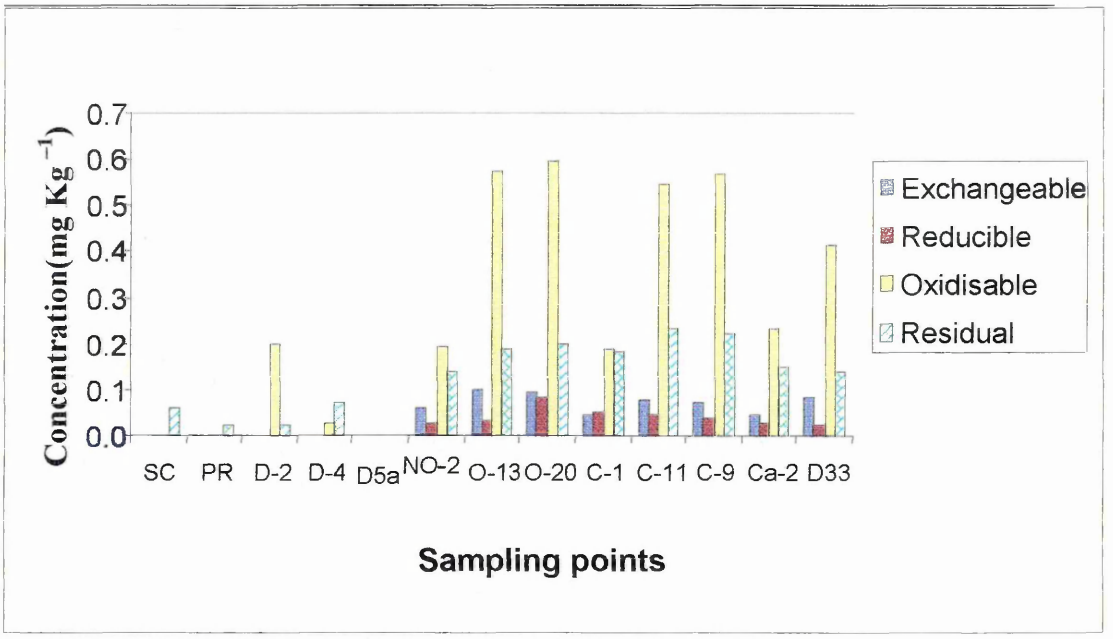
showed that selenium was distributed mainly in the third extraction phase in the zone of the lake which is associated with organic matter and sulphides. This is understandable because the chemistry of selenium is very similar to that of sulphur, including their presence in organic matter. At these sample points was also found the highest concentration of total selenium (ca. 0.85 $\mu g.g^{-1}$), the anoxic conditions favouring enrichment of the sediment by this element.

Table 4.17.: Selenium concentrations ($\mu g\ g^{-1}$) and recoveries in the four sequential extraction steps.

Sample	1	2	3	Residual	Total	Total content	%Recovery
SC	0.00	0.00	0.00	0.06	0.06	0.09	63.8
PR	0.00	0.00	0.00	0.02	0.02	0.02	100.0
D-2	0.00	0.00	0.20	0.02	0.22	0.16	137.5
D-4	0.00	0.00	0.03	0.07	0.10	0.18	55.6
D5a	0.00	0.00	0.00	0.00	0.00	0.00	100.0
NO-2	0.06	0.03	0.20	0.14	0.42	0.59	71.3
O-13	0.10	0.04	0.57	0.19	0.89	0.51	174.6
O-20	0.09	0.08	0.60	0.20	0.97	0.68	142.2
C-1	0.05	0.05	0.19	0.18	0.46	0.69	67.2
C-11	0.08	0.05	0.54	0.23	0.90	0.70	128.0
C-9	0.07	0.04	0.57	0.22	0.90	0.81	111.2
Ca-2	0.05	0.03	0.23	0.15	0.45	0.83	55.1
D33	0.08	0.02	0.41	0.14	0.66	0.85	77.4

The results of the first extraction step (the exchangeable phase, the water- and acid-soluble phase, and the second extraction step associated with Fe/Mn carrier phases, showed that only 0-10 % of selenium was present as the dissolved element in these phases. This behaviour has also been found in studies of other lakes (5).

The recoveries of method used were between 55 to 172 %, the low recovery being possibly due to a redistribution of the element during the extraction method, and the high recovery due to contamination during the different extraction steps.



Figures 4.2.: Distribution of selenium in sediments of Lake Maracaibo.

In the four extraction steps the highest concentrations of selenium were found in the main zone of the lake which is a similar behaviour to sulphur. In the gulf- and strait of Maracaibo-zones, the results of the scheme of sequential extractions for all the extracted phases, exchangeable, reducible, oxidisable and residual, were similar (t-test showed not statistically significant differences, $p<0.05$) and completely different to the lake zone.

The residual fraction of selenium showed correlations with Na_2O ($r=0.6094$, $n=13$, $p<0.027$); Al_2O_3 ($r=0.6091$, $n=13$, $p<0.0271$) and SiO_2 ($r= -0.6949$, $n=13$, $p<0.0084$), from X-ray fluorescence spectrometry results.

4.3.3.3.- Lead:

Inorganic lead arising from a number of industrial and mining sources occurs in water in the +2 oxidation state. Lead can exist in organic form as Pb(II), but less as Pb(IV), and also in organic form (up to 4 Pb-C bonds). The flux of organometallic lead is small compared with that of inorganic lead on a global basis, but on a local basis (e.g. near gasoline stations) it might be a significant factor (36). Inorganic lead is far more

extensively studied than organometallic lead and numerous reviews have discussed speciation and cycling (37-39).

Lead in the centre of Lake Maracaibo was mainly associated with Fe/Mn phases where between 5-90 % is present in this form (Figures 4.3). This behaviour was reported by Lum and Gammon in 1985 (40) in their study of Lake Eries and Lake Detroit, which are similar to Lake Maracaibo. Lead mobility is controlled by Fe oxides. A study on sequential leaching of contaminated reservoirs by Schintu et al (1991)(41) indicated that Fe and Mn oxides were the dominant sorbent for lead. Organic phases showed only a low affinity for lead. The concentration of lead was high at all the points sampled [4.0 – 110.0 $\mu g.g^{-1}$]. The highest concentration was found at the sites D-2 and D-4, possibly due to the traffic over the bridge that joins the two coasts in the strait., The high concentration near to the Venezuelan Gulf (points SC-PR) could be associated with the coal mining port in the north of the lake. In all of these points, lead was associated with the residual phase.

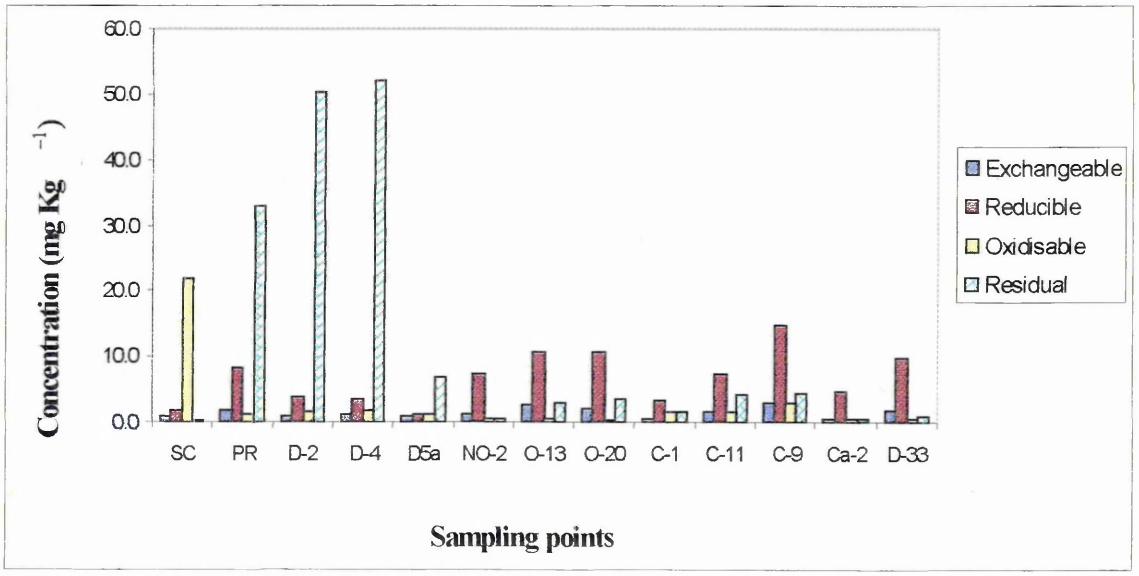


Figure 4.3: Distribution of lead in the sediments of Lake Maracaibo

Table 4.18.- Lead .concentrations (μg g^{-1}) and recoveries in the four sequential extraction steps

Samples	1	2	3	Residual	Total	Total content	%Recovery
SC	0.78	1.81	21.91	0.22	24.72	23.60	104.7
PR	1.71	8.21	1.30	32.71	43.93	43.28	101.5
D-2	0.82	3.84	1.54	50.26	56.46	58.46	96.6
D-4	1.04	3.61	1.79	52.00	58.44	58.50	99.9
D5a	0.80	1.33	1.30	6.70	10.13	10.11	100.2
NO-2	1.32	7.41	0.45	0.51	9.70	6.21	156.2
O-13	2.55	10.73	0.63	3.04	16.95	24.79	68.4
O-20	2.03	10.58	0.39	3.47	16.48	86.03	19.2
C-1	0.48	3.35	1.35	1.57	6.74	3.96	170.2
C-11	1.54	7.39	1.62	4.03	14.57	64.91	22.5
C-9	2.81	14.77	2.92	4.37	24.87	69.56	35.8
Ca-2	0.66	4.65	0.55	0.70	6.57	6.21	105.7
D-33	1.84	9.89	0.45	0.96	13.13	110.02	11.9

The percentage recovery of the sequential extraction method used was good in general (Table 4.18) with the exception of the sample C-11, C-9, O-20 and D-33 which are anaerobic sediments. Possible transformation during the extraction procedure could have affected the results. A similar behaviour between estuary (SC, PR) and strait (D-2, D-4, NO2) in phases exchangeable, reducible and residual was found; and different behaviour for the metal associations between these and the main lake zone. The Pearson product moment correlations between lead in the residual phases and the X-Ray fluorescence spectrometry results did not show any correlations between lead and the other metal oxides determined with this technique.

4.3.3.4.- Tin:

Tin is present in natural waters at concentrations below 50 $\mu g.L^{-1}$ and is generally accepted as being principally Sn(IV) on the basis of thermodynamic equilibria. However, it is possible that kinetic control of steady-state speciation may indeed favour Sn (II), especially in polluted waters, and this is not always readily measurable (42). The major source of tin compounds in the aquatic environment is organotin compounds,

particularly trialkytins, which are extensively used as biocides, algicides, fungicides, and molluscides, in marine antifouling paints, and agriculture.

In the sequential extraction experiments, the tin concentrations in the lake itself were found to be associated to the residual phase and to be at very low concentrations, which means that Sn is not present as a dissolved species. However, this behaviour is different in the estuary and the strait where tin was mainly associated with the organic and Fe/Mn hydroxides phases,(Figure 4.4).

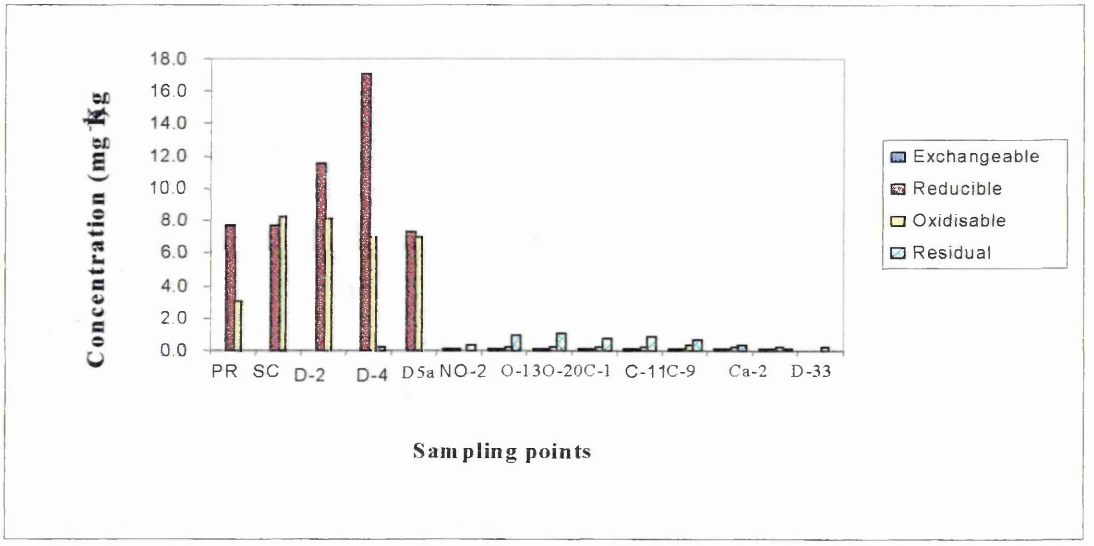


Figure 4.4. : Distribution of tin in the Lake Maracaibo.

Table 4.19: Tin concentrations ($\mu g\ g^{-1}$) and recoveries in the four sequential extraction steps .

Samples	1	2	3	Residual	Total	Total content	% Recovery
PR	0.00	7.70	8.20	0.00	15.90	17.05	93.3
SC	0.00	7.70	8.16	0.00	15.86	17.16	92.4
D-2	0.00	11.53	6.99	0.00	18.52	24.78	74.7
D-4	0.00	17.06	7.00	0.23	24.29	27.14	89.5
D5a	0.00	7.28	0.00	0.00	7.28	7.22	100.8
NO-2	0.09	0.08	0.23	0.26	0.66	0.45	146.4
O-13	0.07	0.07	0.23	0.98	1.35	0.25	548.2
O-20	0.07	0.07	0.23	1.02	1.39	0.23	594.1
C-1	0.07	0.06	0.23	0.69	1.06	0.24	444.6
C-11	0.07	0.08	0.27	0.83	1.24	0.24	523.0
C-9	0.07	0.07	0.24	0.66	1.04	0.25	414.0
Ca-2	0.06	0.06	0.21	0.35	0.68	1.05	65.3
D-33	0.07	0.06	0.22	0.15	0.50	0.84	60.2

The percentage recoveries that were found for the sequential extraction method were high in some places having anaerobic sediments (Table 4.19). In the strait and the gulf the concentrations were higher than the main zone of the lake. The third extraction step results showed that the tin is associated mainly with organic phases or sulphur-containing compounds in the Gulf of Venezuela and in the zone of the Strait of Maracaibo.

The multivariate statistical analysis between the residual tin and the X-ray fluorescence results showed correlations between Sn and Na_2O ($r = 0.5773$, $n=13$, $p<0.0388$), Sn and Al_2O_3($r =0.5636$, $n=13$, $p<0.0449$), Sn and Fe_2O_3 ($r = 0.5990$, $n=13$, $p<0.0305$) and Sn and SiO_2($r = -0.6282$, $n=13$, $p<0.0215$). Tin in the residual phase could be associated with Fe oxides or aluminum oxides.

4.3.3.5.- Vanadium:

Concentrations of vanadium in all the lake sites were high [1.8-113.5 mg Kg^{-1}], especially in the centre where there are 10,000 petroleum extraction towers. Vanadium in this zone is distributed mainly in the Fe/Mn hydroxide carrier phases and in the residual phase (Figure 4.5).

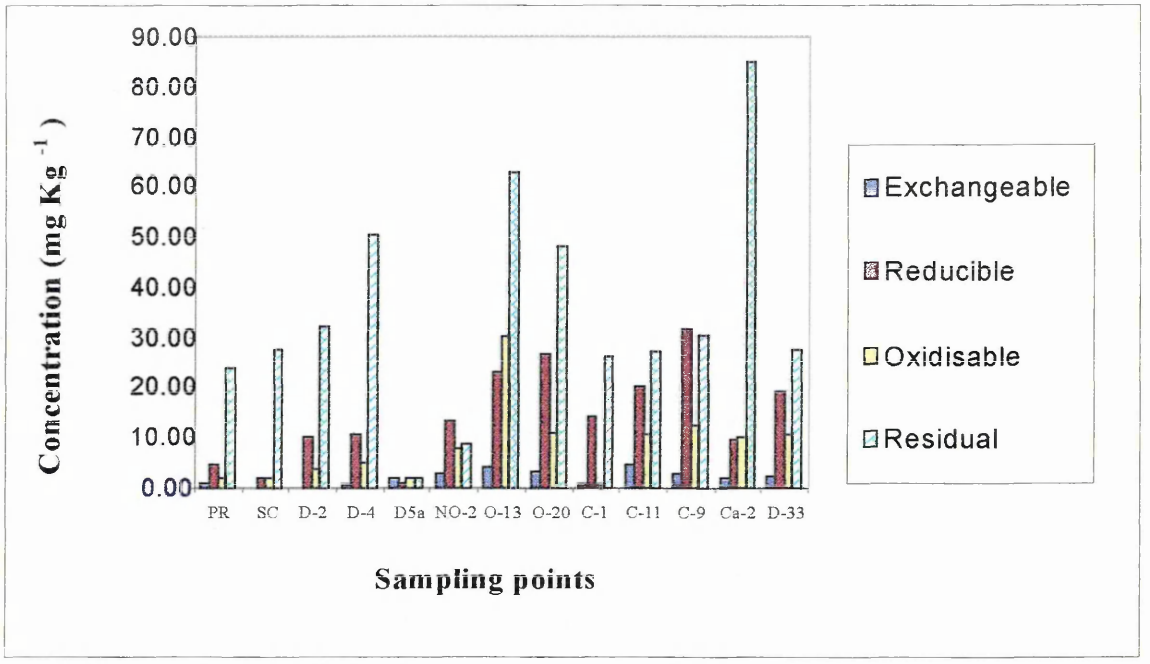


Figure 4.5.: Distribution of vanadium in Lake Maracaibo

Table 4.20: Vanadium concentrations (μg g^{-1}) and recoveries in the four sequential extraction steps.

Samples	1	2	3	Residual	Total	Total Content	%Recovery
PR	0.89	4.46	1.63	23.80	30.78	30.80	99.9
SC	0.14	1.98	1.97	27.50	31.58	31.63	99.9
D-2	0.00	10.13	3.82	32.34	46.29	46.29	100.0
D-4	0.50	10.47	5.10	50.67	66.74	72.73	91.8
D5a	1.91	0.98	1.63	1.77	6.29	1.77	355.4
NO-2	2.87	13.18	7.65	8.55	32.25	50.60	63.7
O-13	4.03	23.15	30.25	63.03	120.46	91.83	131.2
O-20	3.42	26.82	11.00	48.09	89.33	113.49	78.7
C-1	0.46	14.36	0.46	26.33	41.61	47.54	87.5
C-11	4.54	20.17	10.34	27.03	62.07	81.18	76.5
C-9	2.91	31.65	12.39	30.29	77.23	81.07	95.3
Ca-2	1.69	9.57	10.21	84.88	106.35	61.73	172.3
D-33	2.23	19.49	10.68	27.42	59.83	79.01	75.7

The correlations between the residual phase and the X-Ray results showed that there were correlations between the residual phase of vanadium and the Fe_2O_3 results ($r = 0.6435$, $n=13$, $p < 0.0177$). The recovery for the extraction method, seen in Table 4.20,

indicates that high recovery percentages could be associated to contamination during the extraction method.

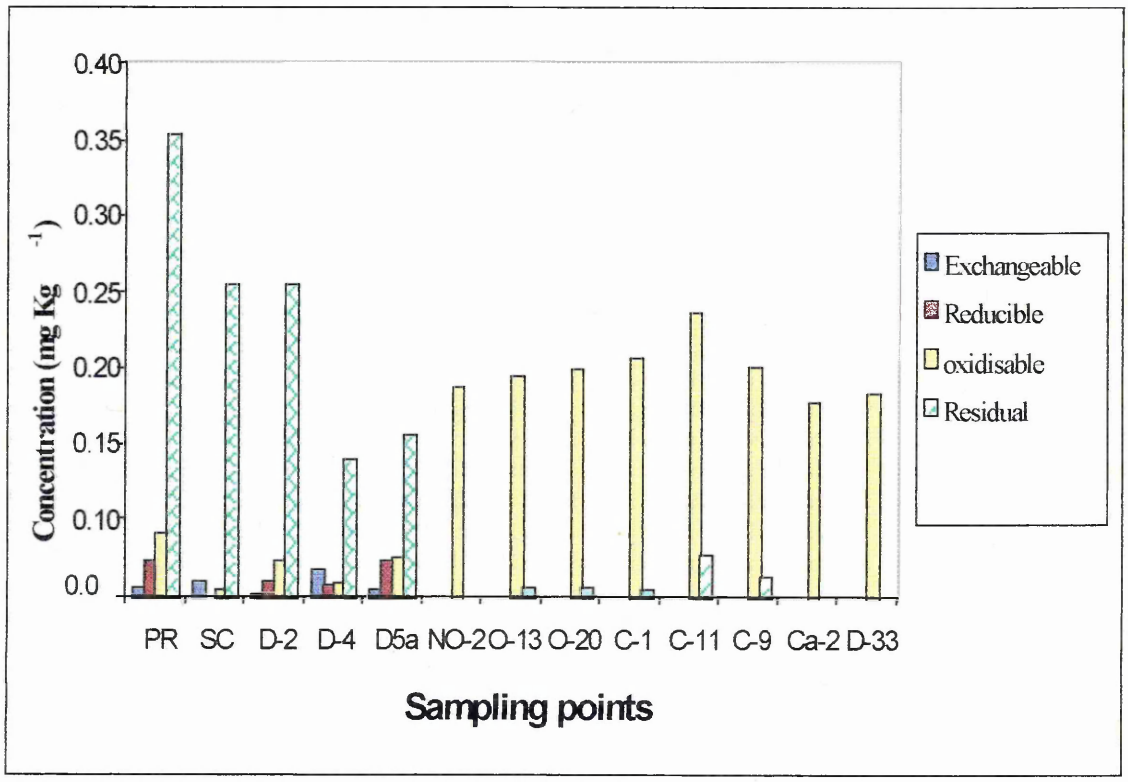
The multivariate analyses between trace elements (total Cu, Ni, Cd, Cr, Ti, Zn, As, Se, Pb, Sn, Hg) and V showed that there were correlations between V and Cu ($r = 0.8682$, $n=13$, $p<0.0001$); V and Cr ($r= 0.5922$; $n=13$, $p<0.0330$) (that behaviour is expected because both can exist as anions in solution); V and Ti ($r= 0.5890$, $n= 13$, $p<0.0342$) (this behaviour is expected because the contamination source is the oil extraction industry for both); Se and V ($r = 0.6791$, $n=13$, $p<0.0107$) and V and Pb ($r= 0.5894$, $n=13$, $p<0.0340$). A coal mine port is one of the sources of contamination for selenium, vanadium and lead. Similar correlations have been found by M. Leivuori (43) for the sediments of the Gulf of Finland and the Gulf of Bothnia.

Correlations also exist between : V_2O_5 and Fe_2O_3 ($r = 0.6019$, $n=13$, $p<0.0295$); Fe_2O_3 and V total ($r = 0.7712$, $n=13$, $p< 0.0020$); Fe oxides can be the carrier phases of vanadium in the sediment as is seen in the sequential extraction results; V total and Cr_2O_3 ($r = 0.7458$, $n=13$, $p<0.0034$) ; TiO_2 and V total ($r=0.6164$, $n=13$, $p<0.0249$) ; SiO_2 and V total ($r=-0.8957$, $n=13$, $p<0.0001$)(this similar correlation was found by A. Shiller and L. Mao in the Mississippi River in 1990 (44)); Al_2O_3 and V total ($r =0.8957$, $n=13$, $p<0.0001$); P_2O_5 and V total ($r=0.5580$, $n=13$, $p<0.0475$).

4.3.3.6.- Mercury:

In the results found for the Lake Maracaibo system, three different zones for the distribution of metals were apparently as previously noted for other metals.. The results of the sequential extractions revealed that most of the mercury in the centre of Lake Maracaibo (sites NO2 to D-33) was extracted in the organic/sulfide phases [95-100 %], indicating the occurence of comparatively stable mercury compounds such as humic

acid – bound mercury or mercury sulfides. In contrast, data from samples taken in the Strait and in the Gulf showed that mercury occurs mainly in the residual phase even after an oxidising step (organic-sulfide fraction),in which mercury was oxidised by HNO_3-H_2O_2 solution. The ocurrence of mercury after the oxidising leaching steps indicates that the mercury found in the residual fraction consists of Hg^0 and matrix-bound mercury, which has not been extracted in the previous steps.



Figures 4.6. : Distribution of mercury in sediments of Lake Maracaibo.

The data for the samples from the Strait are associated with an essentially inorganic mercury contamination source (a clhor-alkali plant located in the point D-2 or near the point). The percentage of recovery of mercury in all the sites sampled were between 70.8 – 264.9 %; high percentages (more than 100%) could appear because the different sequential extraction method might introduce contamination to the sample or because the method used for total concentration determination does not solubilise all the mercury compounds (Table 4.21., mercury concentrations in $\mu g.g^{-1}$).

Table 4.21.: Mercury concentrations ($\mu g\ g^{-1}$) and recoveries in the four sequential extraction steps

Sample	1	2	3	Residual	Total	Total Content	%Recovery
PR	0.01	0.02	0.04	0.30	0.37	0.40	91.9
SC	0.01	0.00	0.00	0.21	0.22	0.26	84.5
D-2	0.00	0.01	0.02	0.20	0.24	0.28	84.7
D-4	0.02	0.01	0.01	0.09	0.12	0.12	100.2
D5a	0.00	0.02	0.02	0.10	0.15	0.19	81.4
NO-2	0.00	0.00	0.14	0.00	0.14	0.05	264.9
O-13	0.00	0.00	0.14	0.01	0.15	0.13	111.7
O-20	0.00	0.00	0.15	0.00	0.15	0.13	122.2
C-1	0.00	0.00	0.16	0.00	0.16	0.19	84.2
C-11	0.00	0.00	0.19	0.03	0.21	0.27	78.8
C-9	0.00	0.00	0.15	0.01	0.16	0.19	87.5
Ca-2	0.00	0.00	0.13	0.00	0.13	0.07	173.4
D-33	0.00	0.00	0.13	0.00	0.13	0.14	98.29

In the Figure 4.6. in which is presented data for the four extraction phases, for all extraction phases determined, the differences between each zone is seen clearly, every zone representing a different system with different physicochemical parameters and different species of mercury. The exchangeable or water soluble fraction is mostly present in the zone near to the Strait and the Gulf of Venezuela (SC, D-4), and it is also near to the former source of inorganic mercury contamination in the Tablazo Bay. Although, this source of contamination was eliminated five years ago, the continous dredging releases the mercury in the water column. These zones also represent the major values of the residual phases. There is clearly a difference between the above two zones and the main lake zone where most of the mercury is bound to organic matter or sulphides. Sulphides can produce a precipitation of mercury in the form of methylated compounds. Similar results where mercury is bound mainly to the organic-sulphide phase and the residual phase have been found by Biester and Scholz (45) in previous laboratory scale studies.

There was not a correlation between the total concentration of mercury and the nutrients; a correlation between Hg (inorganic) and the dissolved oxygen concentration

was found ($r= 0.6601$, $n=13$, $p<0.0141$). There was also a correlation between the residual phase of mercury and the P_2O_5 ($r= -0.5701$, $n=13$, $p<0.0419$), as shown by the X-ray fluorescence spectrometry results.

