The determination of mercury and its organo compounds by high performance liquid chromatography-atomic flourescence spectrometry.

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The Determination of Mercury and its Organo Compounds by High Performance Liquid Chromatography - Atomic Fluorescence Spectrometry

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ABSTRACT

A number of digestion procedures used for the preparation of soil and sediments samples for the determination of total mercury by cold vapour atomic fluorescence spectrometry were investigated. Satisfactory results were obtained from a closed microwave digestion procedure for which 0.5g of soil or sediment sample was taken and heated with 3 mL of nitric acid and 1 ml of hydrogen peroxide. After cooling down of the digest, 0.5 mL of potassium permanganate solution (60 $g.L^{-1}$) was added and left to react for 30 min to 4 hours following the decomposition of the sample. The excess of potassium permanganate was then reduced with 0.5 mL of hydroxyammonium chloride (20 $g.L^{-1}$). After optimisation, this procedure was applied to the analysis of 50 sediment samples.

The accuracy of this method was confirmed by recoveries of total mercury in certified reference material (C74-05) containing 294 $ng.g^{-1}$ mercury and mean recoveries of 101.6 ± 4.4 % were obtained.

The second part of this work covered the speciation of organo mercury compounds in sediments by high performance liquid chromatography - atomic fluorescence spectrometry. Methyl mercury and mercury in standard solutions were successfully separated using a C_{18} loaded silica column. The resolution obtained using a Hypersil column (medium carbon load) was 67. Levels of methyl mercury as low as 2 $ng.g^{-1}$ were detected in aqueous solutions. Two types of extraction procedures were investigated for the determination of organo mercury compounds by HPLC-AFS. Acid leaching extraction procedure was the most promising of the two methods. For this procedure, 1 g of sample was taken and 10 mL of 6M HCl was added, the sample was shaken for at least 15 min before being centrifuged. 5 mL of aliquot was treated and diluted in order to obtain a pH greater than 4. This procedure although allowing qualitative analysis did not allow quantitative determination of mercury and methyl mercury.

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1.1. Introduction

Mercury is the only metallic element which is liquid at normal temperatures and pressures, it has a melting point at -38.9°C. Mercury has three oxidation states, Hg(0), Hg(I), and Hg(II). There are 7 stable and 11 unstable known isotopes of mercury. It occurs in nature as a mixture of the 7 stable isotopes and the average atomic mass of the blend is 200.6.

Mercury vapour, even at room temperature, is almost all monatomic; apart from the noble gases, mercury is the only element to show this behaviour at such low temperatures.

In the environment, mercury can be found under various inorganic forms such as HgS, HgSe, or Hg^{2+}. Some of the reactions which can take place in a natural environment are presented below.

Conversion between inorganic forms:

$Hg^{2+} \rightarrow$ HgS/HgSe: Wherever sulphide and selenide ions are present, mercury sulphide or selenide form, owing to the great affinity of mercury for sulphide sulphur and selenide. HgS seems also to be stable under anaerobic conditions. In excess sulphide ions, the complex HgS_2^{2-} is formed, depending on the pH, a reaction is believed to occur in soils.

$HgS \rightarrow Hg^{2+}$: humic compounds (fulvic-, humic acid) increase the solubility of the HgS by complex formation. It seems likely that an enzymatic reaction oxidises the sulphide to sulphite and sulphate releasing bivalent mercury ions, which undergo further conversion.

$Hg^{2+} \rightarrow$ Hg: The transformation from the cationic to the elemental state can occur chemically under suitable reducing conditions, e.g. in the presence of humic acid or by bacterial cultures (pseudomonas), yeast, and other microflora. As a method of detoxification under strictly anaerobic conditions the reduction of Hg^{2+} to Hg^0 becomes an important consideration.

$Hg^0 \rightarrow Hg^{2+}$: The oxidation depends on the redox potential in a medium.(1)

There are very few natural sources of mercury and most of it comes from the Earth crust and rocks (Table 1). The crustal abundance of mercury is 0.08 mg/kg and is mainly associated with sulphur. The main ore is cinnabar from which mercury is extracted. Other important ores are metacinnabar, conderite, livingstonite, montroydite, terlinguaite and calomel. Mercury deposits are usually from hydrothermal solutions around hot springs or volcanoes where levels can reach up to 200 µg/L (1).

Table 1: Levels of mercury in rocks:

Type of rocks	Concentration ($ng.g^{-1}$)
Basaltic rocks	12
Granitic rocks	80
Igneous rocks	5-26
Shale	400
Limestone	30
Sandstone	16
Metamorphic rocks	2-2500

Source of data: The Heavy Elements: Chemistry, Environmental Impact and Health Effects (1).