4.4.- CONCLUSIONS

In conclusion the distribution of the six metals studied varied in the three systems found in Lake Maracaibo. Mercury and selenium were distributed in the main zone of the lake as the third extraction phase, bonded to organic matter and sulphides, however, for arsenic, the first extraction phase (exchangeable metal, water and acid soluble) and the second extraction of the iron /manganese oxyhydroxides phase were mainly present and higher in the lake zone. Vanadium in this zone was distributed mainly as residual phase and the Fe/Mn hydroxide carrier phases. Lead distribution was controlled by Fe oxides in the main zone of the lake. Tin concentrations were higher in the estuary and the strait where this element was mainly associated with the organic and Fe/Mn hydroxides phases.

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Chapter V

Chemical speciation of arsenic, selenium and chromium in water, fish muscle tissue, mussel and sediment samples from Lake Maracaibo.

5.1.- INTRODUCTION

The environmental levels of trace elements such as arsenic, selenium and chromium are of considerable interest because of the potential toxic and carcinogenic properties of these elements. The toxicological and physiological behaviour of these elements is known to depend on the oxidation state and the chemical form .

5.1.1.- Arsenic

The location of arsenic in the periodic table directly below phosphorus predicts an analogous chemical behaviour for arsenate and phosphate including incorporation into organic molecules (1). Similar to phosphorus, arsenic can occur in numerous oxidation states (+5, +3, +1, 0, -1, -3) and in both inorganic and organic compounds, as is described in the arsenic cycle by Ferguson and Gavis in 1972 (2).

Environmental contamination by arsenic has increased in the world as a result of the application of arsenical herbicides and pesticides, smelting and mining operations, and the burning of fossil fuels. The toxicity and mobility of this element are dependent on the chemical forms in which it exists; the two more toxic species are arsenite (As^{III}) and arsenate (As^{V}), which represent the main forms of arsenic present in soils, sediments and water (3). Methylated arsenic, such as monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMMA) are less toxic and arsenic compounds such as arsenocholine (AsC) and arsenobetaine (AsB) are considered non- toxic (4).

In waters, dissolved arsenic can occur in both inorganic and organic forms. The inorganic forms at natural pHs include anionic ($H_2AsO_4^-$ and $HAsO_4^{2-}$) or neutral

arsenite ($As(OH)_3^0$) (5). In lakes, arsenic can exists in two common oxidation states, As(V) and As(III). Its behaviour is closely linked to that of Fe and to a lesser extent Mn oxides, and it can undergo biological uptake and methylation. Both the oxidation of As(III) and the reduction of As (V) can be microbially mediated (6).

The mobility of arsenic in sediments in Lake Ohakuri (New Zealand) (7) (a stratified lake during the summer) was investigated during 1980 and 1982, including studies of the release of arsenic to the overlying water related to seasonal changes in both water and sediment. The results showed that in shallow areas of the lake, the release of arsenic contributes to the continuous seasonal variation in the arsenic concentration in the lake water. Speciation of arsenic in the interstitial water indicated that in this lake, only inorganic As (V) and As (III) were present, neither methylarsonic acid nor dimethyl-arsinic acid being found in any of the samples, As (III) was usually the major constituent, and in many instances it accounted for > 90% of the total arsenic concentration, although, superficially, variations in the percentage of arsenic present in the interstitial water as As (III) appeared rather large.

The typical concentrations of arsenic in uncontaminated waters are in the range of 1-2 $\mu g\ L^{-1}$ (8). In surface sediments, Seydel (9) reported total concentrations of As in Lake Superior that ranged from 2.8 to 5.4 $\mu g.g^{-1}$. The maximum total concentration of arsenic found in Lake Lansing, studied by Batterson and McNabb (10) was 330 $\mu g.g^{-1}$ whereas studies of Lake Michigan (10) found arsenic in sediments in the range from 7.2 to 28.8 $\mu g.g^{-1}$. Two lakes studied by Wagerman et al (11), contaminated with arsenic as a consequence of gold mining activities, had concentrations between 6 to 3500 $\mu g.g^{-1}$ of As by dry weight.

Figure 5.1. shows the cycling of arsenic in a lake under summer conditions.

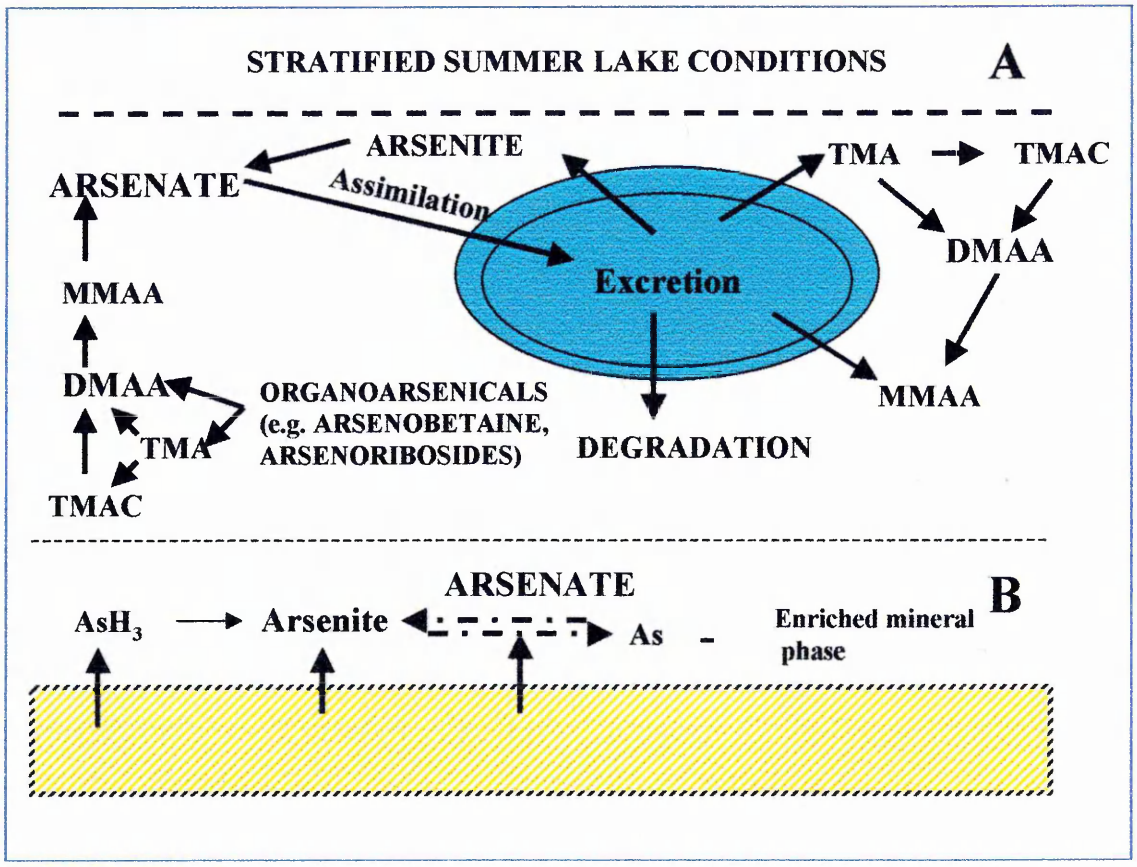

Figure 5.1 : The As cycle. Transformations include oxidation-reduction and ligand exchange. Methylation of arsenic compounds is thermodynamically unfavourable in water and can occur only by biological mediation (5)

In the oxic photic zone, arsenate and DMAA dominate the chemistry in solution. The dominant form in the hypolimnion is arsenate until reducing conditions increase sufficiently to cause the reduction of arsenate to arsenite. DMAA concentrations could arise from direct excretion from algae or microbes or degradation of excreted arsenicals or more complex cellular organoarsenicals. Reduction of first Mn and then Fe compounds results in a release of adsorbed or precipitated total As in the hypolimnion. Under sulphate-reducing conditions arsenate can be reduced to arsenite. Evidence exists for the removal of an As-enriched mineral phase (5).

The transformations presented in the cycle include oxidation- reduction and ligand exchange, tranfers from solution to solid phases, and vice versa. Although, the methylation of arsenic compounds is thermodynamically unfavorable in water and can occur only by biological activity, the presence of monomethylarsenic acid (MMAA) and dimethylarsinic acid (DMAA) in natural waters may be as a result of pesticide residues from agriculture or home use. Studies of an eutrophic lake (Lake Biwa, Japan) presented by Sohrin et al (12), showed that a depletion of oxygen concentrations and increased sulphide concentration in some situations can cause a release of arsenic from the sediments.

A study of bivalve species collected from Miami River (USA) showed concentrations of arsenic ranging between 23. 6 to 37.3 $\mu g.g^{-1}$ (13). Similar determination of arsenic in muscle tissue and inner organs of mullet from Lake Macquarie, Australia (14) revealed concentrations of arsenic between 4.7 to 19.2 $\mu g.g^{-1}$, the concentration in muscle tissue being the lowest. Arsenic in marine organisms is not usually present as inorganic arsenic or simple methylated forms (15). Arsenobetaine has been identified in lobster (16) and other organo-arsenic compounds (trimethylarsine, arsenocholine, tetramethylarsonium ion) in algae and molluscs(17-19)). However, M. Suñer et al (20) found inorganic arsenic (As(III) + As (V)) in a variety of molluscs, shrimp and fish from the Guadalquivir Estuary in Spain.

5.1.2. Selenium

Selenium (Se) has a complicated redox chemistry, closely related to that of S, and is biologically both an essential and a toxic element (21). In natural waters, it can occur in four oxidation states: selenate (VI) as the selenate oxyanion (SeO_4^{2-}) , selenite (IV) as

the selenite oxyanion ($HSeO_3^-$ and SeO_3^{2-}), elemental Se(0) as colloidal elemental selenium. The latter is stable over a wide Eh-pH range, and also as formally zero valent atoms in various inorganic and organic compounds and species. Selenide (-II) as biselenide (HSe^-), and as a variety of organic and inorganic compounds is also known. Organic forms of selenium are analogous to those of sulphur and include seleno amino acids and their derivatives, methylselenides, methyseleninic esters, methylselenones, and methylselenonium ions (21).

Selenate should thermodynamically predominate in well-oxygenated surface waters, but this is frequently not the case. Most of the transformations of Se are microbially mediated and its methylation is of both biological and environmental significance (21-22). Selenate has a low adsorption affinity for common inorganic solids, and therefore tends to exhibit high mobility. Selenite is strongly adsorbed, especially by Fe and Mn oxides. The Fe oxides have the greater affinity for selenite and adsorption increases with decreasing pH (23), as expected for an oxyanion. The reduction of selenate to selenite has been found in lakes such as Katepwa Lake in the Qu'Apelle river basin in Canada, whereas the sediment from Buffalo Pound Lake in the basin promoted the reverse reaction (24).

The biogeochemical cycle of Se in lakes, proposed by Cutter (1991) (20), is shown in Figure 5.2.. The speciation of Se in the inputs varied according to source. Dissolved selenite dominance was associated with coal ash leachates, whereas Se (-II, 0) was the main dissolved forms in normal stream inputs. The Se within the reservoirs exhibited a very dynamic cycle, showing seasonally-based, non-steady-state behaviour. Scavenging of dissolved Se took place mainly in the epilimnion and was maximal during the

summer months, due to uptake by algae. Selenite appeared to be preferentially taken up, and once incorporated into the algae, was converted to Se (-II). These findings are consistent with Se behaviour observed in lake and ocean experiments (25). Rapid regeneration of dissolved Se from the algae in reservoirs has also been demonstrated (26). Se(-II) and Se(0) were the predominant species released mainly as dissolved organic selenide. In the hypolimnia of the various reservoirs, regeneration of dissolved Se from decomposing algae is an important process. During periods of hypolimnetic anoxia, dissolved selenite and selenate concentrations are decreased, with increased concentrations of elemental particulate.

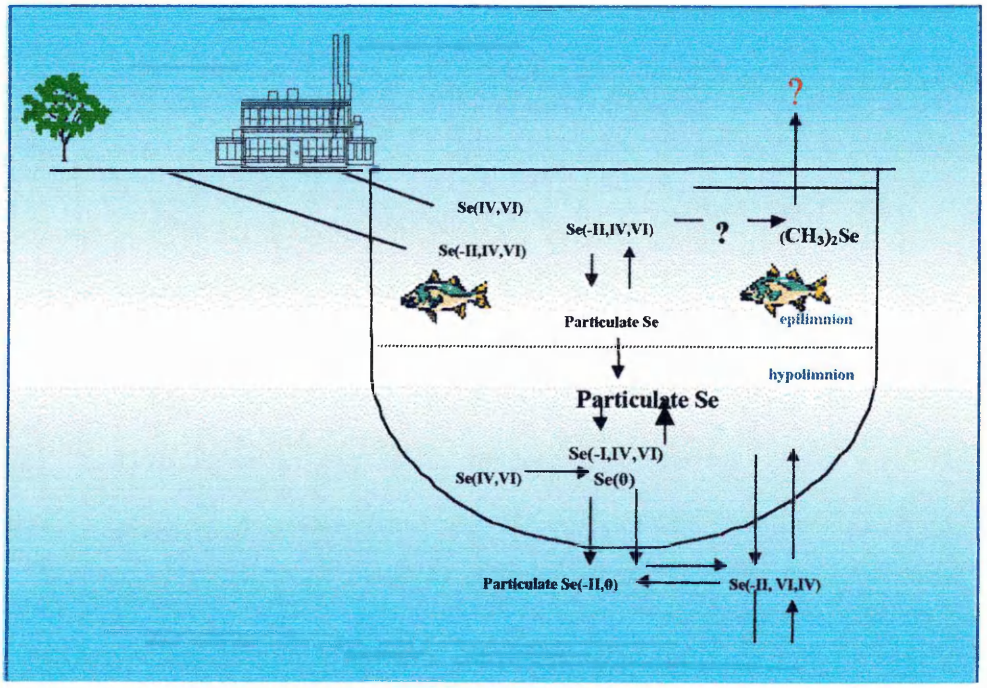

Figure 5.2. Biogeochemical cycle of selenium in lakes (20).

Studies of the Kesterson Reservoir, California, USA(27) which was contaminated with

selenium, showed concentrations in surface water between 200 – 300 μg L^{-1}. A similar investigation reported a distribution of Se in the sediment of 2.216 $mg.m^{-2}$. The concentrations found in Lake Macquarie, Australia showed concentrations between 0.1 and 12 μg g^{-1} in surficial sediment (28).

5.1.3.- Chromium

The wide spread use of chromium and the frequently inadequate disposal of by-products and wastes from industrial processes have created serious environmental pollution problems in urban areas and in other ecosystems. For instance, industrial discharges have resulted in severe Cr(VI) contamination of an aquifer underlying a farming region in southern California (29). Consequently, regulatory agencies have mandated remediation of the site, setting a maximum concentration limit of 50 μg/L for Cr(VI) and 1700 μg/L for Cr(III) (30).

As a transition metal, Cr can occur in several oxidation states, from Cr(0) to Cr(VI). In aqueous environments, however, Cr exists primarily in two oxidation states, trivalent Cr (III) and hexavalent Cr(VI). Cr(VI) exhibits d^0 electron configuration and forms complexes mainly with oxo- or hydroxo-ligands with a tetrahedral configuration. In aqueous solutions Cr(VI) is present in anionic forms whose compounds are generally soluble over a wide pH range. Hydrolysis of Cr(VI) yields a number of pH dependent species, chromic acid (H_2CrO_4), hydrogen chromate ($HCrO_4^-$), chromate (CrO_4^{2-}), dichromate ($Cr_2O_7^{2-}$) , hydrogen dichromate ($HCr_2O_7^-$), trichromate ($Cr_3O_{10}^{2-}$) and tetrachromate ($Cr_4O_{13}^{2-}$). The last three ions have been detected only in solutions of pH<0 or at chromium concentrations greater than 1 mol L^{-1} (31). In natural water (at pH

6-9, Cr(VI) concentration less than 10^{-2}M), however, CrO_4^{2-} ion is the main species of Cr(VI) (32). On the other hand, the main aqueous Cr(III) species are Cr^{3+}, $Cr(OH)_2^+$, $Cr(OH)_2^+$, $Cr(OH)_3^0$ and $Cr(OH)_4^-$ while polymeric species such as $Cr_2(OH)_2^{4+}$, $Cr(OH)_4^{5+}$ and $Cr_4(OH)_6^{6+}$ are insignificant in natural systems. In the pH range encountered in natural waters, most Cr(III) exists in the least soluble form of $Cr(OH)_3$(33).

Chromium oxidation states are directly related to environmental conditions. Cr(VI) ions possess a relatively high oxidizing potential and exhibit toxic and mutagenic effects on biological systems, while Cr(III) in nature is relatively insoluble and considerably less toxic.

Cr (VI) is reduced rapidly by Fe(II) and sulphide (34). Organic matter, including humic substances, is also an effective reductant (35). Cr(III) is more readily scavenged by particles, except possibly at low pH, and exhibits typical metal-like sorption characteristics. Anionic Cr(VI) is adsorbed less readily, with Fe oxide generally being its most important carrier phase.

Studies in seasonally anoxic lakes (36-37) have shown that Cr(VI) is the predominant dissolved species throughout the year, whereas dissolved Cr(III) is invariably undetectable, probably due to its efficient scavenging. The findings of Gunkel and Sztraka (38) in two seasonally anoxic urban lakes are different from those described previously, in that greatly enhanced dissolved Cr (III) concentrations were observed in anoxic bottom waters, a result attributed to reductive remobilization of Fe oxides.

5.1.4. Speciation of arsenic, selenium and chromium.

Several instrumental techniques have been developed that allow selective detection of different species at very low levels. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a technique which allow the determination of the total concentration of many elements with the high sensitivity of mass spectrometry. ICP-MS is well suited as a detector for inorganic and organometallic species separated by High Performance Liquid Chromatography (HPLC) (39), comparing favourably with other atomic spectrometric techniques particularly when an aqueous mobile phase is used. Moreover, sample pre-treatment may be minimised when ICP-MS is used, restricting eventual changes in the relative concentration of individual species.

HPLC-ICP-MS has been applied to As(III), DMAA, MMAA, arsenobetaine, arsenocholine and As(V) studies, mostly in biological matrices (14, 40-41), sediments and soils (42-43) or natural waters (44-45). The direct coupling hyphenated system HPLC-ICP-MS can improve the sensitivity and reduce interferences with the use of hydride generation (HG) (46) but non-hydride forming species such as arsenobetaine and the arsenosugars can not be determined by this technique. Furthermore, conflicting results reported for the determination of arsenic and selenium by the hydride generation method can be attributed to variations in the production of the hydride and its transit into the atomizer. GFAAS using ethylation and on-line trapping detection have been used for the determination of Se(IV)(47) with the disadvantage of a drastic depression in the signal when acid is added to the real samples. Se(IV) and Se(VI) also have been determined in environmental matrices using HPLC-ICP-MS (48-50). HG coupled with AFS (atomic fluorescence spectrometry) has been used for determination of Se(IV) at very low levels (51) and coupled with ICP-MS (52). Organic (seleniomethionine and selenocystine) and inorganic selenium compounds (selenite and selenate) have been

studied using HPLC coupled with UV irradiation and HG in synthetic samples (53). Neutron activation (54) and stripping voltametry (55) have also been used for Se speciation.

The application of the hyphenated technique HPLC-ICP-MS to simultaneous chemical speciation of two or more metal or metalloid species is limited by the different chromatographic conditions required for their resolution. Careful choice of the column, mobile phase and the type of chemical species is essential for success. Simultaneous speciation of As and Se compounds has been realised using emission spectrometry detection (56-57), neutron activation (54) and more recently by the use of ICP-MS as detector (58). Simultaneous determination of arsenic and chromium species in water samples has been developed using ion chromatography and ICP-MS (59), using gradient conditions, with the disadvantage of an increase in the detection limit for chromium.

In this study, an HPLC-ICP-MS method is described for the simultaneous determination of As(III), As(V), Se (IV), Se(VI) and Cr(VI) in water, sediments, fish muscle tissue and mussels . Distribution of these species in water, sediments and biological materials taken from Lake Maracaibo are discussed. Correlations using the Statgraphics statistical program (60) were found between them and the As, Se and Cr speciation results. The variation in the nature and concentration of these metal species and the physicochemical parameters (pH, dissolved oxygen, conductivity, salinity), the variations of the total concentration of selenium with total sulphur, the total concentration of arsenic with the total concentration of phosphorus, and total sulphur were also discussed.

5.2.-MATERIALS AND METHODS

5.2.1.- Reagents

Sodium meta-arsenite ($NaAsO_2$), sodium arsenate heptahydrate ($Na_2HAsO_4.7H_2O$), ammonium carbonate ($(NH_4)_2CO_3$), ammonium bi-carbonate (NH_4HCO_3) were purchased from Sigma. Di-ammonium hydrogen phosphate $(NH_4)_2HPO_4$ and ammonium dihydrogen phosphate $NH_4H_2PO_4$, calcium nitrate $Ca(NO_3)_2$, sodium carbonate (Na_2CO_3) , sodium bi-carbonate ($NaHCO_3$), sodium selenite pentahydrate $Na_2SeO_3.5H_2O$, sodium selenate $NaSeO_4$ (analar) and a solution of 1000 mgL^{-1} of cromium (VI) were purchased from Merck.

A stock solution of arsenic (1000 μg AsL^{-1}) was prepared by dissolving 433.0 mg $NaAsO_2$ and 1041 mg of $Na_2HAsO_4.7H_2O$ in 250 mL. The stock solution of selenium (1000 μgSeL^{-1}) was prepared by dissolving 81.02 mg of sodium selenite and 106.77 of sodium selenate in 250 mL of deionized water (Milli –RO/Milli-Q) system from Millipore, 18 mW). The standards were stored at –4 ^{0}C in the dark.

Five working standards from the stock solution of Se and As (5-100 μgL^{-1}) were prepared daily by dilution. The eluent ammonium dihydrogen phosphate (1.87mM)- di ammonium hydrogen phosphate (1.87 mM) was prepared and filtered (0.20μm) before use; the pH of the eluent solution was 6.5.

5.2.2.- Instruments

The HPLC system for these studies was a DIONEX gradient pump equipped with a Rheodyne Model 7125 injection valve with a 50 μL sample loop and a AS 9 DIONEX

ion exchange column , 10 psi of Helium pressure, flow rate of 1.0 $mL.min^{-1}$.

Arsenic has one isotope ^{75}As (100 % abundance). Selenium has 6 isotopes (74,76,77,78,80 and 82), and it is 35 % ionised in the Ar plasma. Se with mass 82 was used in this study. Chromium has 4 isotopes (masses 50,52,53,54). Cr is over 95 % ionised in the Ar plasma. The main Cr isotopes can suffer interference from Ar, C, Cl and S based species. Therefore Cl^- and S^- based compounds must be avoided in the determination of Cr and care must be taken when determining samples which may have a high organic content. The isotope ^{53}Cr was used for quantification in this study, as it produces less background with the eluent used.

An ICP-MS Hewlett Packard 4500 was used as detector. Instrumental conditions for the ICP-MS are shown in the Table 5.1.

Table 5.1.: Conditions used for the ICP-MS during the As, Se and Cr speciation

Conditions	For As, Se, Cr speciation
Rf Power/W	1200
Carrier gas flow rate	1.25
Sample Depth/mm	6.0
Pump speed/rps	0.30
Uptake speed rps	0.5
Acquisition Mode	Time resolved analysis
Acquisition Time (sec)	2
Torch	Fassel torch
Spray chamber	Cyclonic
Nebulizer	Babington
Coolant gas flow	10 $Lmin^{-1}$
Number of repetitions	3

5.2.3.- Sample preparation

The samples of water were filtered before the analysis (0.2 μm). The samples of sediment, muscle tissue and mussels were extracted with a solution of 1mM of $Ca(NO_3)_2$ (61). An aliquot of approximately 5 g of each was extracted with 25 mL of $Ca(NO_3)_2$ solution , shaken for 2 h, and then centrifuged at 3000 rpm for 10 min and then separated and filtered prior the analysis in the ICP-MS.

The sampling area is described in the Chapter II. The samples of lyophilised mussels (*Polymesoda solida*) and fish muscle tissue (*Cysnocion Acoupa Maracaiboencis*) were supplied by the ICLAM (Institute for the Conservation of Lake Maracaibo, Venezuela).

5.3.- RESULTS AND DISCUSSION

5.3.1.- Method optimisation

ICP-MS sensitivity was optimised by varying one instrumental setting at a time during the analysis of standard solutions of the various species studied`.

The mass spectrometer was set to sample ion intensities using a Time Resolved Analysis mode (TRA) at the following mass-charge ratios (m/z) (^{75}As), (^{82}Se) and (^{53}Cr) during the coupling measurements.

Comparison of ICP-MS signal intensities obtained for each species prepared in water was made using conventional aspiration at 1.0 $mLmin^{-1}$. The following calibration curves were obtained.

As	**r= 0.9999**	**$y = 7.1x10^3X - 3.4x10^3$**
		DL = 0.1 µg .L^{-1}
Se	**r= 1.0000**	**$y= 2.602x10^2\ X + 1.270x10^2$**
		DL= 1.0 µg .L^{-1}
Cr	**r=0.9990**	**$y= 3.316x10^3X - 2.540x10^3$**
		DL= 0.8 µg .L^{-1}

5.3.1.1. Mobile phase optimisation

Three mobile phases with different ranges of concentrations were investigated (Table 5.2). Poor resolution of As (III) was obtained using the Na_2CO_3 / $NaHCO_3$ mixture. In addition, there was a slow build-up of salt deposits in the nebuliser with time. To avoid this , the ammonium salts ($(NH_4)_2CO_3$ /NH_4HCO_3) were used instead.

Table 5.2. Optimisation of the mobile phase

Liquid Chromatography	Column	Mobile Phase
Anion Exchange	AS-9	Na_2CO_3 – $NaHCO_3$ (2.1 to 7mM) (optimised at 3.5 mM) pH=8.5
Anion Exchange	AS-9	$(NH_4)_2CO_3$-NH_4HCO_3 (7mM to 21mM) (optimised at 10.5 mM) pH=8.7
Anion Exchange	AS-9	$(NH_4)_2HPO_4$-$NH_4H_2PO_4$ (0.93 to 7.5 mM) (optimised at 1.87 mM) pH=6.5

However, the resolution of As species did not improve. Good resolution was obtained when ($(NH_4)_2HPO_4$ /$NH_4H_2PO_4$) was used as is shown in the Figure 5.3.

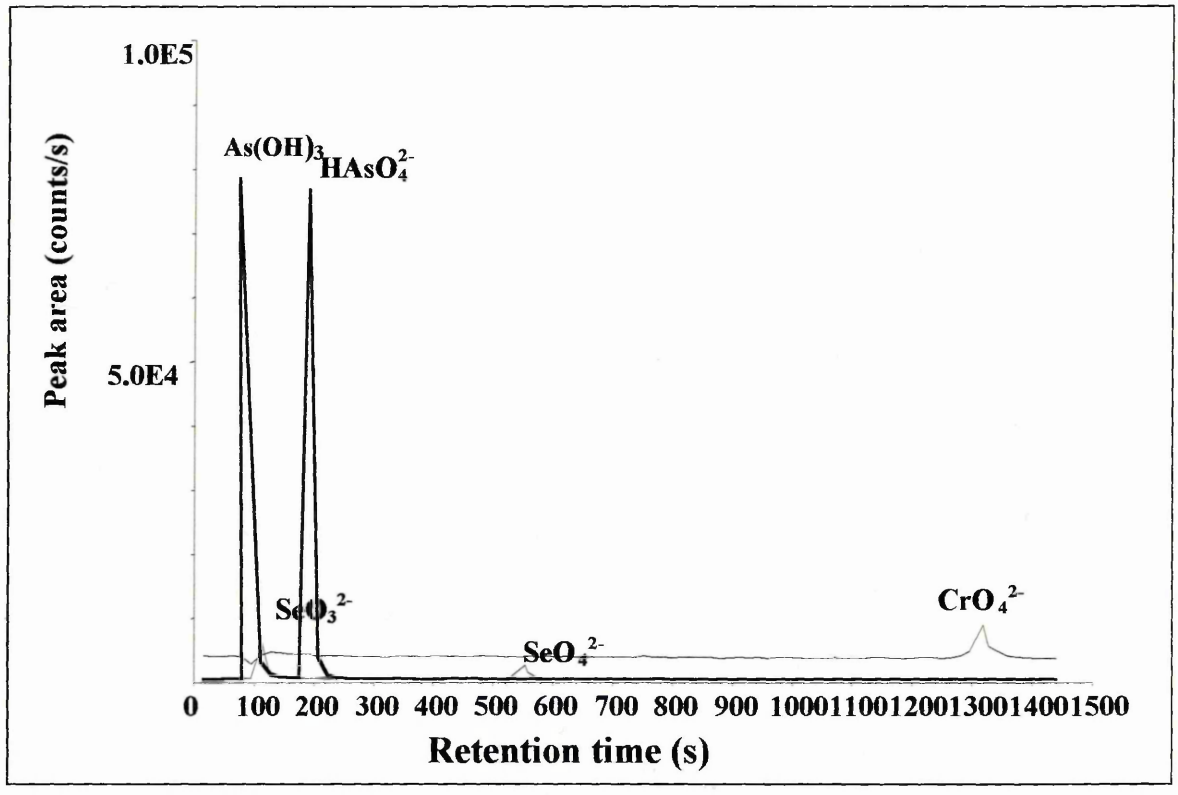

Figure 5.3.: Chromatogram showing the separation of the species from a solution with 100 μgL^{-1} of selenite, selenate, arsenite, arsenate and chromate

Because the long retention time for chromium (VI) (1400 sec), different programs of gradient concentration of the eluent were applied in attempt to eluate the species earlier. The optimised gradient elution program is shown in the Table 5.3.:

Table 5.3.: Gradient program used during the separation of arsenic, selenium and chromium species.

Time	Eluent
0 – 2.5 min	H_2O
2.5 – 3.0 min	change
3.0 – 15 min	7.5 mM eluent ammonium phophate, 7.5 mM di-ammonium phosphate

Although good separations were found for all of the species studied, this program was eliminated because of interference due to the formation of the species N Ar in the ICP when the concentration of the eluent is increased, the resulting high background for the ^{53}Cr affecting its detection limit (Figure 5.4).

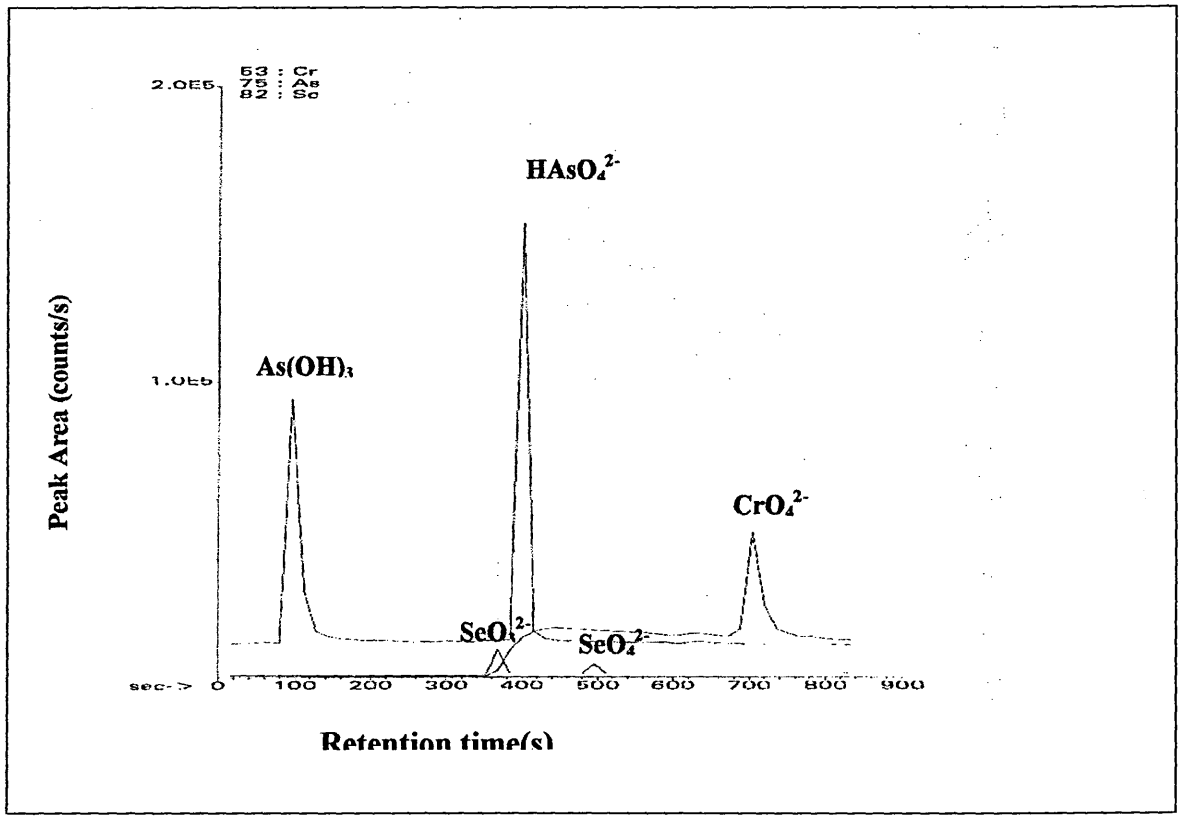

Figure 5.4: Chromatogram showing the separation of the species using the gradient program.

The following calibration curves were used with the developed method without gradient elution:

Regression Curves

$As(OH)_3$

y = 1145.6 X - 57.4 r= 0.9970

DL= 1.3 µg .L^{-1}

$HAsO_4^{2-}$

y = 611.7 X + 2226.0 r= 0.9994

DL= 3.0 µg .L^{-1}

SeO_3^{2-}

y = 100.6 X + 459.1 r = 0.9996

DL= 1.3 µg .L^{-1}

SeO_4^{2-}

y = 29.8 X + 911.6 r = 0.9995

DL= 4.5 . µg .L^{-1}

CrO_4^{2-}

y= 516.5 X + 5742.0 r = 0.9999

DL=2.0 µg .L^{-1}

The method optimised with isocratic conditions was applied to water, sediments, muscle tissue and mussels samples, with the species Cr(VI) included in the analysis. The samples of water were filtered before analysis. Figure 5.5 shows the chromatogram of a sample of water.

Most of the isotopes of Se are subject to interferences from isobaric overlap (masses 74,76,78,80 and 82) or polyatomic interferences, principally Ar_2^+ on masses 76, 78, 80 . ^{77}Se (7.63 % abundance) is one of the most useful isotopes used for the determination of Se but is susceptible to interference of ArCl from Cl matrices such as in sea water and sediments. ^{82}Se (8.73 % of abundance) is also useful for quantification but does suffer from HBr interference in Br-containing samples. In this study ^{82}Se was used as isotope for quantitation.

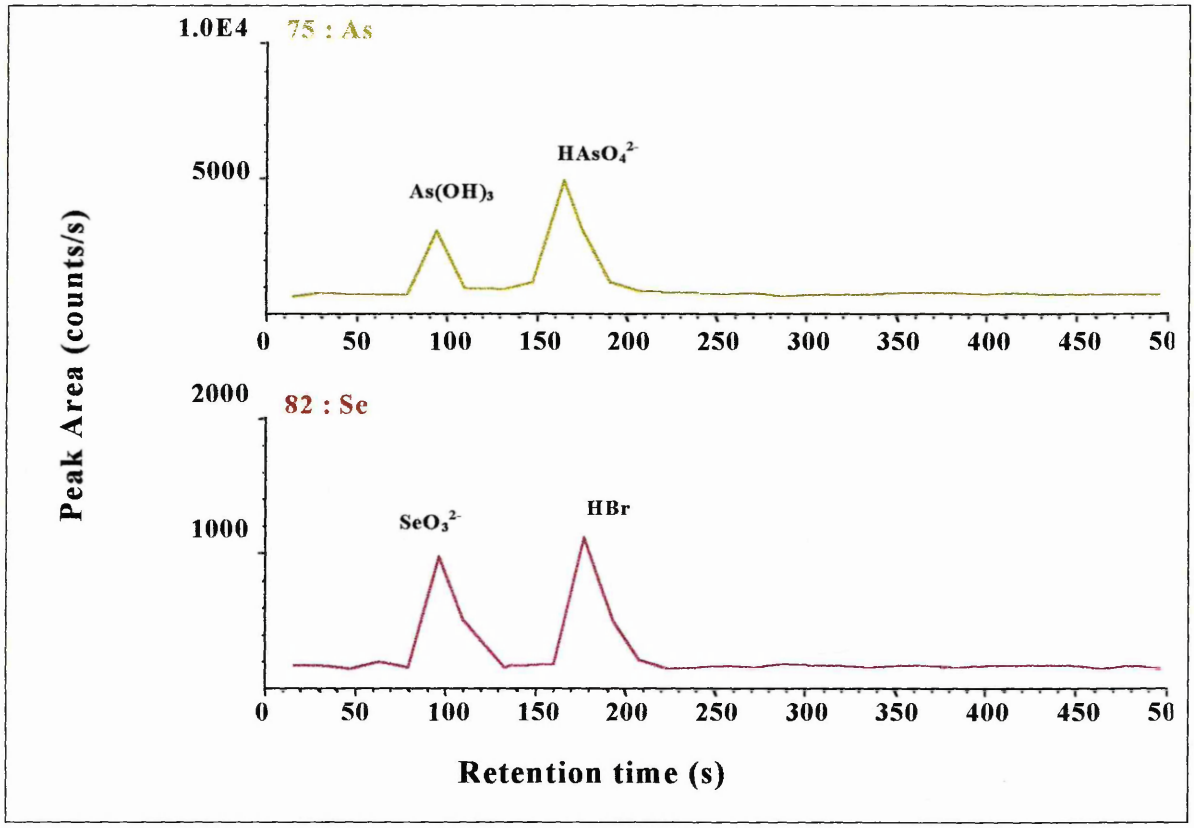

Figure 5.5.: Chromatogram of a sample from Lake Maracaibo showing the species of Se and As.

The following table shows the most common interferences for ^{82}Se:

Compounds	Interference (%)
Kr	11.6
CuO	0.012
ZnO	27.93
BrH	49.30

A spectral interference was found in the samples of water and sediment because the high Br^- concentration leading to the formation of HBr that can interfere with the signal at ^{82}Se, .

Figure 5.6 shows the effect of addition of an aliquot of a solution of 1000 $\mu g.L^{-1}$ of KBr to the water sample from Lake Maracaibo.

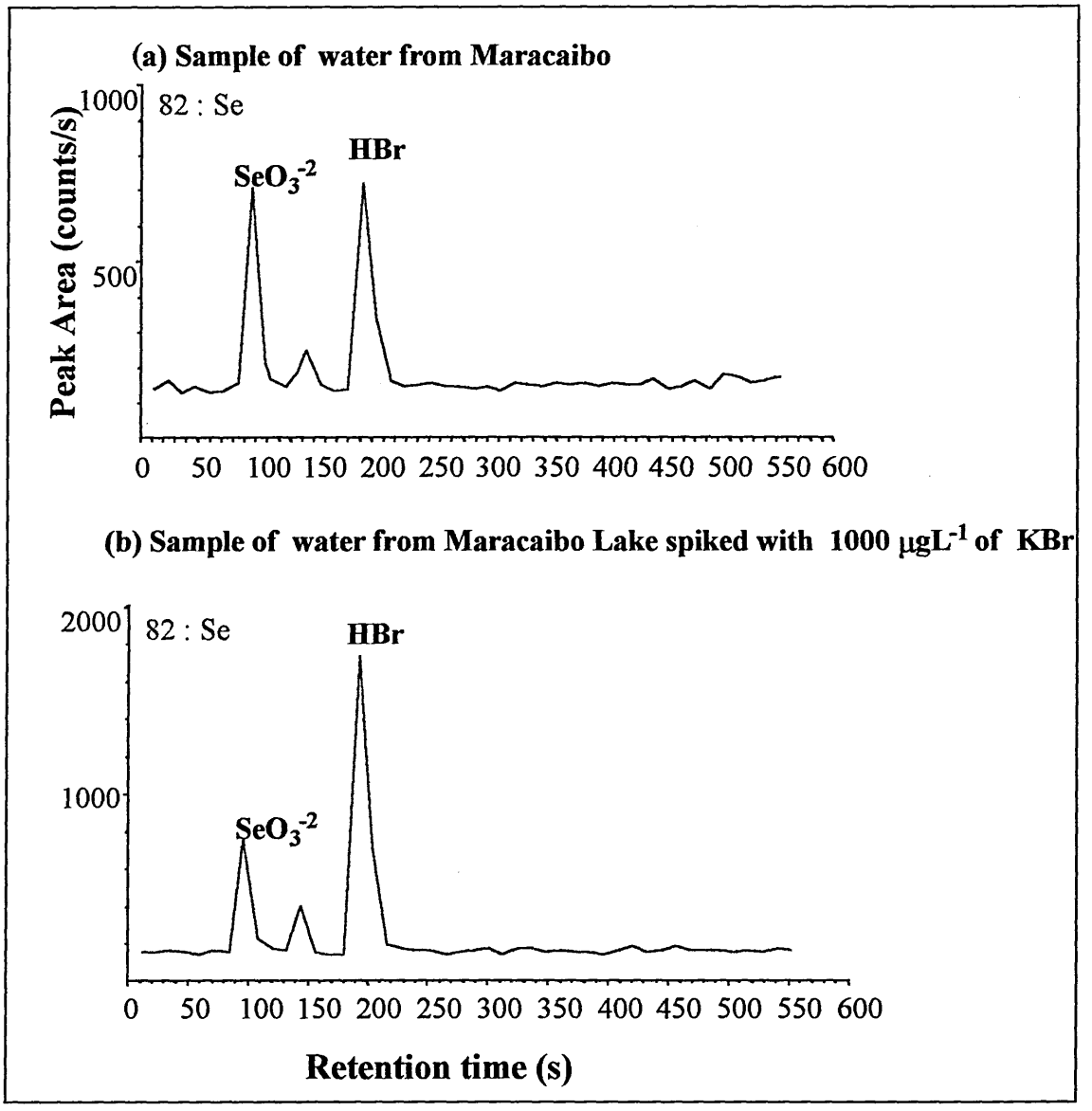

Figure 5.6: Chromatogram of a sample of water with the addition of a spike of KBr showing the interference of the HBr.

Table 5.4 presents the results of the analysis of water samples from Lake Maracaibo. The selenate species was not found at most of the sampling points, although selenate should

predominate thermodynamically in aerated waters. Selenite is more adsorbed by Fe and Mn oxides and this increases with decrease in pH, as is found in zones near to the sediment in the centre of Lake Maracaibo. In addition, selenate reduction to selenite by bacteria has been reported recently (62), and dissolved selenite dominance in lakes has been associated with coal ash leachates.

Table 5.4: Water samples results ($\mu g.L^{-1}$) of the sampling in Lake Maracaibo.

Sampling points	As(III)	As(V)	Se(IV)	Se(VI)	Total As	Total Se
CA-2	4.4 ± 2.74	<3.0	<1.3	<4.5	2.86 ±0.30	2.61 ±0.33
C-1	4.19 ±0.02	<3.0	1.60 ±0.15	<4.5	3.11 ±0.04	3.41 ±0.09
NO-2	4.40 ±0.02	<3.0	<1.3	<4.5	4.13 ±0.06	4.31 ±0.11
D-4	2.82 ±0.04	<3.0	1.72 ±0.15	<4.5	3.56 ±0.11	4.38 ±0.09
PR	6.58 ±0.05	3.08 ±.0.30	<1.3	<4.5	10.56 ±0.11	3.82 ±0.09
D-2	4.40 ±0.12	<3.0	<1.3	<4.5	3.70 ±0.01	3.94 ±0.01
D-114	4.57 ±0.12	<3.0	<1.3	<4.5	4.18 ±0.13	4.64 ±0.19
D-74	4.22 ±0.24	<3.0	<1.3	<4.5	5.32 ± 0.02	2.60 ±0.16
Guam	6.14 ±0.24	<3.0	<1.3	<4.5	10.33 ±0.12	8.11 ± 0.22
S-6	4.07 ± 0.05	<3.0	<1.3	<4.5	2.51 ± 0.00	2.85 ± 0.01
D-119	3.58 ± 0.34	<3.0	<1.3	<4.5	2.97 ±0.06	2.62 ± 0.18
C-11	5.09 ±0.01	3.98 ± 0.61	2.29 ±0.24	<4.5	8.68 ± 0.06	2.84 ± 0.07
D-33	2.93 ± 0.80	<3.0	2.18 ± 0.04	<4.5	4.54 ±0.04	3.27 ± 0.07
0-20 ±	3.96 ± 0.14	<3.0	<1.3	<4.5	3.80 ± 0.06	3.23 ± 0.14
0-13	4.16 ±0.14	<3.0	<1.3	<4.5	4.06 ± 0.05	4.18 ± 0.10
C-9	4.06 ± 0.01	<3.0	<1.3	<4.5	4.02 ± 0.05	2.34 ±0.11
D5a	<1.3	<3.0	<1.3	<4.5	4.19 ± 0.07	4.56 ± 0.09
San Carlos	<1.3	<3.0	<1.3	<4.5	<0.1	<0.1

Two species of arsenic were found in the samples as was expected. Arsenic in lakes (63) normally occurs in the two common oxidation states (+3, +5), these species existing in solution as arsenate ($H_2AsO_4^-$ and $HAsO_4^{-2}$), and arsenite in the form of the neutral species $As(OH)_3$.

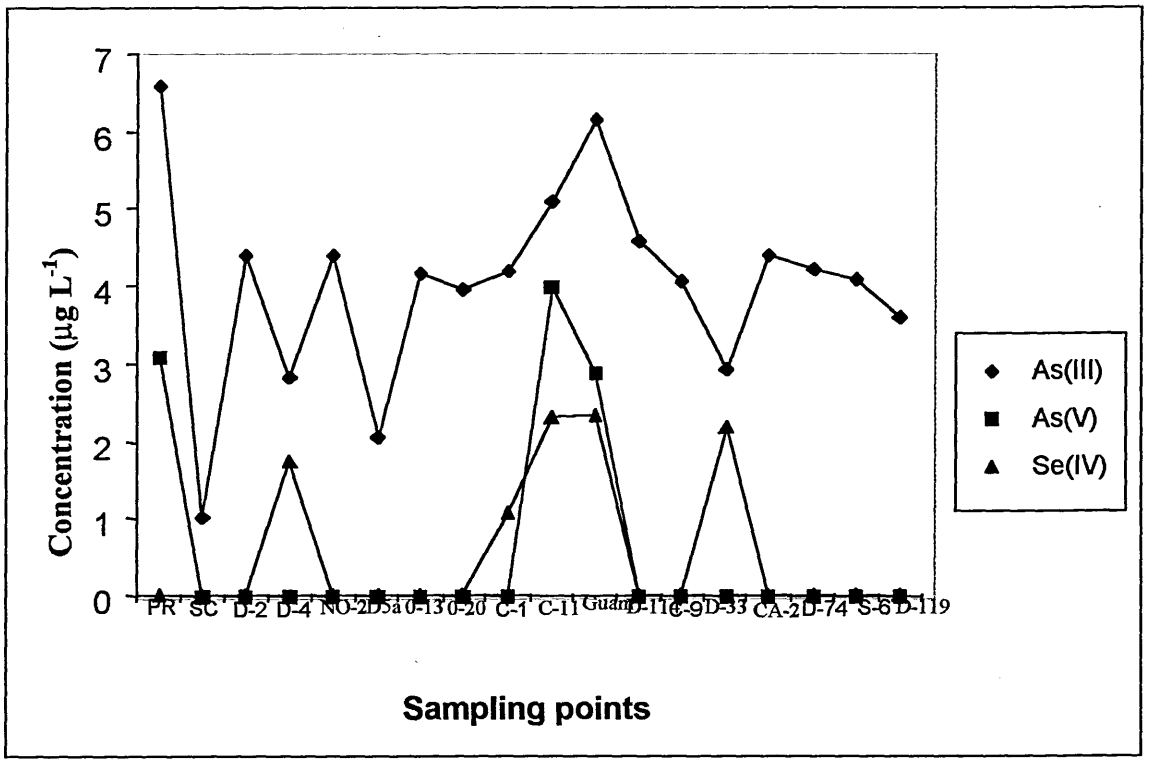

Figure 5.7: Dominance of As(III) species over the other species found in water samples from Lake Maracaibo

The Figure 5.7 shows the dominance of the As(III) specie in all of the places sampled. This can be explained because As(V), remobilised from Fe oxides, can be reduced to As(III) (64), and S(-II) could also act as a reductant (65). In addition, algae and microbiological cultures under aerobic conditions are able to reduce As(V) to As(III). The ecological logic of microbiological As(V) reduction has been explained in terms of a detoxifications strategy aimed at avoiding the consequences of incorporating arsenate instead of phosphate (66).

5.3.2.-Sediment results

The total Se concentrations found in waters and sediments from Lake Maracaibo were in the ranges of 4.8 – 8.1 μg L^{-1} and 286 – 1,890 μg Kg^{-1}, respectively which are low if they are compared with the studies of the Kesterson Reservoir, California, USA, that was contaminated with selenium and showed concentrations in surface water between 200 – 300 μg L^{-1}. Se concentrations in sediments found in a lake in Australia showed concentrations between 100 to 12,000 μg Kg^{-1} in surficial sediment (28).

The concentration ranges of As (III) and As (V) of the sediments were (13.1-200.7 μg g^{-1}) and (26.7 – 237.8 μg g^{-1}), respectively. The levels of Se (IV) range between 8.5 – 38.5 μg g^{-1}. No Se (VI) species was detected. The dominance of As (III) in the sediments of Lake Maracaibo at different pH is shown in the Figure 5.8.

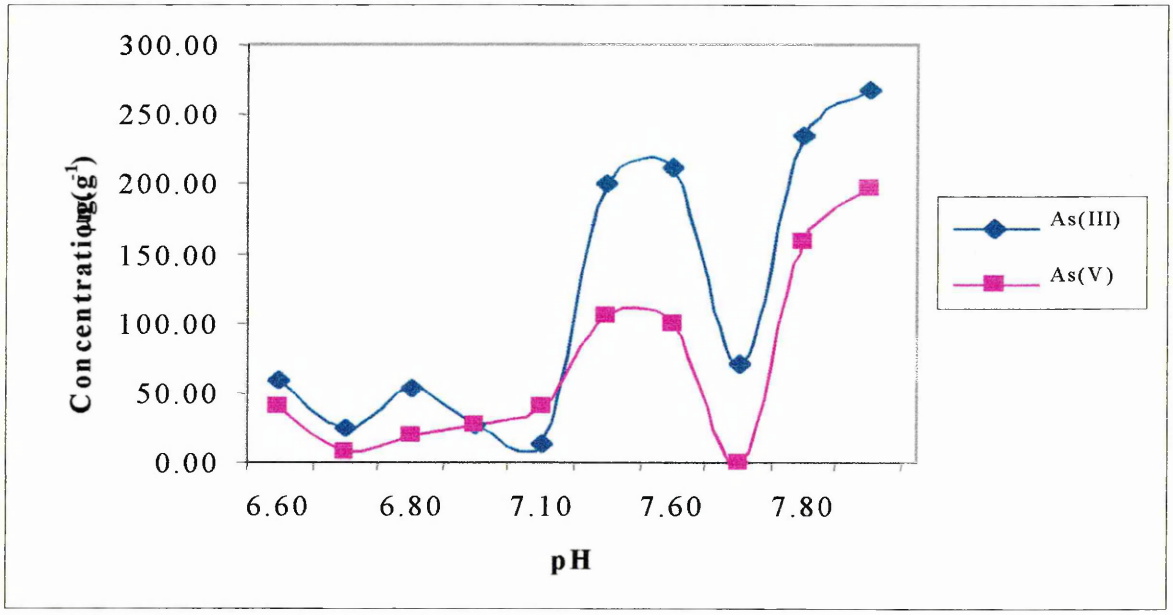

Figure 5.8: The variation of As(III) and As(V)in sediment at different pH values found in Lake Maracaibo.

5.3.3. Biological indicator results

The selenite species was found in samples of muscle tissue of curvine *(Cynoscion Acoupa Maracaiboensis)*, a more abundant fish from Lake Maracaibo. The mean concentration of trace metal species in the samples analysed were As (III) = 764.5 μg Kg^{-1} and Se (IV) = 313.6 μgKg^{-1}, respectively. Inorganic arsenic species have been found by Suñer et al (20) in fish from Guadalquivir estuary, Spain, contaminated with arsenic and heavy metals released from a mine, at concentrations of 260 μg Kg^{-1} , which are lower than the concentrations found in Lake Maracaibo.

The total arsenic found in bivalve species from Miami River (USA) revealed concentrations ranging between 23. 6 to 37.3 μg.g^{-1} . A study of total arsenic in muscle tissue and the inner organs of mullet from Lake Macquarie, Australia (14) showed concentrations of arsenic between in 4.7 to 19.2 μg.g^{-1} , this being a much lower level for muscle tissue than that found in Lake Maracaibo. Valette-Silver *et al* (13) have shown that the most common species of arsenic in bivalves from the Southeast Coast of the USA are monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) . The mean concentrations of the various metallic species found in mussel *(Polymesoda solida)* from Lake Maracaibo were As (III) = 1.3 μg g^{-1}, Se (IV) = 0.3 μg g^{-1} ; Se(VI) = 6.3μg g^{-1} which are solely inorganic species as was also found in the Guadalquivir Estuary in Spain. However, the behaviour of tropical lakes like Lake Maracaibo is different and estuarine chemistry cannot be compared with seasonal lakes which have high differences in temperature. The natural cycles of the elements can produce changes in the binding of these elements and this could be the cause

of the differences between the arsenic species in this lake and the species found in other seasonally variable lakes. Information about selenium species in fish muscle tissue and mussels does not appear to be available.

Although, the total chromium concentration in the waters of Lake Maracaibo ranged between (1.71 – 2.14 $\mu g.L^{-1}$) and in sediments between (0.93-17.20 $mg.Kg^{-1}$), Cr(VI) was not found in the samples. The reduction of Cr(VI) to Cr(III) by bacteria, Fe oxides and algae could be the cause of this effect. Cr(III) cannot be determined by the developed methodology.