One of the main anthropogenic sources is the chlorine and paper industry. Mercury compounds were also widely used in paints, and in agriculture, as bactericides and fungicides. But such uses are now very limited if not stopped. Other sources of mercury are in dentistry (fillings), pharmaceutical and cosmetics industries as well as through combustion (2). Its uses are now declining mainly because of its toxicity and the introduction of strict regulations on authorised emission levels.

Mercury is toxic in its various chemical forms. The biological and toxicological activity of mercury with special regard to inorganic and organic mercury compounds, epidemiology, and genetic effects depends on the form in which it is taken up, the route of entry in the body and on the extent to which mercury is absorbed. Mercury intoxication can lead to neurological problems affecting sensory, visual and auditory functions. Methyl mercury particularly is able to cross the blood/brain and placenta barriers (3).

Methyl mercury chloride discharged into the Minamata river (Japan, 1967) initiated the first disease caused by environmental pollution. As a result, over 100 persons were afflicted, causing 46 deaths and several cases of prenatal intoxication manifesting in characteristic symptoms, e.g. motor disturbance, mainly ataxia, mental symptoms, congenital malformation, and cerebral palsy as a major effect. Disasters such as Minamata have focused attention on mercury and its organo-compounds and, as a result, a lot of research has been devoted to the understanding of its biogeochemical cycle (4).A study of the distribution of the various

chemical forms of mercury is essential since its toxicity is dependent in the form in which it is found (3, 5).

A number of biological studies have been made on mercury in the human body. Matrices such as blood, urine, nails, hair and kidneys have been studied for mercury and/or methyl mercury contents (1, 6). However, it is difficult to estimate the mercury level in a normal individual because its concentration will depend on many external factors such as pollution, time of exposure, length of exposure, dental treatment and diet. However, the study of populations can lead to conclusions on toxicity and long term effect of mercury. Table 2 shows the results obtained for different populations in different matrices.

Table 2: levels of mercury in biological samples (1)

Matrix	Range (μg/g)	Comments
Blood	0.18-2.73	Swedish people
Kidney	0.16-4.42	Swedish people
	3.1-144	Minamata patients
Liver	0.41-1.01	Swedish people
	0.3-70.5	Minamata patients
Hair	0.2-4.29	Swedish people
	2.45-705	Minamata patients
Brain	0-24.8	Minamata patients

1.2. Biogeochemical cycle of mercury

One of the most important factors which determines the behaviour of mercury in the environment is its volatility (Figure 1). Most mercury compounds are relatively volatile. It is the main transport pathway from soil to the atmosphere. Volatilisation of mercury can occur at any stage of the transport process.

Natural weathering transfers some of the mercury present in rocks and the earth's crust to oceans as well as to the atmosphere through land degassing. The mercury reaches the hydrosphere mainly in suspended matter, only a small portion being dissolved in water. In the