Correlations:

The Pearson product moment showed correlations between the following pairs of variables (P-values below 0.05 indicating statistically non-zero correlations at the 95% confidence level): As (III) and Fe_2O_3 ($r = -0.5630$, $n=13$, $p< 0.0451$); As(V) and Fe_2O_3 ($r = -0.6421$, $n=13$, $p< 0.0180$); SiO_2 and As(III) ($r = 0.5823$, $n=13$, $p< 0.0368$) and As(V) ($r= 0.5544$, $n=13$, $p< 0.0493$) ; As(III) and As(V)(($r =0.9304$, $n=13$, $p<0.001$); Se(IV) and Mn_3O_4 ($r = 0.7272$, $n=13$, $p< 0.0049$).

5.4.-CONCLUSIONS

In conclusion, the developed method can be used for the determination of As(III) and As(V), Se(IV) and Se(VI) and Cr(VI) in waters, sediments, fish muscle tissue and mussel. The reducing conditions of the lake sediments could be the cause of the reduction of As, Se and Cr and the fact that they mainly occur as reduced species in the Lake. The method was not suitable for the determination the Cr(III) species.

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Chapter VI

Chemical speciation of mercury and selenium in water, sediment, fish muscle tissue and mussel from Lake Maracaibo, Venezuela

6.1.- INTRODUCTION

6.1.1.-Mercury

Mercury is considered a nonessential but highly toxic element for living organisms. Even at low concentrations, mercury and its compounds present potential hazards due to bioconcentration in the food chain. Poisoning by methylmercury compounds presents a bizarre neurological picture as observed in large scale out-breaks in Japan and Iraq. Damage is chiefly in the cerebellum and sensory pathways with lesions in the cerebral cortex of man (1).

The high toxicity of mercury (II) compounds has long been known . This high toxicity can be explained for the profound capacity of the soft acid (acceptor) such as CH_3Hg^+ to bind soft ligands such as –SH groups of proteins (2). The conversion of inorganic mercury to the more toxic monomethyl and dimethyl mercury was first detected in aquarium sediments. It was discovered that microorganisms are capable of this transformation (3). Thus a pathway was uncovered by which mercury could enter the biological food chain. Monomethylmercury, being a toxic compound, is not tightly bound to sediments is somewhat water-soluble and volatile, and it is rapidly assimilated by living organisms and then retained (4).

A typical biological food chain for mercury is shown Figure 6.1(4). Decay of organic material in the aquatic environment enriched by disposal of sewage and industrial effluents together with detritus formed by natural weathering processes, provides a rich source of nutrients in both the bottom sediments and the overlying water body. Microorganisms and the microflora are capable of incorporating and accumulating

metal species into their living cells from these sources. Subsequently, small fish become enriched with the accumulated substances. Predatory fish, generally display higher levels than their prey. Eventually man, consuming fish, inevitably suffers from the results of bioaccumulation having taken place at each tropic level i.e., where less is excreted than ingested.

With regard to the element mercury, it is generally accepted that large predatory species such as swordfish and tuna usually have higher levels of mercury in their tissue than lower species in the food chain (4) . A study revealed that the position of the fish

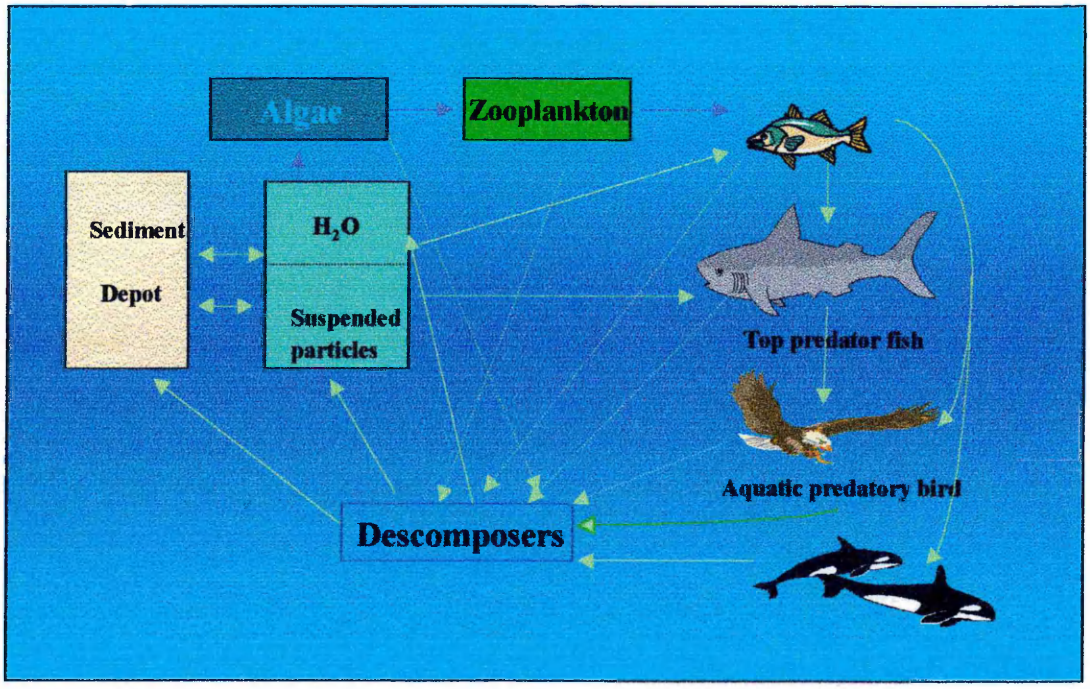

Figure 6.1: Food chain model for mercury (4).

in the food chain appears to be an important factor in determining its mercury content, the 51% of fish species whose diet predominantly consists of other fish, had mercury concentrations in excess of 0.5 $mgKg^{-1}$. In contrast, only 24% of invertebrate predators and 7% of individuals of herbivorous habits had mercury concentrations in excess of

$0.5mgKg^{-1}$.

The largest single source of mercury pollution results from the Hg-electrode process in the chlor-alkali industry; mercury based fungicides formerly contributed extensively toward mercury in the environment (5).

The mercury ion can be converted into methylmercury species by algae (6), humic substances (7) etc via methylation of mercury derivatives by bacteria (8,9,10). It should be stressed that most cases of human poisoning by organometallic compounds have involved the ingestion of methylmercury compounds. This organometallic species is neurotoxic, causes blockage of enzymes binding sites, interferes in protein synthesis, impedes thymidine incorporation into DNA, etc (11) . Reports of such cases have come from many areas of the world, but those from Asia have been most numerous. Particularly disastrous were the widespread methylmercury poisoning cases of Minamata Bay, Japan from which the name of "Minamata Desease" was derived to describe methylmercury poisoning (12).

The high affinity of methylmercury to sulphydryl groups and the lipids of animals would explain its accumulation in living organisms, particularly in lipid tissue of mammals. It appears that sulphide groups in the sediment have influences on the binding and final preconcentration of mercury species in sediments (13)

The rate and the extent of methylation of Hg(II) in waters and sediments depend upon factors such as: precise nature of the inorganic mercury precursor, e.g., mercury acetate is easier to methylate than mercury chloride, the methylating agent (14), the chemical composition of the sediment, its oxygen concentration and the pH (15).

Several interconversions are possible which are catalysed or at least promoted by microorganisms (16). Three characteristic steps are described in the Figure 5.2. in

biological cycle of mercury (17).

(a) Mercury sulphide transformations: (1) precipitation from Hg^{2+} and S^{2-} ions

(2) Take over of sulphide ions from other sulphides like FeS and

(3)Interaction with the equilibria of organomercurials

(b) Hg^{2+}-Hg^{0} Transformations. Reduction of dissolved Hg^{2+} to Hg^{0} by enzymatic reactions.

(c) Organomercurial transformations

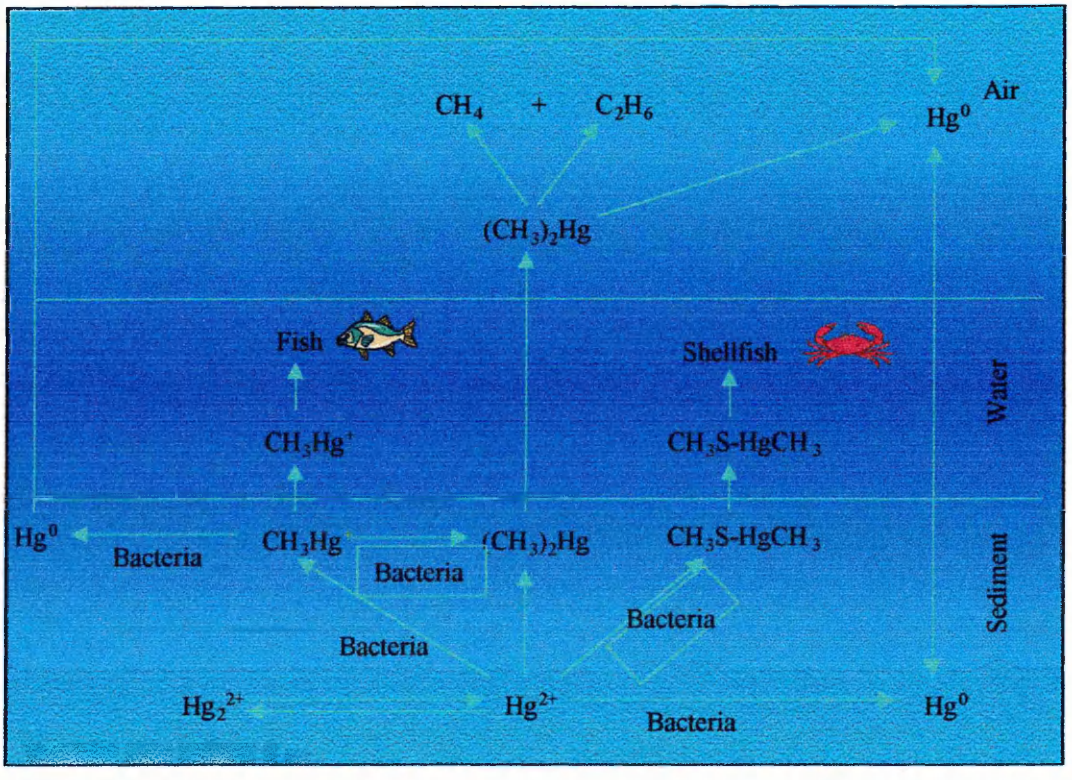

Figure 6.2 : Biological cycles of mercury in the environment (17)

Recent reports have estimated a total mercury concentration in natural waters, ranging from 0.2 to 100 ng $L^{=1}$, while methylmercury levels were much more lower, around 0.05 ng L^{-1} (18- 20). However, higher levels can be found in water from heavily industrialised areas (21-22). The upper limit for total mercury concentration in drinking

water recommended by the EU is 1 μg L^{-1}. Inorganic mercury and methyl mercury can be preconcentrated in sediments and they are found at relatively high levels in fish (23-25). The concentration of Hg in lakes and rivers can vary from a few ng $L^{-1}$to 1000 ng L^{-1} for waters exposed to sewage effluents, industrial contamination or Hg deposits (1). Klein (26) suggested that an average concentration of 55 ng L^{-1} was a reasonable estimate for a natural Hg background in the rivers and lakes of the USA.

Sediment cores from lakes, which have been investigated with respect to an assessment of historical changes in the pollution intensities, mostly exhibit a characteristic increase of the mercury concentrations during the last few centuries even if there is no significant variation of other metals (except in many cases of lead) (27). Industrial and mining activities were sources of contamination which can affect the enrichment of mercury in most aquatic sediments (27). Sediments from lakes in Southern Sweden were examined for mercury pollution from pulp and paper mills and from chlor-alkali plants; the smaller lake (Lake Bjorken) presented a maximum of mercury of 11 $mg.Kg^{-1}$ Hg in the surface sediment (1). Colina and Romero (28) studied the total mercury concentration in muscle and organs of fish (Curvina), shrimp and mussels from Lake Maracaibo where a chlor-alkali plant was located; they reported concentrations that ranged between 0.06 to 1.5 $mg.Kg^{-1}$ of total mercury.

The mercury problem in the North American Great Lakes has aroused public concern because fish in Lake Eric had mercury concentrations of 5 mg Kg^{-1} ie. ten times the current permissible level laid down by the U.S. Food and Drug Administration Act. In the bottom sediment, the highest value was 86 mg Kg^{-1} ; significantly, a chlor-alkali plant was located near to the area(1).

Dredging processes have been investigated because they can also increase the mercury problem, dredging activities dispersing uncovered mercury sediment over large areas, resulting in an increase in the mercury content over a period of time in the flora and fauna of the water course (27, 29).

The physico-chemical state of mercury in water systems is rather complicated, as mercury can form a wide variety of species. The reported partitioning of total Hg between the dissolved and particulate fractions indicates that between 10 to 90 % of the Hg may be associated with particulates (5). Mercury can exist in three stable oxidation states, ie. 0 (elemental), +1 (mercurous) and +2 (mercuric); bivalent mercury has ability to form complexes with many chemical species in solution, commonly referred to as complexing ligands. Complexation of mercury by both inorganic and organic ligands plays an important role in the migration and behaviour of mercury forms in natural waters. In addition to the complexes, bivalent mercury forms an important group of organomercury compounds, where one or two alkyl groups (R or R') are directly linked via their carbon atoms to the mercury atom: R-Hg-X or R-Hg-R' (X is an inorganic ligand)(5).

Hem (30) calculated the soluble inorganic mercury forms in typical fresh waters using chemical equilibrium constants and standard redox potentials published in the literature. This results showed in the form of a Eh-pH diagram how the chemical form of mercury in solution was strongly affected by the redox conditions in the system, characterized by its redox potential Eh, and the pH. The redox potential in natural waters is determined mainly by the concentration of dissolved oxygen and by the organic matter content. In well-aerated, oxygen-containing waters (Eh~0.5 V), the predominant mercury species will be the form of inorganic soluble mercury. On the

other hand, elemental mercury is formed under mildly reducing conditions, unless enough sulphide is present to stabilize hydrosulphide or sulphide complexes of bivalent mercury. A significant abundance of the sulphidic complexes can be expected in sulphide marine waters (8) in the interstitial water of bottom sediments, or in certain types of waste waters.

As mentioned before, the alkyl-mercury compounds representing the most toxic forms of mercury can be divided into two types: those which in the mercury atom is linked to one alkyl group and those in whist the mercury it is attached to two alkyl groups. The first type is rather soluble in water where it is attached to give $R\text{-}Hg^{+}$ cation and an X^{-} anion. The second type of organo-mercurial compound, such as dimethyl-mercury and diphenyl-mercury, are volatile, non-polar and very poorly soluble in water. Thus it is improbable that dimethyl-mercury would represent a significant part of the mercury dissolved in water (18). The occurrence of organo-mercurials in some Canadian lakes was studied by Chau et al (31); methyl mercury was detected in three lakes with a concentration between 0.5 and 1.7 $ng.L^{-1}$. No dimethyl or diphenyl-mercury was found.

An inverse relationship has been observed between dissolved sulphide concentration and the production of methylmercury ($MeHg^{+}$) in sediments from aquatic ecosystems (32-34). However, Benoit and co-workers (35) have recently hypothesised that uptake of inorganic Hg by methylating bacteria is diffusive and that the observed sulphide inhibition arises from a decreasing fraction of neutral Hg complexes with increasing sulphide concentration . Also, it has been shown that at least some neutral complexes such as $HgCl_2$, are lipid soluble and that their uptake by phytoplankton occurs by passive diffusion (36). In contrast, the transport across the blood-brain barrier by

methylmercury appears to involve one of the amino acid transport systems (37).

Complexation of Hg may affect its availability to the bacteria that produce methylmercury (MeHg). Sulphate-reducing bacteria (SBR) mediate methylation of inorganic Hg in aquatic sediments (8), and these organisms produce sulphide as a byproduct of their metabolic activity. Methylation of Hg occurs inside SBR via enzyme-mediated transfer of methyl group from vitamin B_{12} (38), but the Hg uptake mechanism in SBR is unknown. The presence of sulphate both stimulates $MeHg^+$ production and enhances the activity of SBR in sediments (39), except under conditions where sulphide accumulation limits MeHg production (40). Sulphide inhibition has been ascribed to the removal of Hg from solution via enhanced precipitation of HgS(s) (41) or to the formation of volatile dimethylmercury from reaction of $MeHg^+$ with H_2S (42). Recent studies by Tossels (43) concluded that the species HgS is unstable in the presence of H_2O, reacting to form HgS-(H_2O) which subsequently isomerizes to Hg(SH)(OH). This is more stable than HgS(H_2O) at all levels of theory, in both gas phase and solution. When the SH^- concentration increases, $[Hg(SH)_2(OH)]^{1-}$ predominates. This anion has a large hydration energy, and is thus confined to aqueous solution.

6.1.2.- Mercury species determination

The determination of the two main environmentally relevant species of mercury, Hg(II) and CH_3Hg^+ in aqueous solutions is relatively straightforward but is more involved when they are present in solid samples (44). It is essential that the integrity of the mercury species is maintained during the sample pre-treatment and analysis. Sample preparation includes homogenisation, extraction and pre-concentration before the

chemical species are separated and determined.

Cryogenic trapping (45), column chromatography (46), non-chromatographic column (47) and electrochemical methods (48) can be used as pre-concentration techniques. On the other hand, GC (49), HPLC (50-51) and non-chromatograpic (5) methods are normally included during the separation of mercury species. In HPLC-ICP-MS methods the column- atomiser interface is simple, via direct connection with a teflon tube, (52-55). Plasmas, both at atmospheric and reduced pressure (56) offer great analytical potential as detectors. Some of them, particularly ICP-MS, have many of the main desirable features for a detector in hybrid chromatographic techniques. Other methods for mercury species detection include AFS (57) and electrochemical techniques (58).

6.1.3.- Selenium

Selenium (Se) has a complicated redox chemistry, closely related to that of S, and biologically, is both an essential and a toxic element (59).

The aquatic chemistry of selenium is also complicated since it can exist in four different oxidation states and as a variety of inorganic and organic compounds. These are described in Chapter V.

Several techniques have been developed that allow selective detection of different species of Se at very low levels. HPLC-ICP-MS (60-61) or HPLC-ICP-AES (62-63), hydride generation techniques (64) coupled atomic absorption techniques and ICP-MS, fluorimetric methods (65), GC-AED (66) and neutron activation analysis (67) have been used for Se speciation, but in most of the cases, only selenite and selenate have been determined.

Seleno-aminoacids have been determined using GC with element specific atomic emission detection (AED)(68). More recently, Goessler et al (69) have described a method which determined the distribution of eight selenium species by chromatographic separation and inductively coupled plasma-mass spectrometry detection. Another method for chiral speciation and determination of selenomethionine enantiomers in yeast was developed by Sanz-Medel et al (70) using HPLC with methanol-water as mobile phase and ICP-MS as detection. Gonzalez-La Fuente et al developed a method for the determination of selenite, selenate, selenomethionine and selenoethionine in urine using an on-line reversed –phased high performace liquid chromatography sstem with microwave digestion-hydride generation atomic detection (71). The retention time obtained for those species varied between 3-9.0 min and the method is complicated to use. Ion –chromatography coupled with ETAAS (72) has been used to determine inorganic and organic selenium compounds with the disadvantage that it involves matrix modifiers because of the differences of thermal stability of the selenium species. HPLC-ICP-MS, with an anion exchange column, has been used for the determination of selenocystine, selenomethionine, selenite and selenate in fish (73).

This investigation describes a simultaneous method for the separation and determination of the inorganic mercury and methyl mercury, and selenocystine and selenomethionine using HPLC with a reversed phase column and ICP-MS detection. The method was used for the environmental evaluation of mercury and selenium in Lake Maracaibo. Samples of water, sediment, mussel and fish muscle tissue were analysed using this methodology. Mercury species in sediment were correlated with the physicochemical parameters and the availability of nutrients, e.g. nitrogen, phosphorus and sulphur, the total content of which was determined during sampling .

6.2.- MATERIAL AND METHODS

6.2.1. Simultaneous determination of Hg and Se

6.2.1.1.-Reagents

The inorganic mercury solution was prepared from a stock solution of 1000 mg Hg L^{-1} ICP Aristar standard in 2% HNO_3, supplied by Merck (Poole, Dorset, UK), and a stock solution of 1000 mg L^{-1} of the methyl mercury chloride from Reidel-de-Haen (Seelze, Germany) was made by dissolving 0.125 g in 10 ml of 10 %v/v HNO_3. Seleno(dl)-cystine (99 %w/w) and seleno(dl)-methionine (99 %w/w) were purchased from Sigma (Poole, UK) and Fluka (Poole, UK) respectively.

Toluene, hydrochloric acid, L-cysteine, hydrogen peroxide and nitric acid used during the extraction-digestion methods were supplied by Merck.

For the mobile phase, ethylenediaminotetraacetic acid (EDTA) from Fluka, HPLC grade methanol (99.9%) and 2-mercaptoethanol (Analar grade) from Merck and ammonium acetate from Aldrich (Milwuakee, USA) were used.

Reference Materials

Polluted Marine Sediment IAEA 356 Reference Material and Estuarine Sediment LGC 6137 was used to validate the total mercury content and the methylmercury concentration in sediments.

6.2.1.2.- Procedure

Instruments

The HPLC system (isocratic conditions) for these studies was a DIONEX GPM 2 (Dionex Corporation, Sunnyvale, California, USA) gradient pump equipped with a Rheodyne Model 7125 injection valve with a 50 μL sample loop and a C18 reversed phase column (150mmx 3.9 mm, 4 μm) which was comprised of dimethyloctadecylsilyl bonded amorphous silica (Waters HPLC column Milford, MA, USA) , 10 psi of Helium pressure, flow rate of 1.0 $mL.min^{-1}$.

For the determination of mercury, the most abundant ^{202}Hg isotope was used. An ICP-MS Hewlett Packard 4500 was used as detector.

The mobile phase was connected directly into the nebulizer without the need to use ICP-MS pumps.

The method is modified from that previously reported (74) for inorganic mercury and methyl mercury, Table 2 shows the LC conditions standardised during the study.

The water samples were filtered with a 0.2 μm Millipore filter before the injection in the HPLC system for the determination of the mercury species.

Table 6.1 : ICP-MS conditions used during this study

Conditions	For Hg speciation
Rf Power/W	1200
Torch	Fassel torch
Spray chamber	Cyclonic
Nebulizer	Babington
Coolant gas flow	10 $Lmin^{-1}$
Sample introduction	HPLC gradient pump
Carrier gas flow rate ($L.min^{-1}$)	1.25
Sample Depth/mm	6.0
Pump speed/rps	0.30
Acquisition/seconds	2 using time Resolved Analysis

The lyophilised samples of sediment, mussel (*Polymesoda solida*) and fish muscle tissue of curvina (*Cysnocion acoupa Maracaiboencis*) were extracted using two different methods:

(1) Cold digestion procedure(74): 1.5-2.0 g of freeze dried sample mixed with 2 mL of concentrated HNO_3 and 1 mL of H_2O_2 , left to stand for 24 h at room temperature and finally diluted to 15 mL solution. The solution pH was about 1.0 .

(2) Digestion-extraction method : 1.5-5 g of freeze dried sample was mixed with 10 ml of water, 5 mL of HCl and 20 mL of toluene in a 100 mL conical flask and shaken for 10 min, the mixture was then centrifuged at 3000 rpm for 5 min.

12 mL of the extracted organic phase was mixed with 3 mL of L-cysteine and shaken for 2 min. The mixture was then centrifuged for 5 min and 2 mL of the aqueous phase was taken for the mercury species determination, final was about pH=4.

After the extraction or digestion, the samples were adjusted to pH= 6.5 with NaOH 1 %.

The final HPLC conditions used were the following:

Mobile phase: 0.06 M ammonium acetate, 3 % methanol, 0.1 % 2-mercaptoethanol, 2 mM EDTA, pH= 6.5

Column: C_{18} (Reverse Phase)

The mercury and selenium total content method of determination is described in Chapter IV.

The total nitrogen, phosphorus and sulphur content were determined by a previous method reported using microwave digestion and ion chromatography detection (75).

6.3.- RESULTS AND DISCUSSION

6.3.1.- Analytical results

The Table 6.2. shows the optimal chromatographic conditions and mobile phase used to determine inorganic mercury and methylmercury, and selenocystine and selenomethionine were suitable to determine with the method.

Table 6.2. : LC conditions used during this study

Liquid Chromatography	Column	Mobile phase
Reverse phase	C_{18}	0.06 M ammonium acetate, 5% methanol, 0.1 % 2-mercaptoethanol, pH=6.5
Reverse phase	C_{18}	0.045 M ammonium acetate, 2.5 % methanol, 0.1 % 0.075-mercaptoethanol, pH=6.5
Reverse phase	C_{18}	0.06 M ammonium acetate, 3 % methanol, 0.1 % 2-mercaptoethanol, 2 mM EDTA pH=6.5

The Figure 6.3. shows the separation of a 40 μg L^{-1} solution of inorganic mercury and 40 μg L^{-1} methyl mercury with the third mobile phase used. A good separation of the mercury species is shown in the figure. Figure 6.4. shows the separation of a solution of 100 μg L^{-1} of selenocystine and 100 μg L^{-1} of selenomethionine simultaneously with the mercury species determination.

***Calibration Curves*:**

CH_3Hg^+ **retention time: 480 sec** **y= 331.2X + 669.1**

r^2 = 0.99933 detection limit: 2.4 μg L^{-1}

Hg^{2+} **retention time: 680 sec** **y = 129.7 X + 1127.2**

r^2 = 0.99829 detection limit : 4.3 μg L^{-1}

SeCys **retention time: 50 sec** **y = 163.3 X + 1012.9**

r^2 = 0.99997 detection limit : 3.0 μg L^{-1}

SeMe **retention time: 100 sec** **y= 194.4 X + 1454.7**

r^2 =0..99999 detection limit: 6.8 μg L^{-1}

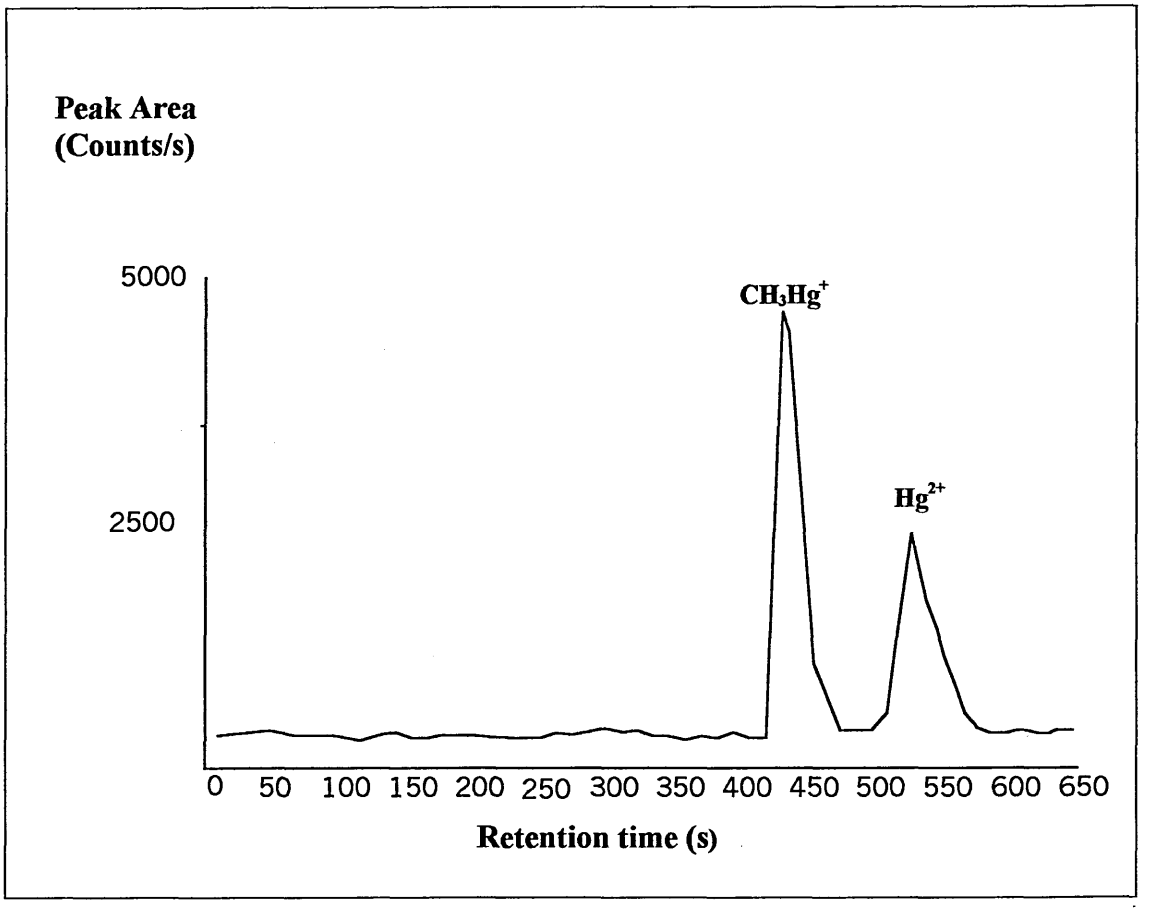

Figure 6.3: Chromatogram of a solution of 40 μg.L^{-1} of methyl mercury and 40 μg.L^{-1} of inorganic mercury using the proposed method by HPLC-ICP-MS.

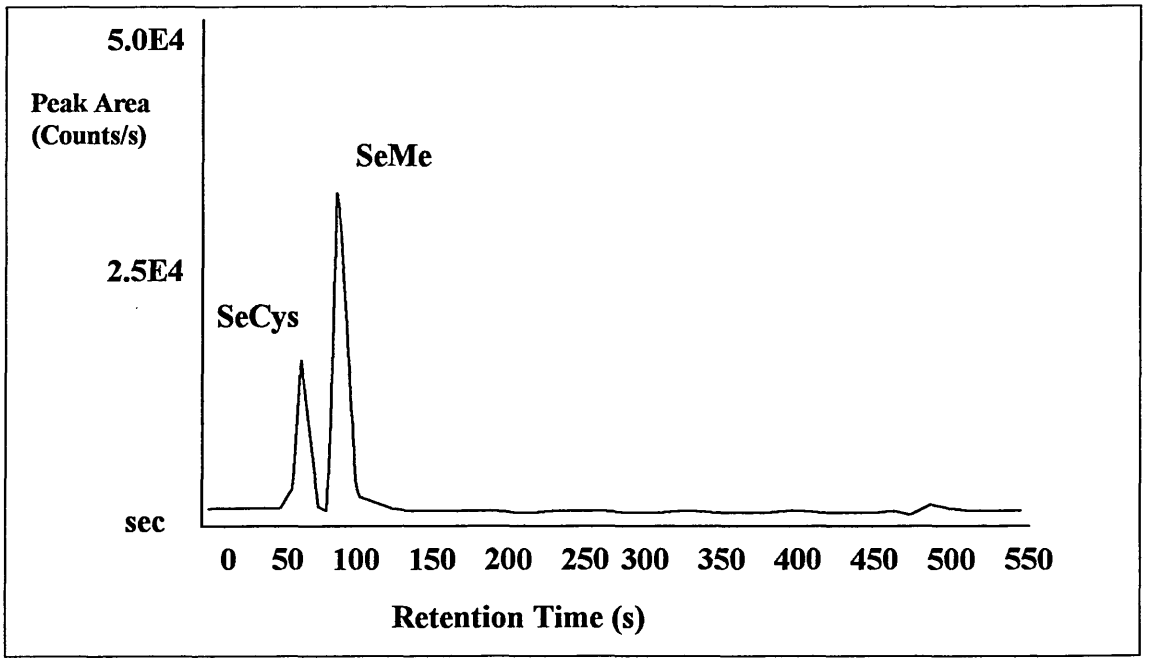

Figure 6.4.: Chromatogram of a solution with 100 μg.L^{-1} of selenocystine and 100μg.L^{-1} of selenomethionine

The methodologies were checked for methyl mercury, mercury total content and selenium total content in sediment using a Reference Material IAEA 356 and LGC 6137. Table 6.3 and 6.4 shown the results:

Table 6.3: Comparison of the results obtained with the reference material IAEA-356

Metal	Found ($\mu g.g^{-1}$)	Certified($\mu g.g^{-1}$)**
Hg (total content)	7.4 ± 0.7	7.62 [6.74-7.98]
Se (total content)*	0.54 ± 0.02	0.76 [0.40-1.58]
MeHg (Methylmercury) ($\mu g.Kg^{-1}$)	5.5 ± 0.7	5.46 [5.07- 5.84]

**Information value*

***Median*

Table 6.4: Comparison of the results obtained with reference material Estuarine Sediment LGC 6137.

Metal	Found	Certified
Hg	0.38 ±0.05	0.34±0.05

6.3.2.. Mercury environmental results

6.3.2.1. Mercury in waters:

In the waters, it is interesting to note that levels of methyl mercury are usually lower than those of inorganic mercury. This is due to the difficulty of methylation reactions in aqueous phases, and to the easy decomposition by solar UV light (17-18) of organo - mercury compounds . The results obtained during the sampling of Lake Maracaibo show a range between 1.1 to 7.8 $\mu g\ L^{-1}$ for total mercury concentration in waters,

which are under the detection limit of the developed determination method for mercury species but higher than the EC, WHO(World Health Organisation) and TVO-D (German drinking water standards) drinking water recommended limit (1 μg L^{-1})(76).

6.3.2.2 Mercury in sediments:

In sediments and biota, the levels of methyl mercury normally are higher than in the waters because of accumulative phenomena (17); inorganic mercury and methyl mercury is pre-concentrated in sediments. The Figure 6.5 shows the chromatogram of the mercury species from a sediment sample from the centre of Lake Maracaibo.

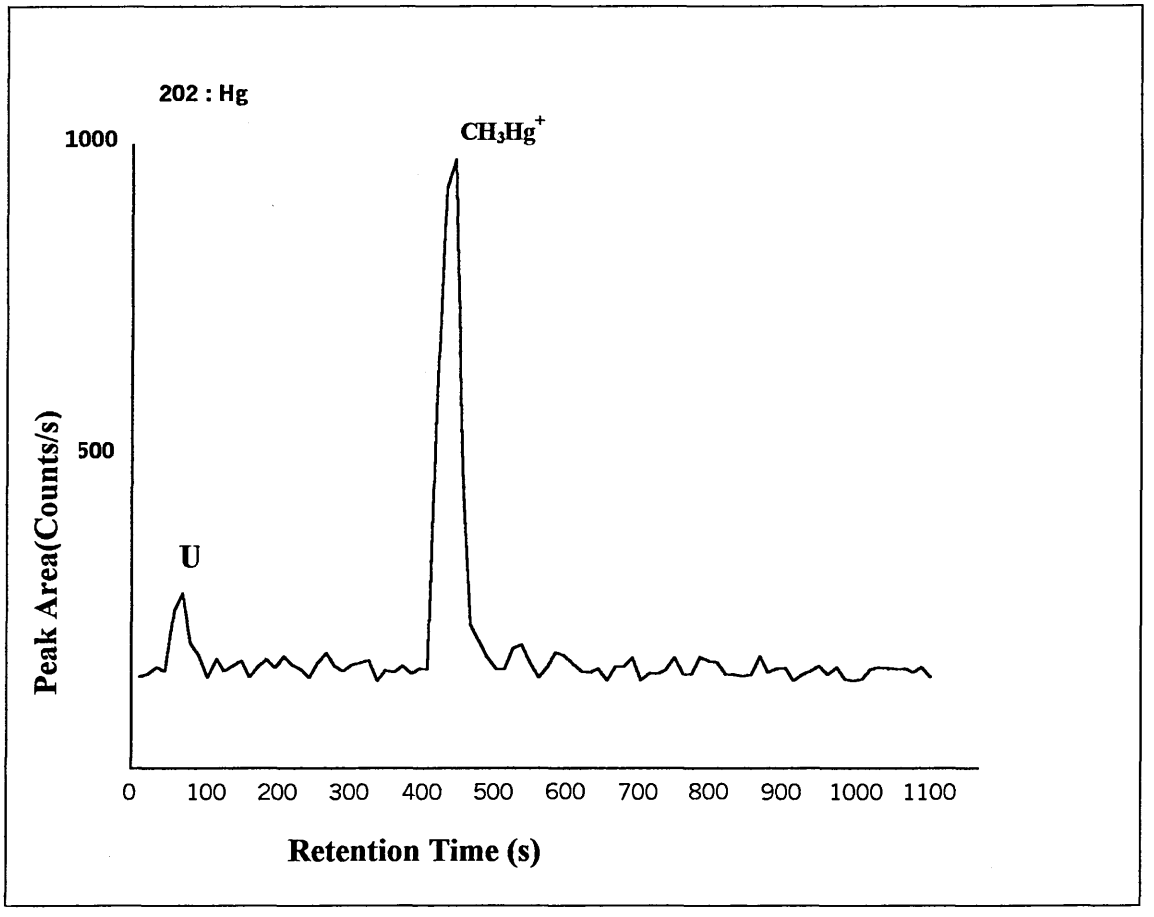

Figure 6.5: Chromatogram of a sediment sample from the centre of Lake Maracaibo (U:unidentified)

There are three systems in Lake Maracaibo that are clearly different, as the sequential

extraction results showed in Chapter IV. The behaviour of the mercury species in the Gulf (PR- SC points) zone corresponds to a mixture of the two species methyl mercury [18.6 – 39.7 μg Kg^{-1}]and inorganic mercury [50.2 – 79.2 μg.Kg^{-1}]; in this zone, the pH is high, salinity and sulphur concentrations are low, and the dissolved oxygen concentration and redox potential are high. In the Strait of Maracaibo, only inorganic mercury was found [30.4 – 85.5 μg.Kg^{-1}]; this zone is near to a petrochemical complex which had a chlor-alkali plant and coal from an open mine is transported through the lake.

The multivariant analysis correlations between Hg^{2+}, total mercury and $MeHg^{+}$ and the nutrients N, P and S, showed no correlations. However, in the main zone of the lake there is a cone in the centre which has zero oxygen content and high salinity in the bottom, Figures 6.6 and Figure 6.7 show the variation of the methylmercury concentration with the physicochemical parameters and total sulphur concentration; the zone with a high concentration of methylmercury corresponds to a zone with very low concentration of dissolved oxygen, lower pH, negative redox potential, high salinity and high concentration of sulphur (under reduced conditions). Sites with high concentrations of total sulphur but high dissolved oxygen, or low dissolved oxygen and low total sulphur concentration, have low methyl mercury concentrations.

Multivariable analysis correlations of the mercury species and the physicochemical parameters showed correlations between $MeHg^{+}$ and salinity ($r= 0.885$, $n=13$, $p<0.0286$) and Hg^{2+} and dissolved oxygen concentrations ($r=0.6601$, $n=13$, $p <0.0141$), taking into account all the zones in the lake.

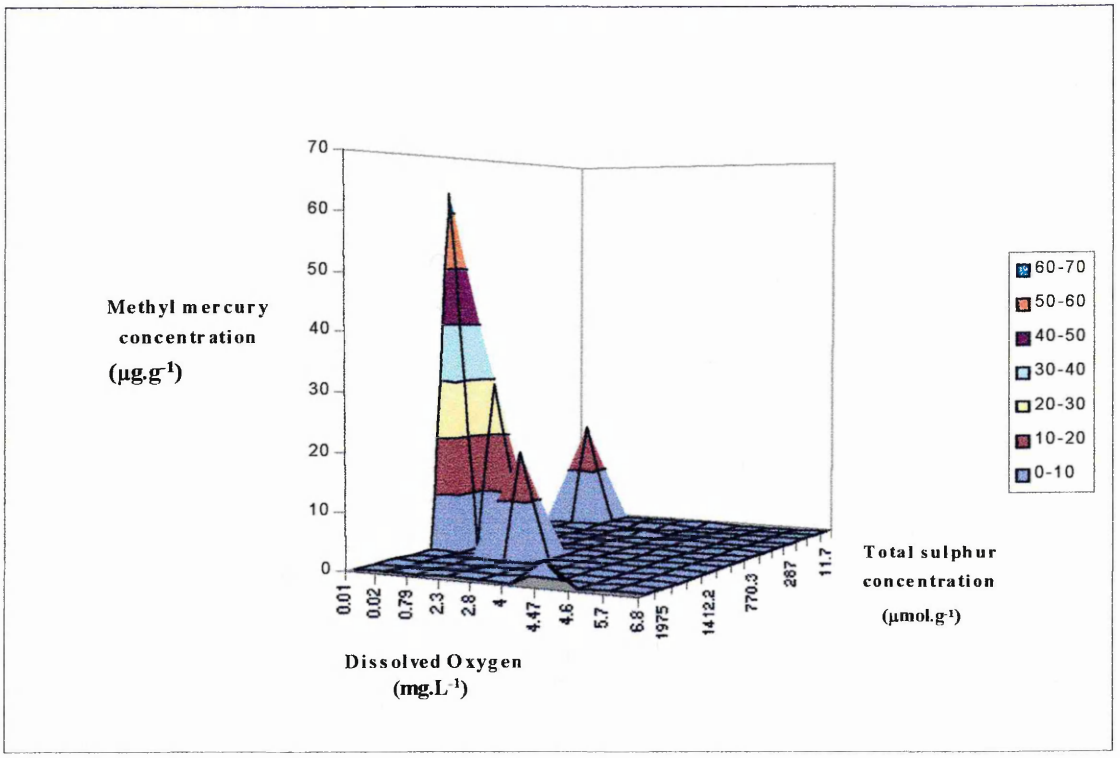

Figure 6.6.: Variation of the concentration of methylmercury (µg.Kg^{-1}), total sulphur concentration, and dissolved oxygen concentration.

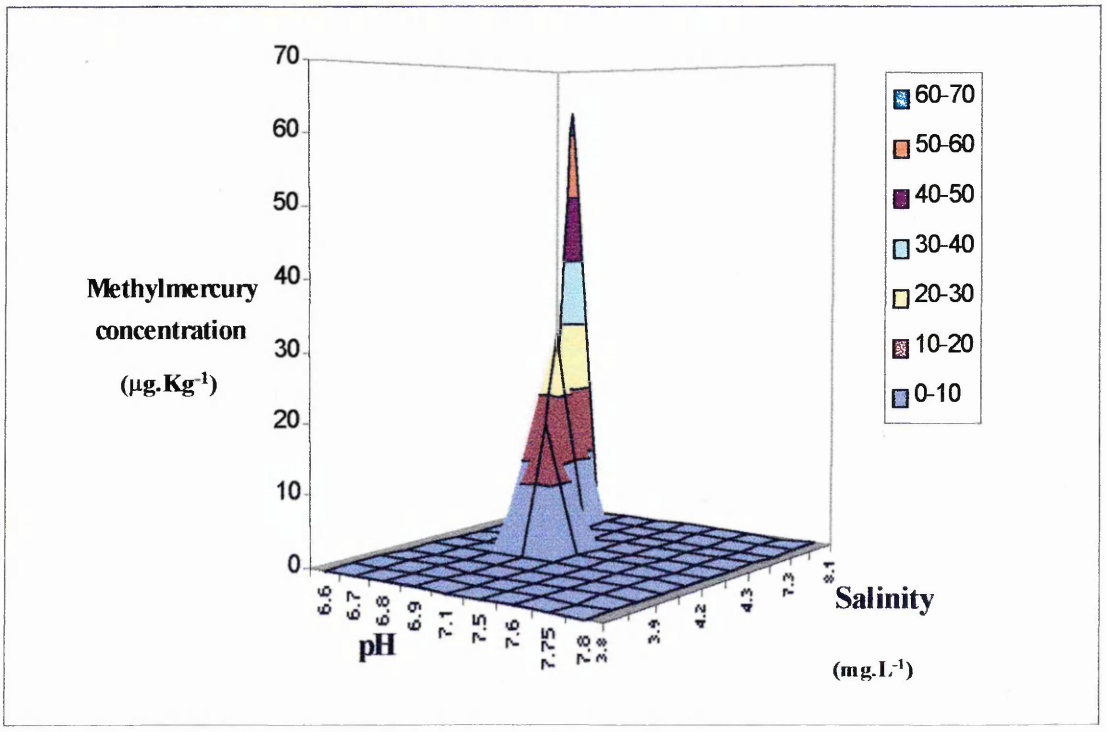

Figure 6.7: Variation of the methyl mercury (µg.Kg^{-1}) concentration, pH, and salinity at the surface sediments from the centre of the Lake Maracaibo.

The behaviour of $MeHg^{+}$ and sulphur can be explained in terms of coordination chemistry. Binding constants for inorganic ligands show that $MeHg^{+}$, like Hg^{2+} , is a B class acceptor (19,77). Methylmercury forms extremely stable complexes with anionic sulphur ligands. Furthermore, sulphide is known to occur in anoxic environments where the rate of oxygen removal is close to or exceeds its rate of supply; in such environments the residual oxygen is present at low or non-detectable levels. At these levels, sulphate is used by anaerobic bacteria as an electron acceptor, leading to the reduction of sulphate to sulphide. These environments are highly dynamic regions and remarkable variations in the dissolved concentration of metals have been found at the oxic-anoxic boundary in anoxic lakes (78).

The results from Lake Maracaibo showed that the methylmercury concentrations can increase with increasing sulphur concentrations under certain conditions. On the other hand, the correlation between methylmercury and salinity could be a signal of that, because in Lake Maracaibo the salinity increases with the depth, the maximum in salinity corresponds to the centre of the Lake. There is also high concentration of sulphur from sulphate intrusions from the Caribbean Sea.

6.3.2.3 Mercury total content

The sediments from Lake Maracaibo reveal a total mercury concentration in the range of 126.3 to 277.5 μg. Kg^{-1}. These concentrations are low when compared with Lake Bjorken (Sweeden) which has 11 mg Hg .Kg^{-1} (1). However, the EC threshold values of total mercury concentration in soil are 1-1.5 mg.Kg^{-1} and 0.3-10 in NL (Dutch standards). The general conditions of Lake Maracaibo as a eutrophic lake do not favour the methylation process. Eutrophic lakes have a high productivity and thus a

large biomass in which methyl mercury will be diluted. The pH in eutrophic lakes is usually high, which favours the formation of the volatile dimethyl-mercury which may escape from the system (5). The high productivity in eutrophic lakes gives rise to large amounts of complexing agents and also to high sedimentation rates. In addition Lake Maracaibo is a tropical lake which has high rate of productivity because of its temperature (79). However, the Lake Maracaibo is continuously dredged to maintain a shipping channel, and these dredging activities will disperse uncovered mercury sediment over large areas and are likely to result in an increase in the mercury content in the flora and fauna over a period of time.

6.3.2.4. Mercury in fish and mussel

The high affinity of methyl mercury for sulphydryl groups and animal lipids would explain its accumulation in living organisms, particularly in lipid tissue of mammals. Figures 6.8 and 6.9 show the chromatograms from a fish muscle tissue (*Cysnoscion Maracaiboensis*) and mussels (*Polymesoda solida*) from Lake Maracaibo, determined with the proposed method. In the muscle tissue of fish, only methylmercury was found (25.1 $\mu g.Kg^{-1}$). Previous investigations by Westoö (80) found that mercury in fish is mainly in the form of methylmercury, as is also the case for other types of aquatic organisms. The value found in Lake Maracaibo is low compared with data from Lake Mississipi (USA), where the concentration ranged from 0.634 mg Hg Kg^{-1} to 1.89 mg Hg Kg^{-1} (81).

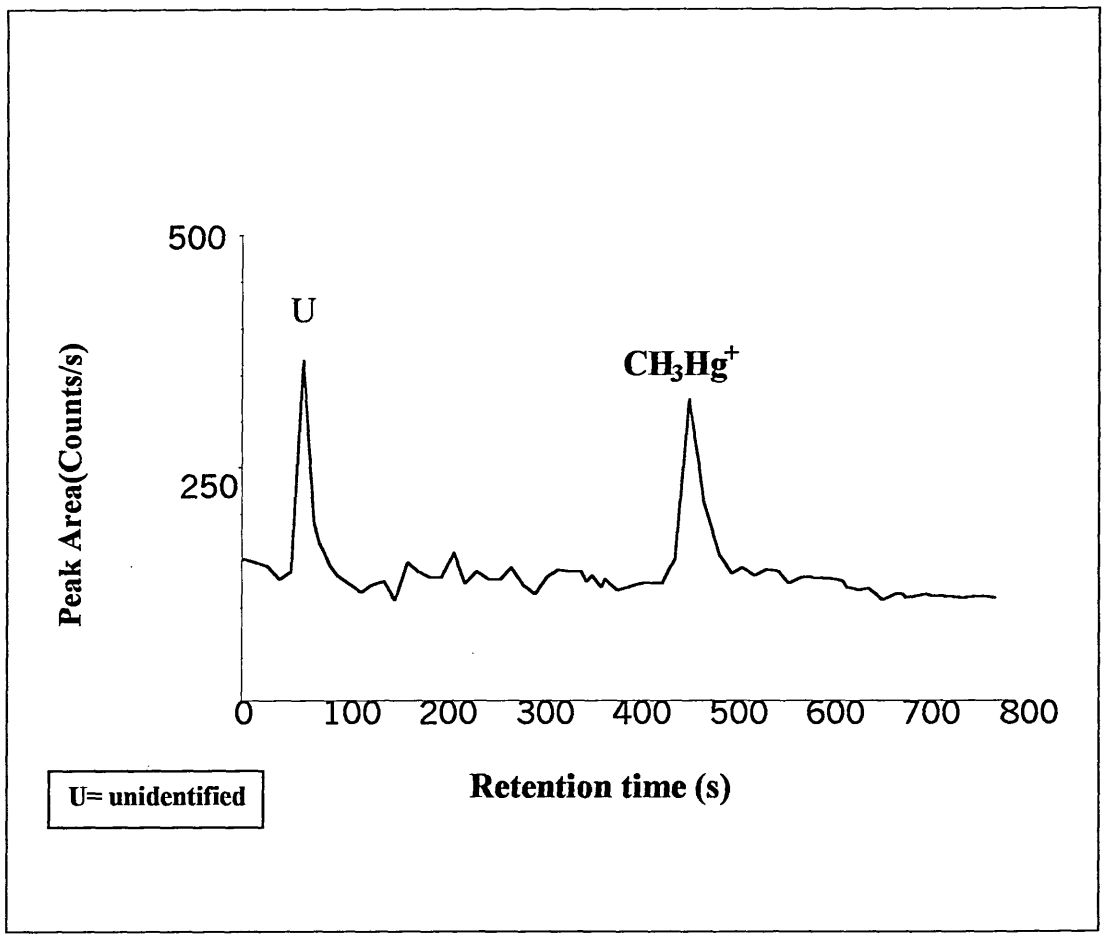

Figure 6.8: Chromatogram of a sample of fish muscle tissue (Curvina, Cysnoscion Maracaiboencis) from Lake Maracaibo using the proposed method for the determination of mercury species.

The mussel samples (*Polymesoda solida)* showed both species, methylmercury (mean: 101 $\mu g.Kg^{-1}$) and inorganic mercury (mean: 73.4 $\mu g.Kg^{-1}$). The mean of the total mercury concentration in this sample was 178.0 $\mu g.Kg^{-1}$. The total value is similar to the values found in estuaries (82) and in oysters from Cartagena Bay(Colombia) where a chlor-alkali plant is located (83).

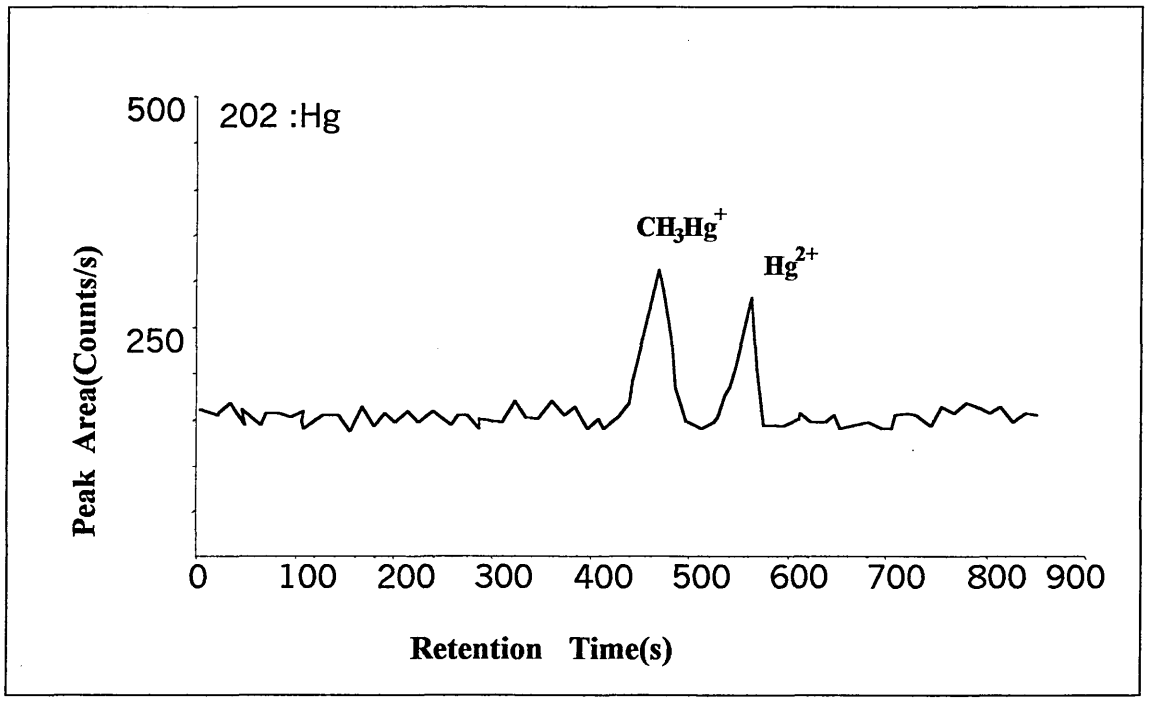

Figure 6.9: Chromatogram of a sample of mussel (Polymesoda solida) from Lake Maracaibo.

6.3.3.- Principal components analysis

The purpose of the analysis is to obtain a small number of linear combinations of the 10 variables which account for most of the variability in the data (84). In this case, 4 components have been extracted, since 1888 components had eigenvalues greater than or equal to 1.0. Together they account for 88.9% of the variability in the original data.

The variables taken in to account are : Nitrogen, phosphorus, sulphur, methylmercury, inorganic mercury, total mercury, depth, dissolved oxygen, pH and salinity, at 13 sampling points.