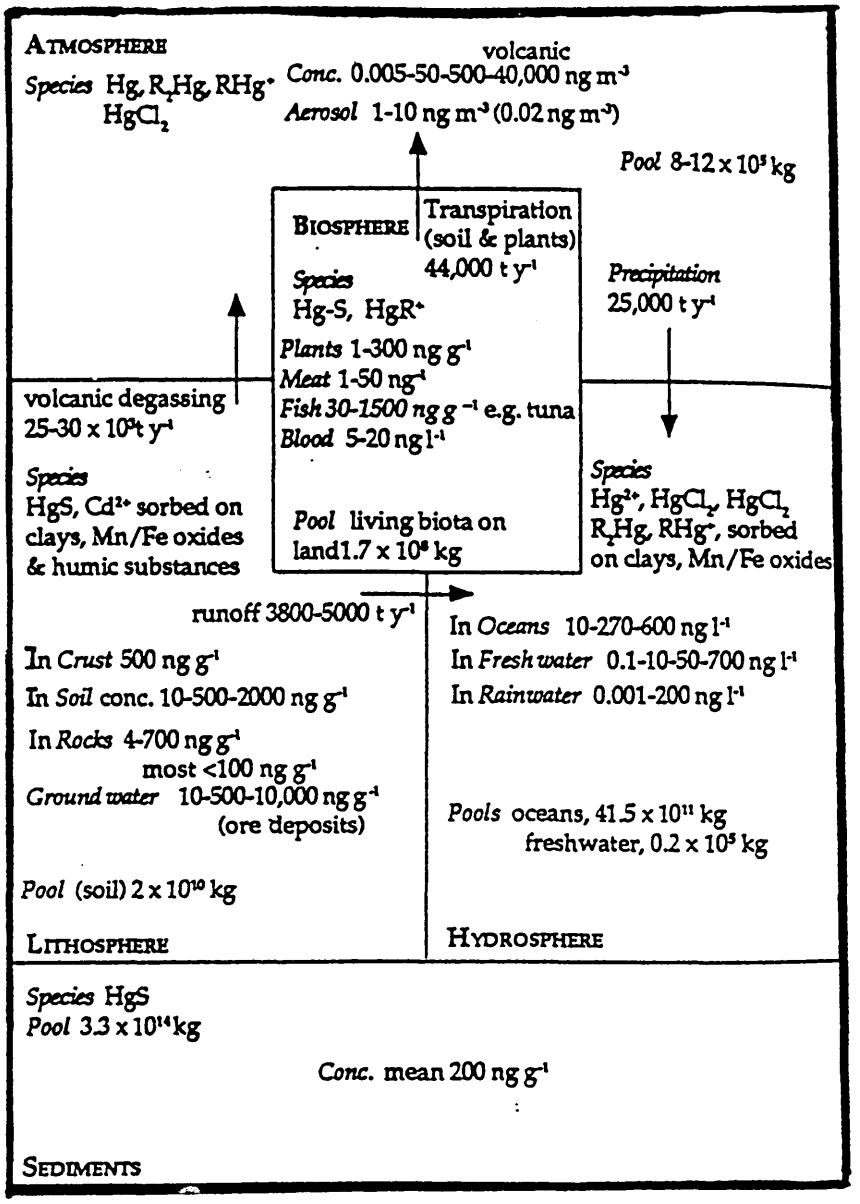

Figure 1: The biogeochemical cycle of mercury

Source: The Heavy Elements: Chemistry, Environmental Impacts and Health Effects (ref. 1)

hydrosphere, it partly deposits in sediments and partly incorporates in soils. Some of it is also removed by fish. Finally the uptake of mercury by plants from soils is weak but animals can then bio-accumulate methyl mercury (food chain magnification). (2, 7)

1.2.1. Atmosphere:

The atmosphere is the main transport pathway for mercury: elemental form (Hg^0: 49%) is the main form present, mercuric ion halide represents 25%, methyl mercury 21%, dimethyl mercury 1% and 4% is particle bound. Both chemical and photochemical reactions are responsible for the abiotic interconversion of the various mercury species.

1.2.2. Hydrosphere:

Mercury enters the hydrosphere mainly by deposition (rain water) and as silted or dissolved emissions from natural weathering and anthropogenic spills. The concentration of mercury and the presence of different species depends on the oxygen content, pH and biological activity. The affinity of mercury for sulphur is the main pathway for removal of the element from water: mercury strongly binds to sulphur sites on the surface of soils and sediment as well as to sites on enzymes and proteins. Microbial methylation followed by bioaccumulation of the lipophilic methyl mercury formed is another way for mercury removal.

1.2.3. Biosphere:

In the biosphere, plankton is the main mercury accumulator. In fish, 90% of the total mercury content is in the methylated form. Further increase in organo mercury occurs along the food chain due to the biological persistence and slow excretion of methyl mercury by organisms.

When the sediments and soils are rich in humus, mercury forms colloids with humic material and is transferred to water where it is available to biota.

1.2.4. Mercury in the soil/sediment environment:

Three aspects of mercury chemistry influences its chemistry in soil, and distinguishes it from the other heavy elements. These are the volatility of elemental mercury, an accessible redox chemistry whereby free mercury can be produced in soils, and the biomethylation of mercury producing very toxic, and often volatile compounds (e.g. CH_3Hg^+). The key materials are HgS, Hg/OH species and Hg-organo compounds, and some of the more important reactions are methylation, oxidation/ reduction, hydrolysis and precipitation.

Mercury distribution in soils has a characteristic profile. Strong adsorption and slow desorption of mercury means that the surface layers are richer in mercury with the highest concentrations in the upper 5 to 20 cm. Its mobility appears to be influenced by the redox potential, pH, drainage and the type of soil. Sulphur-containing amino acids and proteins form very strong soluble complexes and humic acids form strong complexes of relatively low solubility. Results obtained after selective extraction procedures suggest that both metallic and ionic are adsorbed in the form of a humate since none of the common and stable mercury compounds including HgS were found. (1, 2, 8)

Sediments collect most of the mercury moving in the hydrosphere: because reduced sulphur has a high affinity for mercury; both inorganic and organic SH compounds remove mercury from solution. Background levels of mercury in uncontaminated lake and ocean sediments are 0.05 and 0.1 to 1 $\mu g.g^{-1}$ (dry weight) respectively. Anthropogenic releases have contributed to a significant increase of concentrations. The mercury content in recent sediment layers in the Lake Ontario, in lakes of Switzerland, and in the River Rhine near Koblenz (Germany) is 0.31 to 1, 0.01 to 2.23, and 4.5 $\mu g.g^{-1}$, respectively.