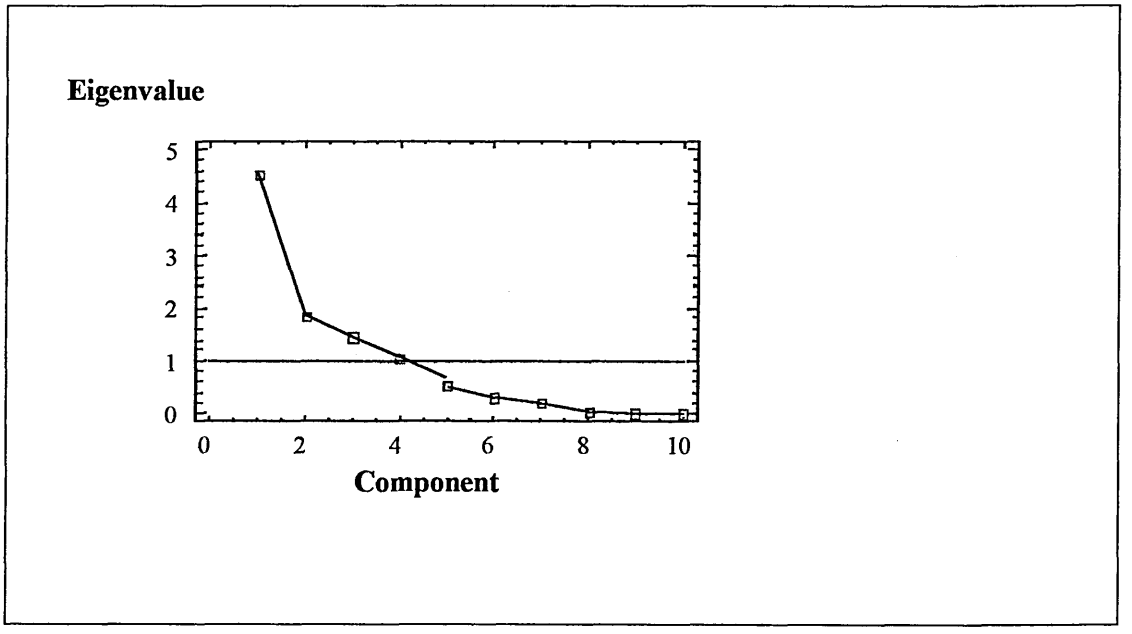

Figure 6.10: Curve of the eigenvalues and the component which shows the four principal components of the experiment

Figure 6.10 and the Table 6.6 shows that there are four components that control 88.9 % of the variability of the data. The parameters that affect most of these components are methylmercury, total mercury, salinity, sulphur, depth and nitrogen (Figure 6.11). Table 6.7 shows the coefficients that could be taken to make equations with these variables.

For example, the first principal component has the equation

0.316*N(μmol/g) – 0.094*P(μmol/g) + 0.299*S(μmol/g) + 0,228*CH_3Hg(μg/Kg) – 0.301*Hg(μg/Kg) + 0.043*Total Hg + 0.386*Depth(m) – 0.455*DO (mg/L)- 0.444*pH + 0.348*Salinity(mg/L)= first principal component

where the values of the variables in the equation are standardized by subtracting their means and dividing by their standard deviations.

Table 6.6: Percentage of variability of each component of the analysis and the eigenvalues

Principal Components Analysis			
Component Number	**Eigenvalue**	**Percent of Variance**	**Cumulative Percentage**
1	4.543	45.4	45.4
2	1.838	18.4	63.8
3	1.449	14.5	78.3
4	1.058	10.6	88.9
5	0.526	5.3	94.2
6	0.303	3.0	97.2
7	0.215	2.2	99.3
8	0.052	0.5	99.8
9	0.014	0.1	99.9
10	0.004	0.1	100.0

Table 6.7: This table shows the coeficients for the equations of the principal components.

Table of Component Weights

	Component 1	**Component 2**	**Component 3**	**Component 4**
N	0.316	-0.064	0.153	-0.596
P	-0.094	-0.248	0.662	0.324
S	0.269	-0.353	-0.414	0.192
Me Hg	0.228	0.520	-0.016	-0.051
Hg^{2+}	-0.301	-0.033	-0.564	-0.175
Total Hg	0.043	0.563	-0.119	0.559
Depth	0.386	-0.301	-0.106	0.262
DO	-0.455	-0.010	-0.012	-0.001
pH	-0.444	0.145	0.094	-0.187
Salinity	0.348	0.323	0.120	-0.235

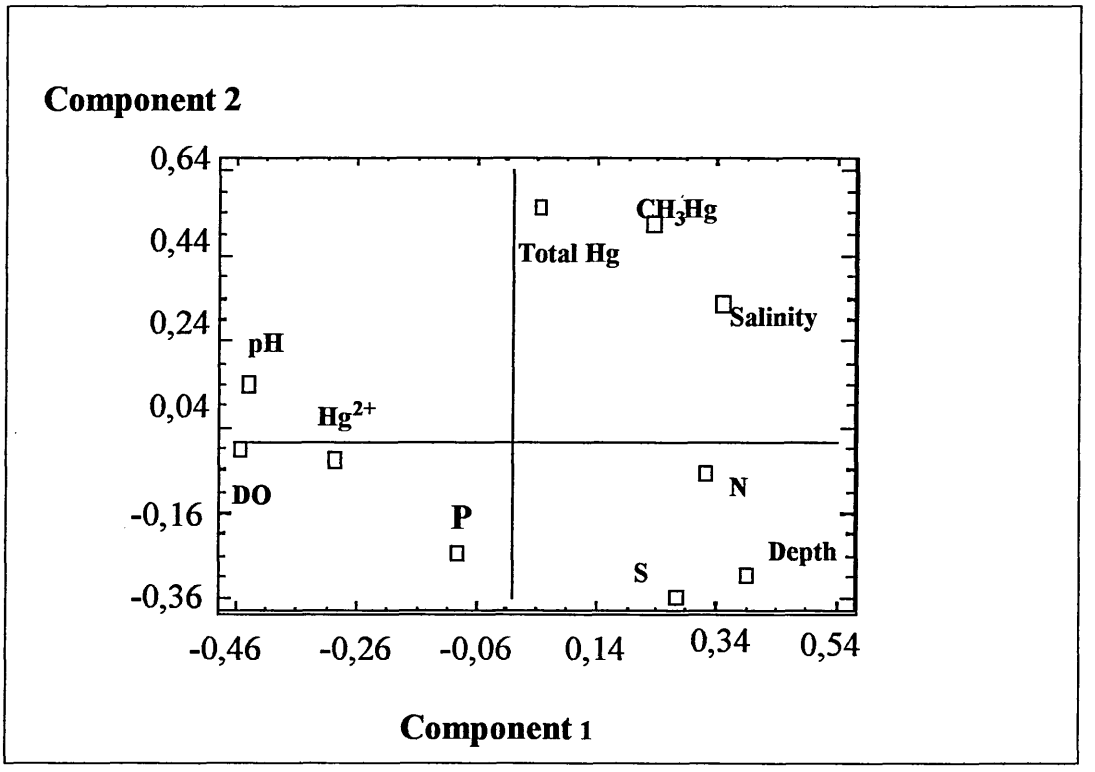

Figure 6.11: Plot of the component weightings of the parameters for the principal components 1 and 2 that produce more variabilty of the data.

Figure 6.11 shows the parameters that have more influence of the variability of the data. The relatively high eigen values for the components 1 and 2 of the principal component analysis showed that salinity, methylmercury and total mercury were affecting all of the results in the Lake Maracaibo system for this experiment, this can be explained because Lake Maracaibo system is formed from an estuary, a strait and the lake. In these three ecosystems salinity variations can affect the distribution of methyl mercury and inorganic mercury. Also, the depth and the sulphur concentration in general can contribute to the variation of data for he component 1, but are affecting negatively the component 2 values. These results can be explained because methylmercury increases with the sulphur concentration under certain conditions that

are affected by the dissolved oxygen concentration and the pH.

6.3.4. Selenium results

J. Marchante et al (59) developed a method for the determination of selenium species using ion pair chromatography-HPLC-ICP-MS, having similar retention times for selenomethionine and selenocystine to the method described in this thesis. In the samples taken in Lake Maracaibo, these species were not found in water and sediment, but this is not surprising because, as it is reported in the results of Chapter V the dominant species of Se in water and sediments was selenite. Only in fish muscle tissue were the two species selenomethionine (8.8μg.Kg^{-1}) and selenocystine (24.0 μg.Kg^{-1}) found as shown the Figure 6.12.

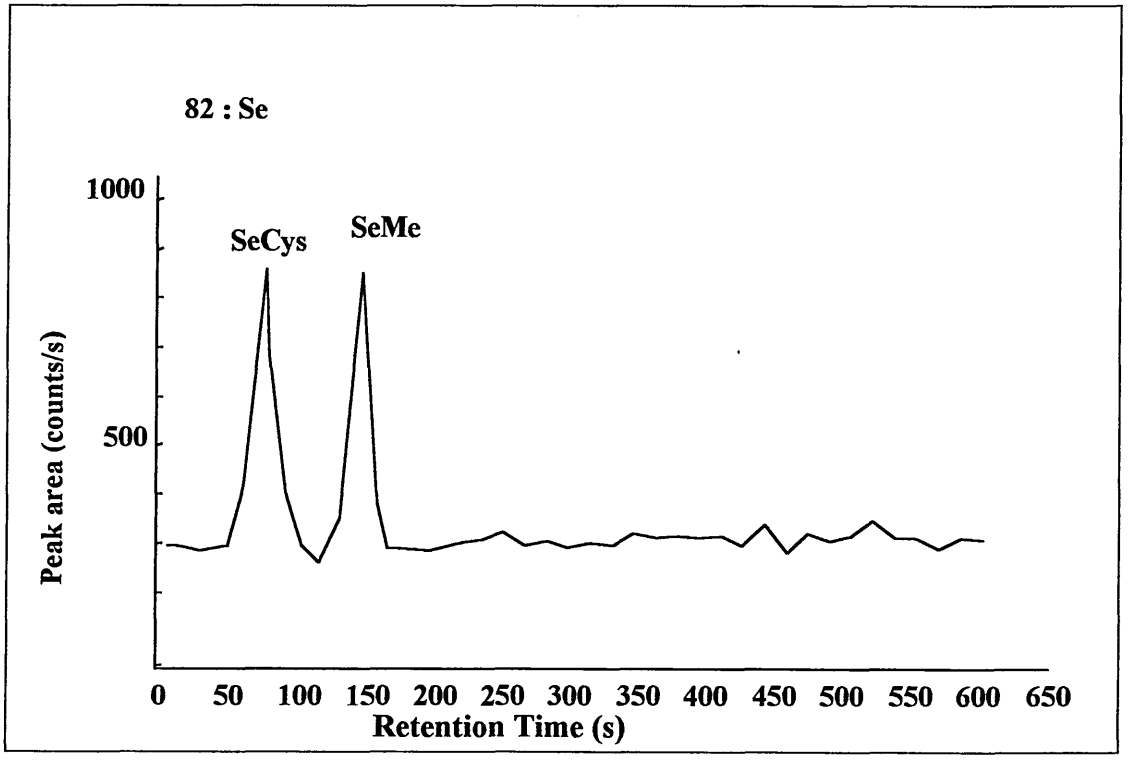

Figure 6.12: Chromatogram of a sample of fish muscle tissue with the two species of Selenium selenocystine (24.0 μg.Kg^{-1}) and selenomethionine (8.8μg.Kg^{-1})

There is no information about organoselenium compounds in fish or mussel. The total

selenium concentrations reported for fish and bivalves ranged between 0.05 to 3.9$\mu g\ g^{-1}$ in contaminated zones of India (85), which are higher than those reported for Lake Maracaibo.

6.4.- CONCLUSIONS

In conclusion, the proposed method for the determination of mercury species is useful in the analysis of environmental samples such as sediment, fish and mussels. The results from Lake Maracaibo show accumulation of the methylmercury species in sediment and biological materials. There is evidence of some methylation of the inorganic mercury in the centre of the Lake. Certain sulphur species appear to influence the methylation of mercury in sediments and it is desirable that further investigations into these effects are carried out.

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Chapter VII

Chemical speciation of vanadium in water, sediment, fish muscle tissue and mussel from Lake Maracaibo, Venezuela.

7.1.- INTRODUCTION

7.1.1.- *Vanadium*

Environmental pollution caused by vanadium is almost entirely due to industrial activities such as the production of steel, pigments, photographic materials and insecticides. Vanadium is released on oil combustion. (1) The toxicity of its species is well documented (2). Vanadium in trace amounts is beneficial to normal cell growth, being one of the so-called essential elements. However, toxicity arises when vanadium concentrations are increased to a higher level. The V^{v} oxidation state ion is more toxic than V^{IV} ion (3). Vanadium is absorbed from a variety of foods with a relatively low efficiency but in sufficient quantities to be absorbed at detectable levels in many body tissues (4).

The chemistry of vanadium is complex because this element can exist in oxidation states from –1 to +5 and frequently forms polymers (5). V^{IV} and V^{v} form many complexes in water that change in accordance with the solution pH, and their concentrations. It is known that in the pH range 2-6 the main species of V^{v} is the orange decavanadate anion $V_{10}O_{29}^{6-}$, which can exist in several protonated forms, and which changes to the dioxovanadium (v) anion VO_2^{+} below pH 2 (6). In contrast, V^{IV} exits as the blue oxovanadium(iv) in acidic solution and this cation readily changes to the anion $V_{10}O_{42}^{12-}$ at about pH 4 (6).

The concentration of vanadium is natural waters is very low and usually in the range 0.5-2.5 $\mu g\ L^{-1}$ (6,7). The vanadium concentrations found in sediments from near the shores of Kuwait varied from 24.8 to 179.4 $\mu g\ g^{-1}$ with an average concentration of $108.3 \pm 34\ \mu g\ g^{-1}$ (8). Vanadium and nickel porphyrins dominate those porphyrins found in the petroleum and bitumen extracted from shales (9). Frequently, V is found in largest concentration in

crude oil, for example, values presented for some Venezuelan crude oils (1) as well as Middle East crude oils (10) have a predominance of V and Ni content. Vanadium concentrations can be as high as 2,000 $\mu g.g^{-1}$ (11). It is expected that, upon extraction of the crude oil and accidental deposition on surface sediments, bacterial decomposition, dissolution and oxidation of most of the organic components and remineralization of the organic matrix, trace elements such as vanadium can be incorporated in the sediment load, increasing the background levels of metal content of the local sediment (11).

The redox chemistry of vanadium leads to a decrease in the solubility of this element on going from an oxic a reducing environment (12). Thus, the fluvial dissolved vanadium, concentrations might be an indicator of inputs from reducing sources within a river drainage (12). Furthermore, various workers have examined vanadium in oceanic sediments and sedimentary components as a possible indicator of the redox history of specific ocean areas as well as of the ocean as a whole (13). However, explaining oceanic changes in dissolved vanadium may also require an understanding of the processes affecting the majority of oceanic sources of this element (i.e., rivers). Crude oil is enriched in vanadium relative to many other trace elements with concentrations occasionally exceeding 1000 $\mu g.g^{-1}$ (12). The high vanadium enrichment found in the North Atlantic, but not in Antarctica, is probably due to vanadium produced by the burning of heavy fuel oil (containing high concentrations of vanadium porphyrin complexes) along the east coast of North America (14). This element, like nickel, does not cause high metal levels in airbrone particles; however, exceptionally high metal concentrations are found in residues.

During the extraction of oil, produced water is found in sub-seafloor sedimentary formations from which offshore oil and gas are derived (15). This water is piped to the surface along with oil and gas during the production process. Then produced water is separated from oil and gas on the platform by depressurization and gravity separation techniques and either discharged back down the well to increase oil recovery or to adjacent surface water. In the Gulf of Mexico (which has 3,000 production platforms, there are 10,000 production platforms in Lake Maracaibo) discharge to the surface water amounts to more than 140 million m^3 per year. Sometimes waste water (produced water) is called oil brine, which contain total dissolved solid (TSD) at levels as high as 300 g/Kg along with elevated concentrations of selected heavy metals and petroleum hydrocarbons(16-17).

7.1.2.- Vanadium determination

The total content of vanadium has been determined by atomic spectroscopy or spectrophotometry (18,19). Some authors have studied vanadium speciation using a flow injection system that incorporated a strong anion-exchange resin and was coupled to a flame atomic absorption spectrometry (AAS)(20) or UV (21) detector. Hirayama et al (22) used a two-column system and inductively coupled plasma atomic emission spectrometry to determine V^{IV} and V^{v} ions. R. Wuilloud *et al* (23) used an on line vanadium pre-concentration system with a knotted reactor and ultrasonic nebulizer with an inductively coupled plasma optical emission spectrometer (ICP-OES) to determine V^{v} in drinking water. Recently the same authors have reported a method for the simultaneous determination of V^{IV} and V^{V}, using a similar flow injection system with an amberlite XAD-7 resin to retain the species (24).

Sugiyama et al (5) applied air-segmented continuous-flow analysis based on a catalytic reaction to determine V^{IV}; this method has the disadvantage that the analytical peaks broaden and overlap with each other due to the dispersion of the sample in the mobile phase in the reaction coil, and the sensitivity decreases. A method using Fe(II) as catalyst was developed by Safavi *et al* for the determination of V^{IV} (25) . However, these methods still do not satisfy the requirements for routine analyses because of their complicated process design. Methods for the determination of the speciation of metal ions using liquid chromatography (LC) have increased rapidly. Komarova et al (26) have studied the ion chromatographic behaviour of EDTA complexes of V^{IV} and V^{v} using aqueous sodium carbonate as eluent. The simultaneous determination of V^{IV} and V^{v} as EDTA complexes has been reported, involving reversed- phase ion pair LC with a conventional UV detector

(21). Complexation of EDTA with V^{iv} and V^{v} forms $[VOY]^{2-}$ (log K_f = 18.8) and $[VO_2Y]^{3-}$ (log K_f = 15.55) (27) (where Y represents the deprotonated EDTA species), respectively. This makes possible separate ion of vanadium species as anionic EDTA, complexes by ion chromatography (26). As vanadium species are separated as a EDTA complex, the retention of the former depends on the extent of the chelation. The pH is the most important factor in the chelation because the condensation of vanadium species occurs at pH > 10 and the protonation of V^{v} chelates occurs at pH < 5 (21). Therefore, the pH chelation and the ion elution have to be maintained at pH 6.

7.2.- MATERIAL AND METHODS

7.2.1..- Instruments

The HPLC system for these studies was a DIONEX gradient pump equipped with a Rheodyne Model 7125 injection valve with a 50 μL sample loop and a Zorbax HICHROM C_8 (150 mmx4.6 mm) reversed phase column , 10 psi of Helium pressure, flow rate of 1.2 $mL.min^{-1}$ (Figure 7.1).

V has two isotopes, 50 and 51, with 0.25 % and 99.75% abundance respectively. V is over 95 % ionized in the argon plasma. ^{51}V should be used as isotope for the ICP determination. ^{50}V is both low abundance and subject to interference from Cr and Ti isotopes. In this study ^{51}V was used for the determination of vanadium.

An ICP-MS Hewlett Packard 4500 was used as detector. The following Table 7.1. shows the conditions for the ICP-MS.

Table 7.1: Conditions for the ICP-MS

Conditions	For V speciation
Torch	Fassel torch
Spray chamber	Cyclonic
Nebulizer	Babington
Sample introduction	HPLC gradient pump
Solution uptake	1.0 ml. ml^{-1}
Rf Power	1200 W
Carrier gas flow rate	1.25 $L.min^{-1}$
Coolant gas flow rate	10 L. min^{-1}
Sample Depth	6.0 mm
Pump speed	0.40 rps
Acquisition	2 sec using time Resolved Analysis

7.2.2.- Reagents

Deionized distilled water was used to prepare all solutions. Stock standard solutions 1000 of mg L^{-1} VO^{2+} and VO_2^+ were prepared by dissolving 0.42 g of analytical grade $VOSO_4.3H_2O$ (Fluka, Dorset, UK) in 100 mL of water and 0.229 g of analytical reagent NH_4VO_3 (Fluka, Dorset, UK) in 5 mL of concentrated H_2SO_4 and dilution to 100 mL with water. Fresh working standard solutions of VO^{2+} and VO_2^+ (single and mixed) were prepared daily by dilution of the stock solution.

Ethylenediaminotetraacetic acid (EDTA) from Fluka (Dorset, UK) , tetrabutylammonium hydroxide (TBAOH) from Aldrich (Dorset, UK), ammonium acetate (analar grade) and di-

ammonium orthophosphate $(NH_4)_2HPO_4$ from BDH(Poole, Dorset, UK) were used to prepare the eluent.

7.2.3.- Procedure

A 25 ml volume of sample solution containing VO^{2+} and/or VO_2^+ was pipetted into a 50 mL beaker and the pH adjusted with NaOH 1%w/v to pH=6.0. After adding excess of EDTA (1.5 times equimolar proportions), the solution was kept for 20 min for the reaction to go to completion, and then transferred into 25 mL volumetric flask, followed by dilution with water. The working standard solutions were prepared from the 10 mg L^{-1} solution. After filtration through a membrane filter (0.2 μm), 50 μL of sample was injected onto the column.

The mobile phase was a solution of 0.06 M of ammonium acetate, 10 mM of TBAOH, 2.5mM EDTA and 10 mM of $(NH_4)_2HPO_4$ at pH=6.0.

The eluent composition and the elution conditions were adjusted in order to obtain optimum separation of the vanadium complexes.

7.2.4.- Sample preparation

The vanadium species were extracted from 0.2 g of lyophilised sediment, mussel and fish muscle tissue samples respectively, using 15 mL of EDTA 2.5 mM with 1 h shaking.

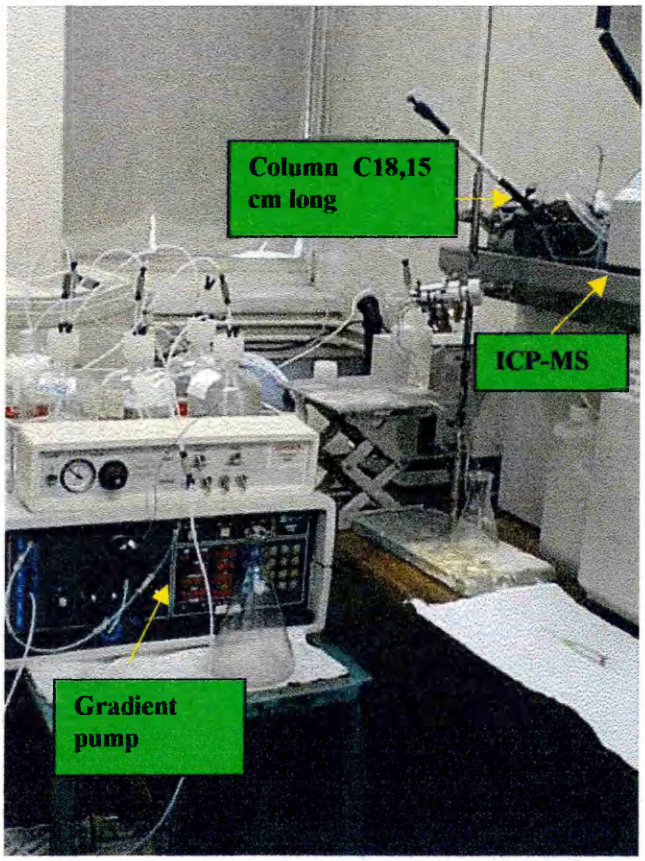


Figure 7.1: A photograph of the chromatographic system coupled to the ICP-MS.

7.3.- RESULTS AND DISCUSSION

7.3.1.- Separation and identification of the species:

A summary of the chromatographic systems investigated in the development of the vanadium speciation method is given in Table 7.2. In the Na-DDTC solution only one vanadium peak was detected, in contrast two species of vanadium were detected using

Table 7.2: Coupling HPLC-systems used during the development of the methodology.

Liquid Chromatography	Column	Eluent
Anion exchange EDTA complexes	AS-9 anion DIONEX	1.87mM ammonium phosphate-1.87 mM di-ammonium phosphate
Anion exchange NaDDTC complexes	AS-9 anion DIONEX	1.87mM ammonium phosphate-1.87 mM di-ammonium phosphate
Reverse phase EDTA complexes	C-18 Waters (15mmX 4.6mm)	0.06 M ammonium acetate,3% methanol, 0.1 % ,2-mercaptoethanol 2mM EDTA
Reverse phase NaDDTC complexes	C-18 Waters (15mmX 4.6mm)	0.06 M ammonium acetate,3% methanol, 0.1 % ,2-mercaptoethanol, 2mM EDTA
Reverse phase EDTA complexes	C-8 (250mmX4.6mm)	0.06 M ammonium acetate, 3% ethanol, 0.1 % 2-mercaptoethanol, 2mM EDTA
Reverse phase EDTA complexes	C-8 (250mmX4.6mm)	0.06M ammonium acetate, 7 mM to 70 mM , TBAOH 7mM to 70 mM di-ammonium phosphate, 2.5 mM EDTA
Reverse phase-Ion pair EDTA complexes	C-8(250mmX4.6mm)	5% to 10 % acetonitrile, 0.05 M TBAOH2mM EDTA
Reverse phase-Ion pair EDTA complexes	C-8(150mmX4.6mm)	5 % to 10 % acetonitrile, 0.05 M TBAOH, 2mM EDTA
Reverse phase-Ion pair EDTA complexes	C-8(150mmX4.6mm)	0.06 M ammonium acetate, 7mM to 70 mM TBAOH, 2.5 mM EDTA, 7 to 70 mM di-ammonium phosphate

The dissociation of TBA aids ion-pair formation, which enhances the retention of vanadium species. Conversely, the ionisation of EDTA increases the ionic strength of the eluent and shortens the retention times of species. At pH values greater than 10, V^{IV} and V^{V} , in the form of $V_{10}O_{42}^{12-}$ and $V_{10}O_{28}^{6-}$, respectively, can precipitate out of solution. In order to prevent this, the pH of the mobile phase, standards and samples was kept at pH 6.

In a reversed phase ion pair chromatographic column, the sorption of TBA^{+} offers dynamic ion-exchage sites. Thus, the retention of $[VO_2Y]^{2-}$ and $[VOY]^{3-}$ is directly related to the surface charge arising from the adsorbed TBA^{+} (TBA_s^{+}) and an adsorption equilibrium of TBA is established between the eluent and the stationary phase; Jen *et al*(21) has reported that retention of $[VO_2Y]^{3-}$ only increases at low TBA concentration ranges and then decrease after an optimun addition. Figure 7.6 shows that above a concentration of 7.5 mM of TBAOH the two species can be separated and the retention times of both species increase with the addition of the ion pair solution.

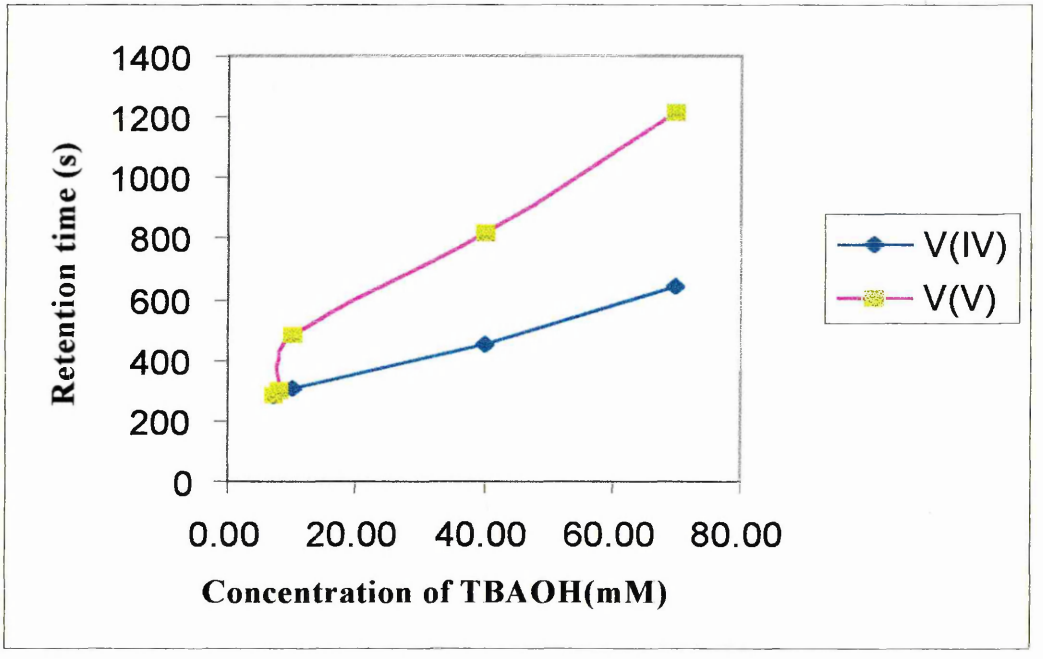


Figure 7.6: Variation of the retention times of the vanadium complexes with the TBAOH concentration

The size of the column was changed from 250mm to 150mm to have a better separation and shorter retention times, Table 7.3.

Table 7.3.: Effect of column length on retention times.

Size (mm)	Retention time(sec) V^{IV}	Retention time(sec) V^{V}
150	308	479
250	930	1501

Because the matrix of the sediment samples are complex, serious tailing occurred in the separation using acetonitrile solvent. Ammonium acetate was instead added to decrease peak tailing and the flow rate of the mobile phase was also increased to reduce all retention times (Table 7.4)

Table 7.4: Effect of flow rates on retention times, using a 100 μg L^{-1} solution of V-EDTA complexes.

Flow rate(mLmin^{-1})	Retention time (sec) V^{IV}	Retention time (sec) V^{V}
0.8	505	813
1.0	393	645
1.1	365	589
1.2	336	547

7.3.2- Calibration curves and detection limits

Calibration graphs were linear in the range of 50 μg $L^{-1}$and 500 μg L^{-1} . The equation for the two species were the following:

V^{IV} =
y= 6528.9 X + 123484.5 (X= concentration µg L^{-1})
r= 0.9965 (Y= Peak area (counts.s^{-1})
DL= 59.1 µg L^{-1}
V^{V}
y= 5475.8 X+ 233247.2
r=0.9986 DL= 113.1 µg L^{-1}

7.3.3- Interferences

EDTA can form complexes with other metals ions and these species could form ion pairs in the mobile phase. The following table shows the most common spectral interferences for the isotope ^{51}V. V readly suffers interference from Cl O^+ and SOH species. Therefore, Cl and S compounds should be avoided in the determination of V.

Compounds	Interference (%)
ClO	75.590
BAr	79.780
NCl	24.141

In the case of vanadium 51, a peak of Cl O^+ appeared at the beginning of the chromatogram, but this does not interfere with the peak due to the vanadium-EDTA complex. Figure 7.7 shows the EDTA solution peak alone and Figures 7.8a and b the peaks following an addition of 10 µL of a solution 1000 mgL^{-1} of Cl^- to a sea water synthetic sample showing the enhanced ClO^+ peak.

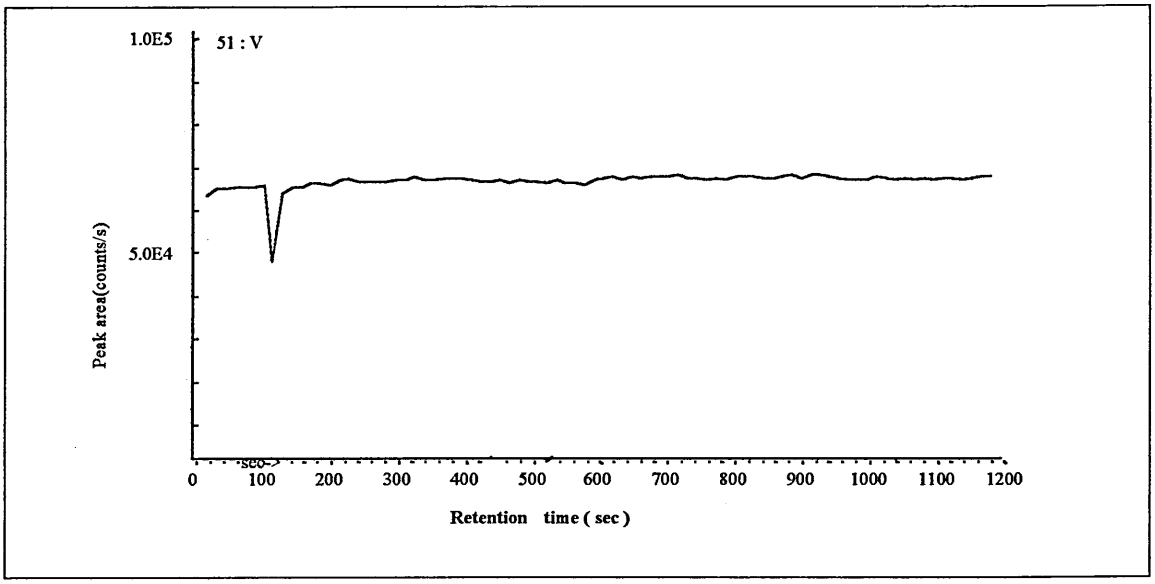


Figure 7.7: Chromatogram of solution of EDTA 2.5 mM.

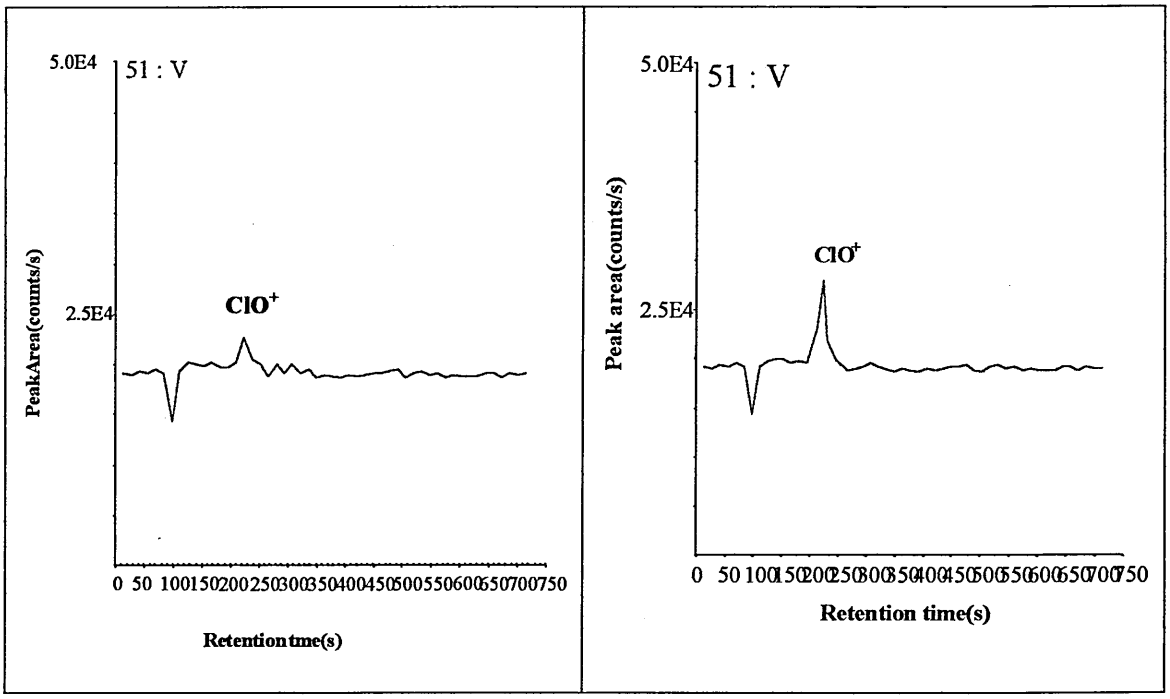


Figure 7.8(a and b) : a) Synthetic water chromatogram b) Chromatogram synthetic water plus 10 µL of solution of 1000 mg L^{-1} Cl^-

7.3.4.- Distribution of vanadium species in environmental samples

The total content of vanadium in water samples ranged between 9-15 µg L^{-1}. However, because these values were under the detection limit of the method of speciation, the

water samples could not be further investigated. In Figure 7.9 is shown the chromatogram from a sample of sediment from Lake Maracaibo. V^{IV} was the predominant species in all of the samples investigated (see Figure 7.9), the distribution of V^{IV} and V^{V} in sediments ranging from 0.4 -25.8 and 0 – 9.2 μg g^{-1} , respectively. The total vanadium concentration ranged between 1.7 – 113. 5 μg g^{-1} ; these are similar to the total vanadium concentration found in Kuwait after the Gulf War [25-119μg g^{-1}](8) . V^{V} is the normal predominant species in well-aerated waters (5) but this is not the case for Lake Maracaibo, which has levels of oxygen nearly at zero in the centre near the source of vanadium pollution. Oil extraction is the probable source of vanadium in the lake; the dominance of the V^{IV} species in sediments can be attributed to the presence of the vanadium-porphyrin complexes in crude petroleum (Figure 7.10).

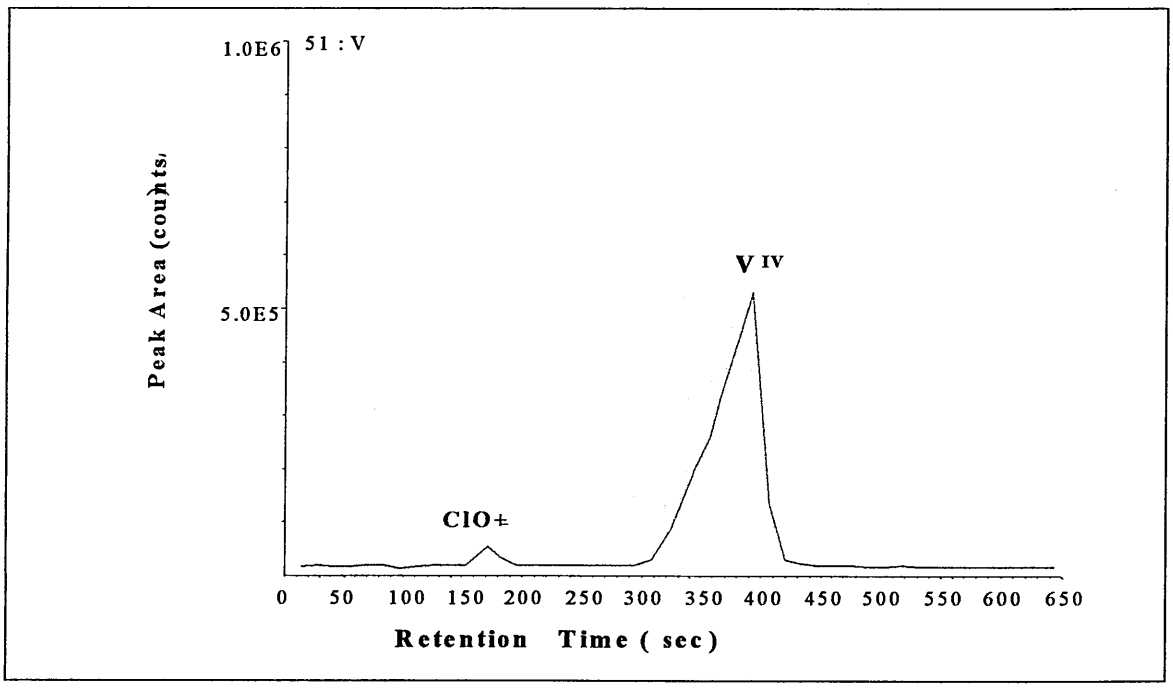


Figure 7.9: Chromatogram from a sediment sample.

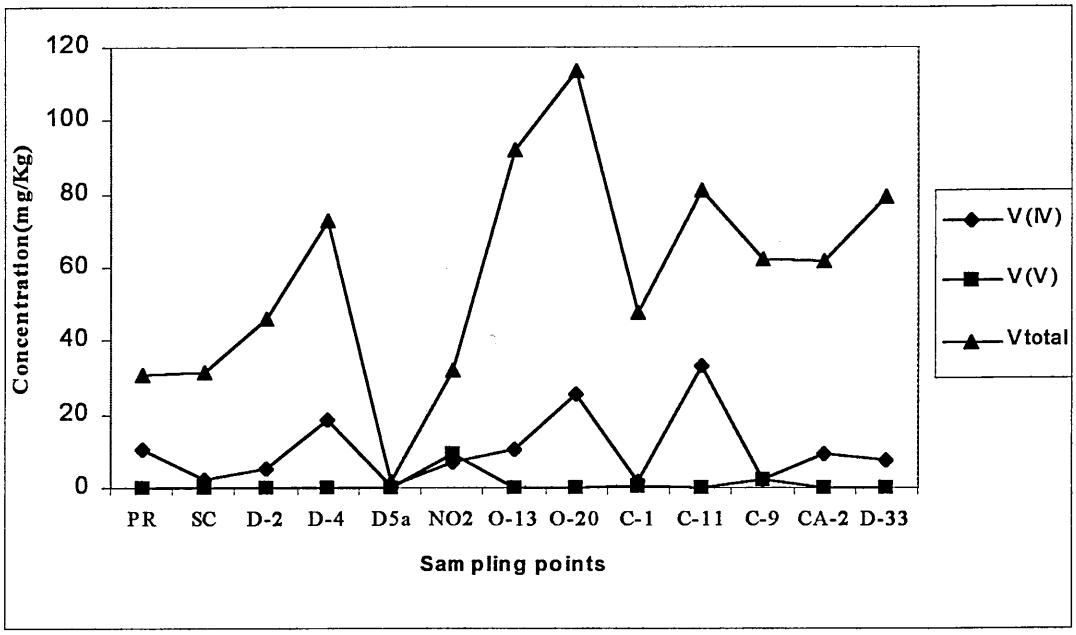


Figure 7.10: Distribution of V^{IV} species in sediment samples taken from the lake .

Metalloporphyrins in sediments have been investigated by Deanton et al (9) who has shown that vanadium and nickel porphyrin complexes dominate those porphyrins found in petroleum. In addittion, vanadyl porphyrin (VO-porphyrin) is the most common vanadium porphyrin, especially those from Canada ,Venezuela (28) and Mexico(29) crude oils. These porphyrins have been isolated from sediments (30) but their manner of formation is still unclear. Marquéz *et al* (31) studied the porphyrin from crude oil from Lake Maracaibo and they found vanadyl porphyrins, using three different methods of determination. Salcedo *et al* (32) and Fujii *et al* (33) have studied the structures of the vanadyl porphyrin ; porphyrinate rings present a great affinity for the VO group with the unit binding to the N atoms (33); Figure 7.11.

It is expected that a simple complex of porphyrin and vanadium, being so reactive, would capture any oxygen atom near it, although the vanadyl group can be formed

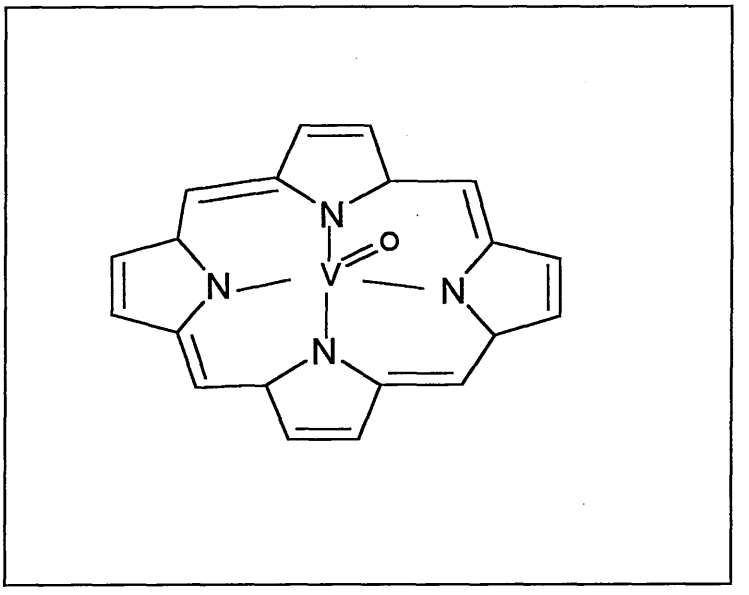
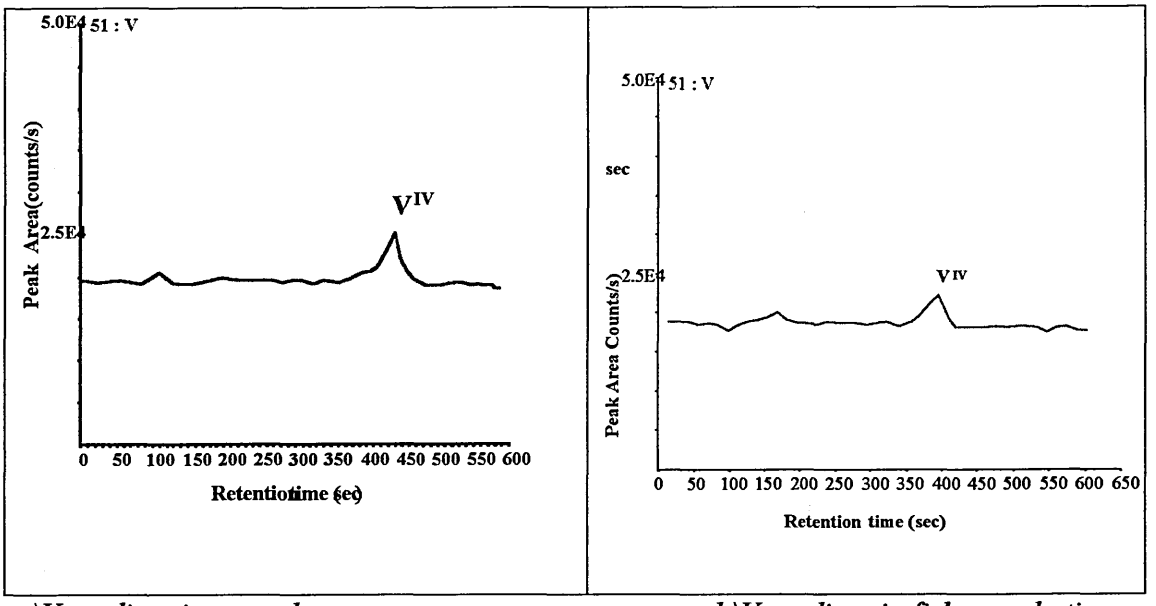


Figure 7.11: Vanadyl specie bonded to porphyrin group.

before any other coordination. More relevant to the study of Fujii (33) is the nature of the complex formed. It is an open shell species, because of the electronic configuration of the V(IV)ion. Bonding of oxo-vanadium (IV) porphyrins to zeolites can occur via a zeolite oxygen atom to vanadyl group and this is one of the dopand effects on catalytic processes (31).

The multivariate analysis of total and species of vanadium in sediment from Lake Maracaibo, and the X-Ray fluorescence spectrometry results, showed correlations between K_2O and V^{IV} ($r= 0.8679$, $n=13$, $p<0.0001$); and K_2O and V total, V^{IV} and Al_2O_3 ($r=0.6117$, $n=13$, $p<0.05$). The nutrients and physicochemical parameters showed correlations between N and V^{IV};P and V^{V}; S and V total; Depth and V total; and DO and V total.

Figure 7.12 (a and b) shows the chromatograms for V^{IV} found in fish muscle tissue (0.92 μg. g^{-1}) and mussel (1.52 μg. g^{-1}). The levels of vanadium



a)Vanadium in mussel *b)Vanadium in fish muscle tissue*

Figure 7.12: Species of vanadium found in mussel and fish muscle tissue.

found in fish muscle tissue from species taken on the Kuwait coast showed concentrations between [0.1-15.6 μg. g^{-1}](34). Studies in seafood in the same area (35) showed the highest concentrations of 1.48 μg. g^{-1}, which is lower than the concentrations in mussels found in Lake Maracaibo. There is no information available to be referred about vanadium species in fish and mussels.

7.4. CONCLUSION

In conclusion, the method for vanadium speciation described here can be used to study the distribution of V^{IV} and V^{V} in sediments, fish muscle tissue and mussels. The dominance of V(IV) at all the sampling points and in the biological indicators could be associated with the source of crude oil, involving a vanadyl porphyrin complex. The results found in Lake Maracaibo can be used as a reference for further studies on vanadium species in tropical lakes or ecosystems which receive pollution from crude oils.

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Chapter VIII

General conclusions, future work and recommendations

8.1.-General Conclusions

The proposed method for the oxidation of N, P and S followed by the determination of the nitrate, phosphate and sulphate ion by ion chromatography gave satisfactory results for the compounds tested. The effectiveness of this procedure was demonstrated by the good recoveries obtained for the two SRMs, oyster tissue and Buffalo river sediment. However, this work was extended and modified in order to determine nitrogen, phosphorus and sulphur in more recalcitrant compounds where the nitrogen, phosphorus and sulphur atoms are in ring systems. For a biological material, sediment or soil, a three step programme could be applied and as a result, three total quantities of each oxidised element in each step (the third step is the quantity of total nitrogen, phosphorus and sulphur in the sample); on this basis a kind of speciation of these elements could be achieved and in a further research using a numeric method may serve to improve this approach.

High levels of nitrogen, phosphorus and sulphur were presented in water and sediments samples collected from the sampling points that indicate that Lake Maracaibo is a hyper-eutrophic lake.

Concentration of sulphur in the sediment at the centre of the Lake with anoxic conditions are responsible for the reduction and precipitation of some metal such as arsenic, mercury and selenium. The concentration of methyl mercury is seen to be affected by the reduced state of sulphur .

The distribution of arsenic and lead in sediments is related to the Fe/Mn concentrations, however, vanadium was distributed mainly in the residual phases and selenium and mercury in the organic-sulphide phase.

The arsenic species found in the lake was mainly reduced arsenite, vanadium was presented as vanadyl and selenium as selenite. Mercury was found in the estuary and the strait as inorganic mercury and methylmercury but is in the latter form in the main zone of the lake .

The three methods developed were satisfactory for the determination of mercury, arsenic , selenium and vanadium species in water, sediment, fish muscle tissue and mussels.

The results of Lake Maracaibo System showed that the distribution of metals in lakes and estuaries are different, variation in salinity and dissolved oxygen can cause changes in the association of metals to the sediments.

8.2.-Future Work

A further work with the nitrogen, phosphorus and sulphur method to improve the methodology can be achieved using hydrogen peroxide and UV light for the digestion of the samples. The identification of the intermediate species of nitrogen, phosphorus and sulphur formed during the digestion with hydrogen peroxide and formic acid could be used to investigate oxidation processes. Further refinement of the speciation of nitrogen, phosphorus and sulphur is required .

To evaluate how sulphur affects the concentration of methylmercury, speciation of the sulphur will be necessary. This information will be useful for understanding the behaviour of arsenic and selenium.

Sampling for fish and mussel in the various zones of Lake Maracaibo could improve understanding of the extent of pollution in the food chain.

The method for vanadium speciation could be used as the basis for the further development of a faster and simple method for the study of the element in the samples.

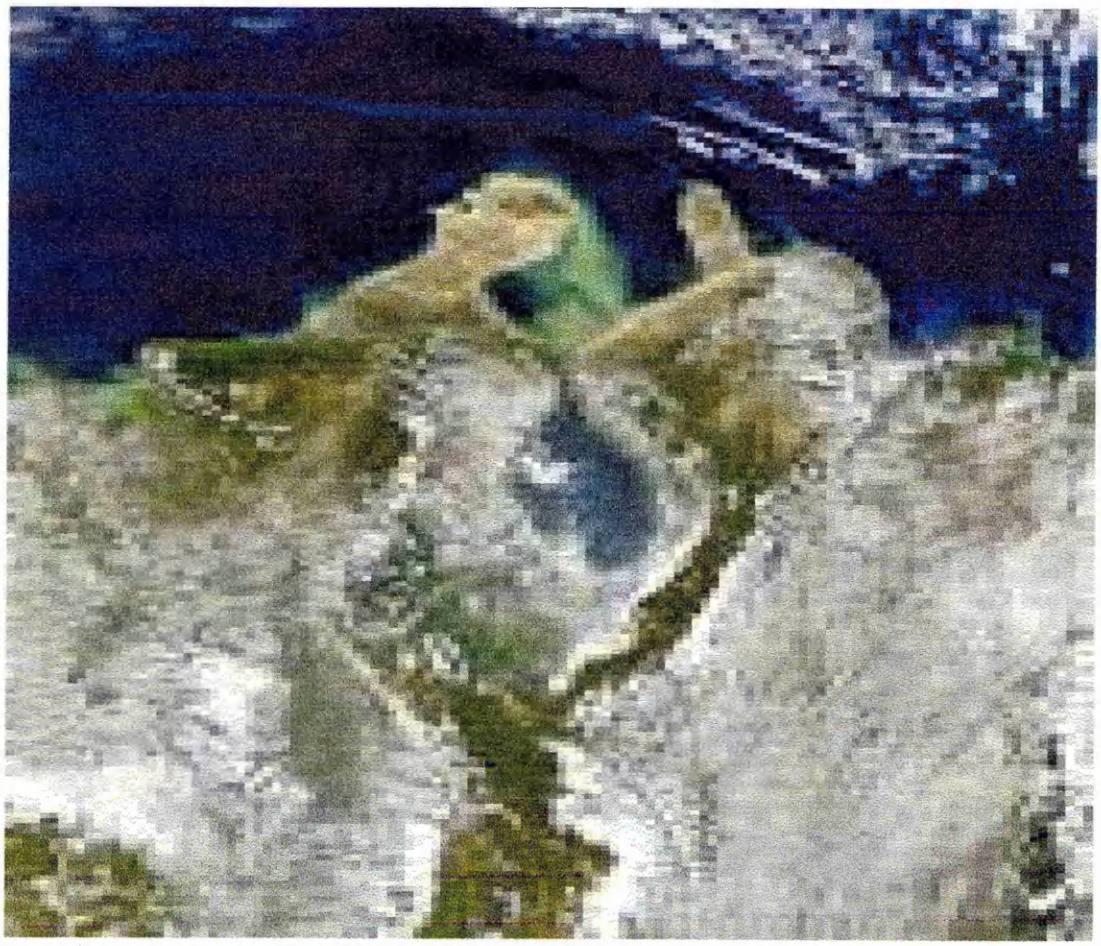
8.3.- Recommendations

To avoid losses of arsenic and selenium in samples of water, the analysis of these samples should be made immediately after the sampling campaign.

Appendix A

Satellite photograph of Lake Maracaibo, Venezuela

Provided by the SeaWiFS Project, NASA/Goddard Space Flight Center, and ORBIMAGE



Satellite photograph of Lake Maracaibo

Appendix B
Paper published in Journal of Chromatography, an oral presentation and two posters presented at the 6th Rio Symposium on atomic spectroscopy,2000.

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Journal of Chromatography A, 847 (1999) 285–290

JOURNAL OF CHROMATOGRAPHY A

Simultaneous determination of total nitrogen, phosphorus and sulphur by means of microwave digestion and ion chromatography

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Abstract

A method for the oxidation of nitrogen, phosphorus and sulphur to nitrate, phosphate and sulphate ions using 22% (v/v) hydrogen peroxide and closed-vessel microwave assisted digestion in two stages is described. Solutions of a variety of nitrogen-, phosphorus- and sulphur-containing compounds with formic acid added to prevent hydrolysis were used to test the efficiency of the procedure. The products of oxidation were determined by ion chromatography. Good recoveries of nitrogen, phosphorus and sulphur were obtained. The results for the NIST reference materials, oyster tissue and Buffalo River sediments agree well with the certified values.

Keywords: Nitrogen; Phosphorus; Inorganic anions; Sulphur

1. Introduction

The nitrogen, phosphorus and sulphur cycles are of particular significance to a number of biological and non-biological processes in the environment [1]. Natural and anthropogenic effects can cause localised inter-related changes to the cycles. In order to assess the impact and extent of the changes, it is essential to develop analytical methods which allow the simultaneous determination of two or all of the three constituents in a wide variety of environmental samples.

In one of the first attempts at simultaneous determination, Ebina et al. [2] developed a method of oxidizing nitrogen and phosphorus to nitrate and phosphate, respectively using alkaline potassium peroxodisulphate. The composition of the oxidizing solution was carefully chosen so that its pH changed from basic to acidic during the oxidation step. The change in pH was necessary because oxidation with potassium peroxodisulphate of nitrogen and phosphorus occurs under basic and acidic conditions, respectively. The nitrate and phosphate ions were then determined colorimetrically.

In a different approach, Nygaard and Sotera [3] used inductively coupled plasma atomic emission spectrometry (ICP-AES) to determine water-soluble nitrogen and phosphorus in fertilisers. More recently, Matilainen and Tummavuori [4] investigated the application of ICP-AES to the determination of water soluble sulphur in fertilisers and reported on spectral and interelement effects.

To be able to analyse both bound and water soluble fractions, samples have to be digested. However, existing digestion methods are not easily adapted to simultaneous determinations because the use of oxidants such as nitric and sulphuric acids and potassium peroxodisulphate precludes the determination of one or more of the analytes.

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PII: S0021-9673(99)00024-2

In addition, water is the main product when the oxidizing strength of hydrogen peroxide is spent, and as a result the digest is amenable to analysis by techniques such ion chromatography (IC) and ion selective potentiometry. UV-induced photoxidation using hydrogen peroxide has been applied successfully to the oxidation of nitrogen, phosphorus and carbon in sea water [5].

In this study, we report results from an investigation of the use of hydrogen peroxide at low pH in combination with closed-vessel microwave assisted digestion for the oxidation of various nitrogen-, phopshorus- and sulphur-containing compounds. The nitrate, phosphate and sulphate ions formed were determined by IC.

2. Experimental

2.1. Apparatus

A Dionex QIC analyser ion chromatograph equipped with a Dionex AG4A guard column, a Dionex AS4A anion separation column, and a Dionex AMMS-II suppressor and conductivity detector was used. The sample was injected into the chromatograph via a 100-μl sample loop, and eluted with a solution of 1.8 m*M* sodium carbonate–1.7 m*M* sodium hydrogencarbonate at a flow-rate of a flow-rate of 1 ml min^{-1}. A chart speed of 0.5 cm s^{-1}, conductivity range setting of 30 μS, and conductivity suppressor solution of 12.5 m*M* H_2SO_4 were used throughout.

A Milestone Model MLS-1200 Mega microwave system (24010 Sorisole, Italy) was used for the digestion of the samples. The digestion programme was as follows:

Step	Power (W)	Time (min)
1	250	5
2	0	15
3	600	10
4	Ventilation	10

2.2. Reagents

The column eluent was prepared from reagent grade sodium carbonate and bicarbonate, and distilled deionized water (18 MΩ cm, nanopure, Millipore, MA, USA) The suppressor solution was prepared from 1.4 ml AristaR grade sulphuric acid (Merck, Poole, UK) and made up to 2 l with distilled deionised water. The following analytical grade compounds were subjected to the digestion treatment: sodium nitrite, urea, L-cysteine and ammonium nitrate (all supplied by Merck), L-lysine and sodium pyrophosphate (both supplied by Aldrich, Gillingham, UK), sodium sulphite (East Anglia Chemicals, UK).

A 22% (v/v) solution was prepared from AristaR grade 30% (v/v) hydrogen peroxide.

2.3. Sample preparation

To test the efficiency of the oxidation procedure, solutions containing 50 μl of formic acid and 40–100 mg l^{-1} in nitrogen, phosphorus or sulphur were prepared.

Standard reference materials oyster tissue (NIST, SRM 1566a) and Buffalo River sediment (NIST SRM 2704) were used to validate the digestion procedure.

2.4. Stock standard solutions

Individual 1000 mg l^{-1} stock standard solutions of nitrate (N), phosphate (P), sulphate (S) and nitrite (N) were prepared from Aristar grade reagents (supplied by Merck) by dissolving 6.0679 g $NaNO_3$, 4.3937 g KH_2PO_4, 1.8141 g K_2SO_4 and 0.2020 g of $NaNO_2$, respectively, in distilled deionised water.

Mixed anion standard solutions of 1.0, 2.5, 5.0 and 10.0 mg l^{-1}, respectively, were used to calibrate the ion chromatograph.

2.5. Sample digestion

Ten ml of hydrogen peroxide solution were added to 5 ml of sample or 0.2 g of a reference material and 50 μl of formic acid in the microwave sample vessel. The mixture was capped and the microwave programme initiated. At the end of the first run, the

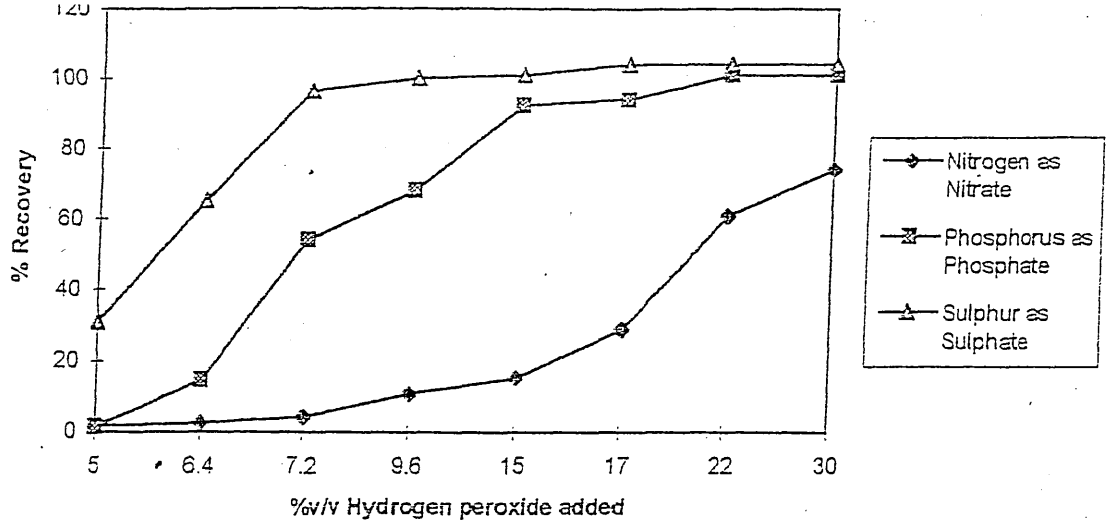

Fig. 1. Effect of varying hydrogen peroxide concentration on the recovery of nitrogen, phosphorus and sulphur from urea, sodium pyrophosphate, and L-cysteine, respectively after the first digestion.

sample was allowed to cool to room temperature, a further 10 ml of the same strength hydrogen peroxide solution was added and then the same programme was repeated. After oxidation, the digest was cooled to room temperature, made up to 100 ml with distilled deionised water, and analysed on the ion chromatograph. Each compound was digested and analysed at least five times.

3. Results and discussion

3.1. Concentration of the oxidizing solution

Fig. 1 represents the effect in percent recovery by varying hydrogen peroxide concentrations on the conversion of urea, sodium pyrophosphate and L-cysteine in the presence of formic acid to nitrate,

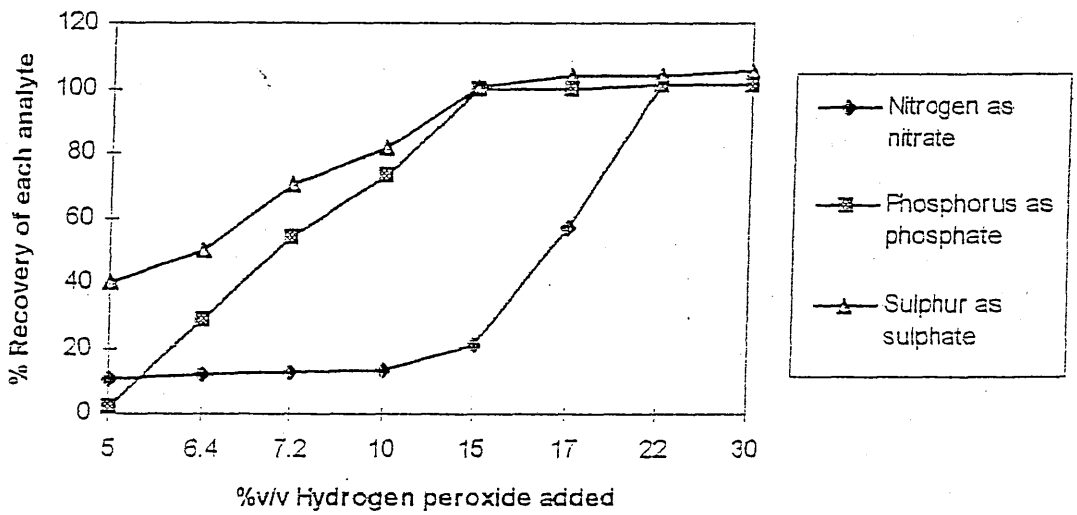

Fig. 2. Effect of varying hydrogen peroxide concentration on the recovery of nitrogen, phosphorus and sulphur from urea, sodium pyrophosphate and L-cysteine, respectively after the second digestion.

Recoveries of N, P and S as nitrate, phosphate and sulphate ions from different concentrations (mg l^{-1}) of pure compounds after digestion with 22% (v/v) hydrogen peroxide ($n=5$)

Compound	$N\text{-}NO_3^{-1}$ expected	$N\text{-}NO_3^{-1}$ found	$P\text{-}PO_4^{-3}$ expected	$P\text{-}PO_4^{-3}$ found	$S\text{-}SO_4^{-2}$ expected	$S\text{-}SO_4^{-2}$ found
Urea	9.93	9.96±0.62				
L-Lysine	4.00	4.01±0.04				
Ammonium nitrate	6.49	6.68±0.06				
Sodium nitrite	10.0	10.02±0.08				
L-Cysteine	2.26	2.12±0.01			5.17	6.10 ± 0.01
Sodium pyrophosphate			9.78	9.80±0.15		
Sodium sulphite					5.38	5.35±0.04
Mix L-cysteine and sodium pyrophosphate	2.26	2.12±0.01	9.78	9.65±0.26	5.17	6.11±0.02

phosphate and sulphate, respectively. Formic acid was added to the samples in order to prevent the hydrolysis of the compounds. However, it has been suggested that the oxidizing power of hydrogen peroxide is enhanced when it is activated by either acid, metal ions or is exposed to UV light [6]. This aspect was not investigated.

The extent of conversion of urea to nitrate was much improved (Fig. 2) when a second 10-ml aliquot of the same concentration hydrogen peroxide solution was added and the sample subjected to the microwave programme for a second time. In subsequent experiments, 22% (v/v) hydrogen peroxide and the two-stage digestion procedure were used to test the efficiency of the oxidation process on a variety of compounds. Tables 1 and 2 summarise the extent of oxidation expressed as recoveries of total nitrogen, phosphorus and sulphur. Varying the amounts of urea, L-cysteine and sodium pyrophosphate did not affect the extent of oxidation (see Table 2). The very good recovery values indicate that the oxidation process is efficient at converting N, P and S in the form they occur in the compounds. The efficiency of the procedure in oxidizing compounds containing nitrogen–nitrogen bonds or amide groups, and condensed polyphosphates is currently being assessed. A comparison of the expected and found values for N, P, S (Table 1) using a paired-t test was found not to be statistically significant at the 95% confidence limits except for the L-cysteine for which

Table 2
Recoveries of nitrogen, phosphorus and sulphur using different concentrations of analyte and 22% (v/v) hydrogen peroxide

Compounds	Concentration expected (mg l^{-1})	Concentration found (mg l^{-1})	Recovery (%)
Urea ($N\text{-}NO_3^{-1}$)	5.00	4.85	97.0
	9.93	10.40	104.7
	6.00	5.42	90.3
	6.24	5.50	88.1
	8.00	7.45	93.1
L-Cysteine ($S\text{-}SO_4^{-2}$)	23.10	22.59	97.7
	11.48	12.11	105.4
	15.11	14.44	95.5
	10.00	10.50	105.0
	5.17	6.10	117.0
Sodium pyrophosphate ($P\text{-}PO_4^{-3}$)	10.00	9.56	95.6
	20.50	22.19	108.2
	6.49	6.72	103.5
	31.8	30.13	94.7
	9.78	9.70	99.2

high recoveries were obtained. The difference in the results could be due to the poorer sensitivity for the determination of sulphate ions at low concentrations.

3.2. Analytical performance

A chromatogram of a mixture of L-cysteine and sodium pyrophosphate after oxidation is shown in Fig. 3. The mean±SD retention times for nitrate, phosphate and sulphate ions were: 4.11±0.14, 6.60±0.05 and 8.65±0.24 min, respectively. The three peaks are very well resolved and as a result samples containing widely different proportions of the analytes can be analysed without interferences.

Calibration graphs obtained from mixed anion standards gave the following highly linear best-fit equations: nitrate: $y=1.18\cdot10^{7}x-7.18\cdot10^{6}$ ($r^2=$ 0.9970); phosphate: $y=4.71\cdot10^{7}x-3.78\cdot10^{6}$ ($r^2=$

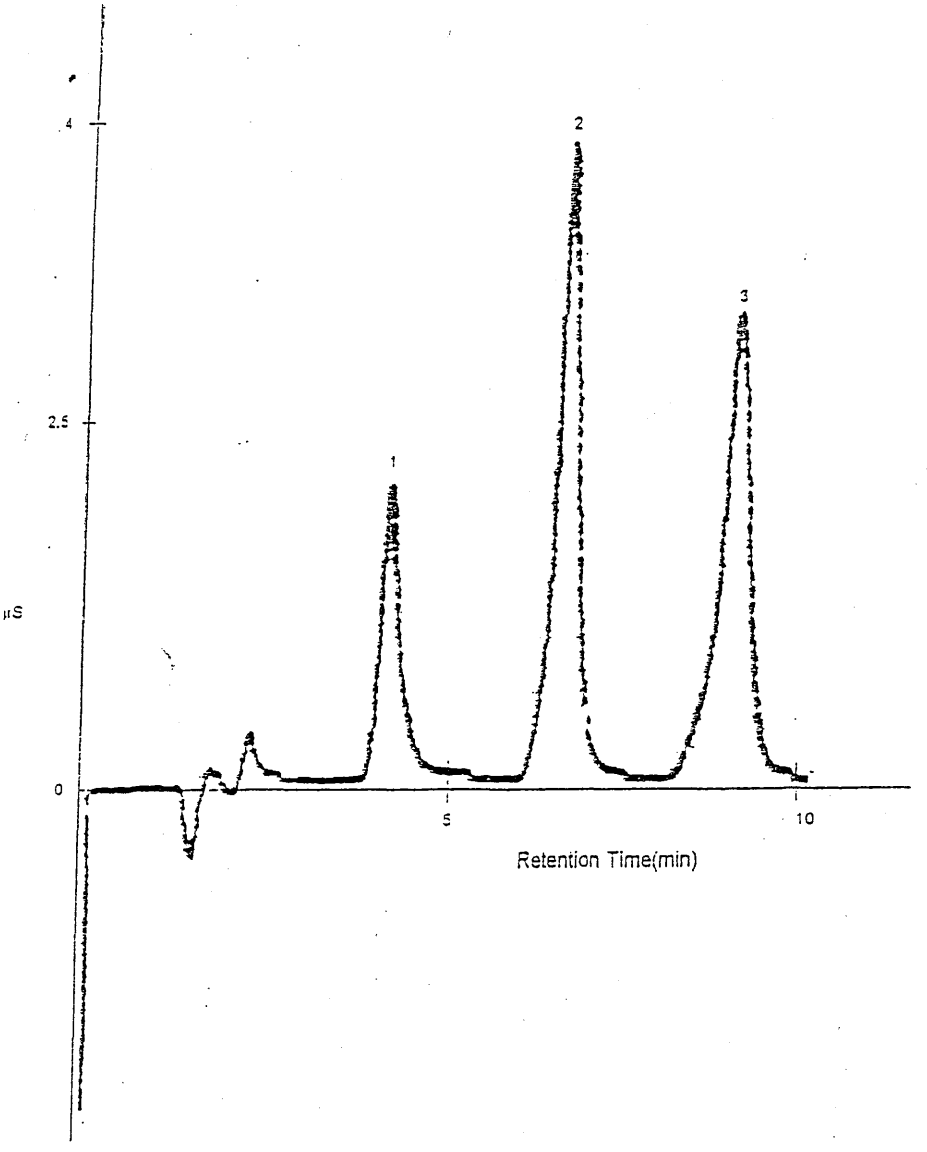

Fig. 3. Chromatogram of a sample containing L-cysteine and sodium pyrophosphate after oxidation to nitrate (1), phosphate (2) and sulphate (3).

Table 3
Comparison of the quantities of N, P and S found using the proposed method and the reported values for the standard reference materials ($n=3$)

Element		Oyster tissue (%, w/w± 95% confidence limit)	Buffalo River (%, w/w± 95% confidence limit)
N	Found	6.62±0.28	
	Reference value	6.31	
P	Found	0.619±0.020	0.0888±0.0125
	Certified	0.623±0.020	0.0998±0.0003
S	Found	0.872±0.011	0.432±0.045
	Certified	0.862±0.021	0.397±0.0005

0.9886); sulphate: $y=4.37\cdot10^{6}x-3.76\cdot10^{6}$ ($r^2=0.9865$) where y=peak area (arbitrary units) and x=anion concentration (mg l^{-1}).

Detection limits were calculated from the calibration graphs using the method of Miller and Miller [7]. The results were 0.123 mg/l nitrate, 0.251 mg/l phosphate and 0.850 mg/l sulphate. The detection limits based on 0.2 g of sediment were 0.006% (w/w) N, 0.012% (w/w) P and 0.042% (w/w) S.

3.3. Method validation

The N, P and S contents for NIST SRM 1566a oyster tissue and NIST SRM 2704 Buffalo River sediment samples digested with 22% (v/v) hydrogen peroxide are given in Table 3. Satisfactory agreement with the certified values was obtained. The presence of a sample matrix did not have an adverse effect on the recoveries.

4. Conclusions

The proposed method for the oxidation of N, P and S followed by the determination of the nitrate, phosphate and sulphate ion by IC gave satisfactory results for the compounds tested. The effectiveness of this procedure is demonstrated by the good recoveries obtained for the two SRMs, oyster tissue and Buffalo River sediment. Current work is focused on the application of the method to more recalcitrant compounds where the N, P and S atoms are in ring systems.

Acknowledgements

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RIO SYMPOSIUM ON ATOMIC SPECTROMETRY

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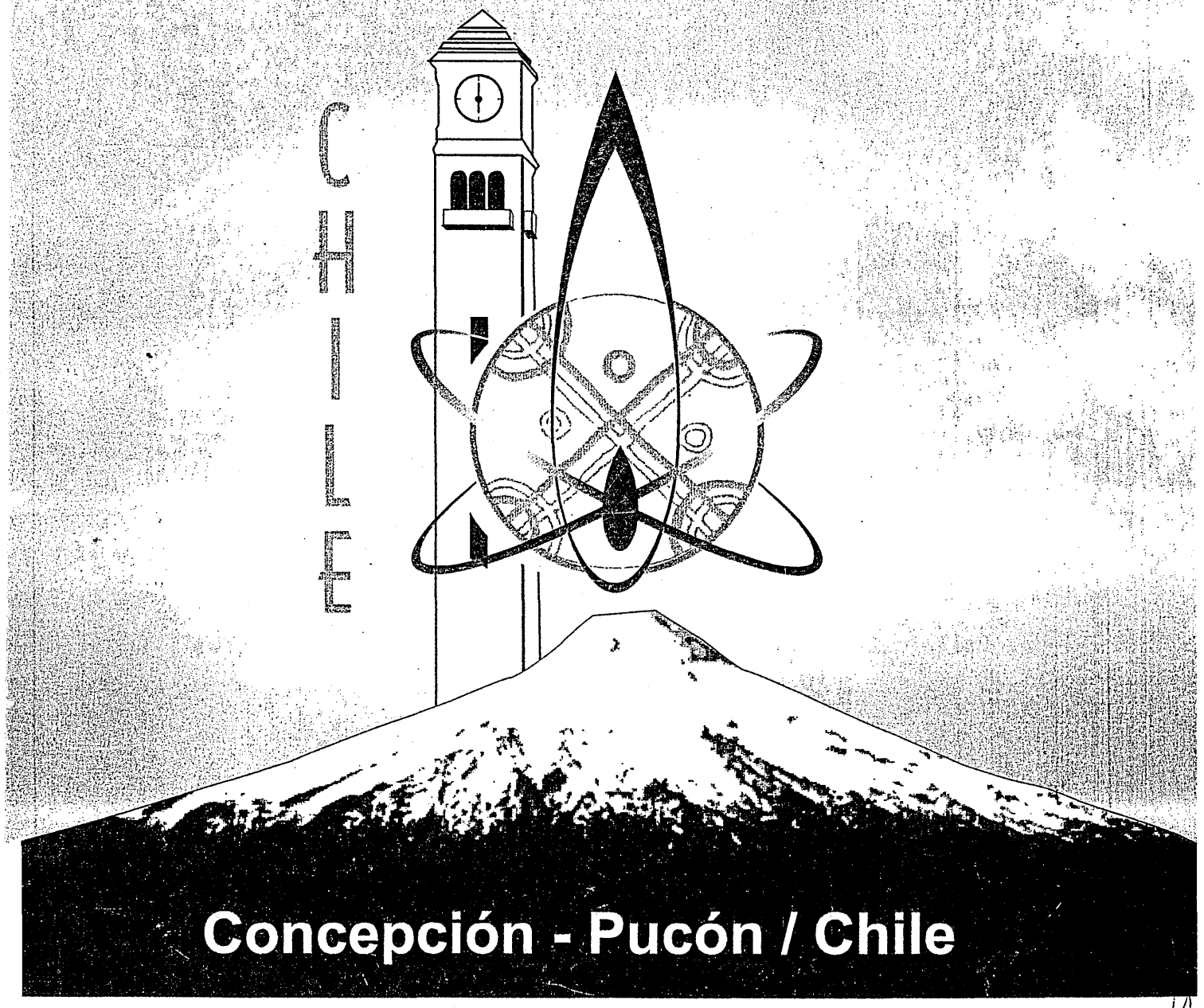
December 3 - 9, 2000



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BOOK OF ABSTRACTS

FINAL PROGRAM

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O 20

CHEMICAL SPECIATION OF MERCURY AND SELENIUM IN WATER, MUSSEL, FISH MUSCLE TISSUE AND SEDIMENT SAMPLES FROM LAKE MARACAIBO, VENEZUELA

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A method for the determination of methylmercury, inorganic mercury, seleno-*dl*- cystine and seleno-*l*- methionine in environmental samples was developed using a Reverse phase C_{18} column which was comprised of dimethyloctadecylsilyl bonded amorphous silica (Waters HPLC column) and connected to a Dionex GPM 2 gradient pump equipped with a Reodyne Model 7125 injection valve with a 50 µL sample loop. Mercury and selenium species separated on the column using a mobile phase containing a mixture of 0.06 ammonium acetate, 3 % methanol, 0.1 % 2-mercaptoethanol, 2 mM EDTA with pH adjusted to 6.5 were detected by ICP-MS (Hewlett Packcard 4500).

The mercury and selenium species were extracted from the lyophilised samples of sediment, mussels and fish muscle tissue using two different methods: a) A modified digestion-extraction method extracting mercury species from the toluene organic phase with L-Cysteine; b) Cold digestion procedure using hydrogen peroxide and nitric acid.

After the extraction or digestion method used the samples were adjusted to pH= 6.5 with NaOH 1 % w/v.

The water samples were filtered with a 0.2 µm Millipore filter before the injection onto the column.

The methods were validated using the following Reference Materials: IAEA-356 sediment and Dogfish Muscle DORM-2 . .

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SIMULTANEOUS CHEMICAL SPECIATION OF ARSENIC, SELENIUM AND CHROMIUM IN ENVIRONMENTAL SAMPLES USING ION CHROMATOGRAPHY AND ICP-MS

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A method for the simultaneous separation and speciation of As(III), As(V), Se (IV), Se (VI) and Cr(VI) levels in water, sediment, mussel and fish muscle tissue using Ion Chromatography with ICP-MS detection is reported. An HPLC system with a Dionex GPM 2 gradient pump, a Dionex AS-9 anion column, a Reodyne Model 7125 injection valve with a 50 μL sample loop and coupled directly to the ICP-MS (HP-4500) was used for the separation and detection. the various chemical species.

The following isotopes ^{75}As (100 % abundance), ^{82}Se and ^{53}Cr were used for detection. and interference effects of ArCl and HBr with the As and Se, respectively were investigated.

Water samples were filtered using 0.2 μm filters before injection to the column. A 5 g aliquot of either sediment, fish muscle tissue and mussels was added to 25 mL of 1mM of $Ca(NO_3)_2$ and shaken for 2 h. The supernatant obtained after the sample is centrifuged, filtered and a 50 μL injected onto the column.In order to optimise the column conditions three different mobile phase mixtures: a) Na_2CO_3 – $NaHCO_3$ b) $(NH_4)_2CO_3$ –NH_4HCO_3 c) $(NH_4)_2PO_4$ –NH_4HPO_4 were studied varying the concentrations of each of the constituents. Both gradient and isocratic conditions were used and the effect on the resolution of the species investigated. Optimum resolution was obtained with the buffer 1.87 mM $(NH_4)_2PO_4$ – NH_4HPO_4) pH= 6.5 under isocratic conditions.

The developed method was used to study the distribution of the chemical species in environmental samples collected from Lake Maracaibo, Venezuela.

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DETERMINATION OF VANADIUM SPECIES USING LIQUID CHROMATOGRAPHY AND ICP-MS DETECTION.

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Environmental pollution caused by vanadium is almost entirely due to industrial activities such as steels, insecticides, oil combustion etc. In the surface waters vanadium exits as V^{V} and V^{IV}; the V^{V} as vanadate is more toxic than V^{IV} present as vanadyl ions. In this work, a liquid chromatographic method for the simultaneous determination of V^{IV} and V^{V} as EDTA complexes was developed using reversed phase ion- pair liquid chromatography. The method was applied to the quantification of the species by ICP-MS. Ammonium acetate 0.06 M, tetrabutylammonium hydroxide 10mM ammonium di-phosphate 10 mM and EDTA 2.5mM at pH=6 was used as eluent to avoid the use of organic solvents that can reduce the sensitivity of the instrument. A C8 reversed phase column; 15 cm long was used to separate the species. Standards and complexed samples should be recently prepared for the vanadium species determination. The application of the method to water and sediment samples from Maracaibo Lake, Venezuela is also discussed. The concentration ranges of sediment samples were [0.7– 61 μg/g] and [0.0 – 2.3 μg/g] for V^{+4} and V^{v} respectively .The method is simple and has adequate sensitivity for these practical applications.

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