Terrestrial soil strongly adsorbs the deposited, agriculturally applied, and waste originated mercury. The global average concentration of mercury in soils is estimated to be somewhere between 50 and 100 $ng.g^{-1}$. Locally, close to strong polluters such as chloroalkali plants and coal fired power plants, the mercury levels can build up to as high as 10 $\mu g.g^{-1}$ and more (Table 3). The residence time for readily leached mercury in soils is 500-1000 years. Mercury sulphide found in rocks is resistant to solubilisation through weathering, and enter the geochemical cycle mostly in the form of mechanically degraded matter. In this form, it may however undergo chemical and microbial transformation to the elemental form. When passing

through the soil, further transformation, e.g. into organo mercurials with aid of bacteria, enable mercury to reach the atmosphere.

Consequently, increased pollution leads to increased mercury deposition, which accumulates in surface layers due to extremely slow leaching of mercury in the soil column.

There is a number of difficulties in estimating the rates of elemental distribution in soil for several reasons. Rock weathering is difficult to estimate because it depends on the type of rocks and climatic conditions. Fall out from rain and dust varies greatly with rainfall and proximity to industrial areas, volcanoes and oceans. Fertilisers, of which the use has been significantly reduced, have different application rates depending on the place in the world and the type of crop. Removal by plants depends on the type of plants (e.g. Cereals remove mercury much less than tobacco or cabbage would do). And finally there are no satisfactory ways of measuring volatilisation which is an important process of mercury removal from soil.

Table 3: Levels of mercury: in non contaminated and contaminated surface soils in Canada and England

Country	Range($\mu g.g^{-1}$)	Comments	Country	Range($\mu g.g^{-1}$)	Source of contamination
Canada	0.02-0.2	various soils	Canada	0.32-5.7	chloroalkali
	0.01-0.70	Podsols		9.4-11.5	fungicides
	0.018-0.22	Gleysols	England	0.21-3.4	Mining areas
	0.05-1.11	organic soils		0.25-15	Gardens and
	0.01-0.09	various soils			orchards
England	0.008-0.19	various soils			

Data source: The Heavy Elements: Chemistry, Environmental Impact and Health Effects (1).

1.3. Methylation/Demethylation:

Methylation is a very important process in the cycle of mercury in the environment. Methylated species are the main forms of mercury in the biota (fish, and subsequently human). However the levels of methyl mercury vary greatly. In sediments, methyl mercury represents

0.1 to 1.5% of the total mercury content, in sea-water about 2% of the total mercury is in the methylated form, and in fish up to 80 % of the total mercury is methyl mercury (1, 17).

The measured concentration of methyl mercury in a sample is the result of an equilibrium between methylation and demethylation (9, 11, 12). Methylation occurs mainly in sediments and fish. A number of factors which include temperature, pH, organic enrichment, oxygen content and depth determine the extent of methylation (11, 12).

Two mechanisms have been identified for methylation of mercury: an enzymatic microbial methylation and a non enzymatic transfer of methyl group to mercuric ion in a biological system. The role of bacteria in the methylation process is known to be very important (1, 7, 9, 11, 13, 18).

Demethylation, like methylation, can occur aerobically and anaerobically. The processes of demethylation can be chemical or involve micro-organisms. Both methylation and demethylation are believed to be ways for bacteria to detoxify their environment (12, 13).

1.3.1 Mechanisms of methylation:

Three naturally occurring methylating agents are known: methylcobalamine, S-adenosylmethionine, N_5-methyltetrahydrate. Methylcobalamine is thought to be the main methylating agent for mercury (1).

1.3.1.1 Methylcobalamine:

Methylcobalamine can be formed from vitamin B_{12}. This vitamin occurs in most living organisms and can be transformed into methylcobalamine by methane producing bacteria.

Processes involving methylcobalamine can be enzymatic or non enzymatic. In the non enzymatic process, methylcobalamine is free to form other compounds and acts as a chemical transfer agent, transferring a methyl group onto mercury. In the enzymatic pathway, methylcobalamine is associated with enzymes such as methionine synthetase, acetate synthetase and methane synthetase and can interfere with the cell metabolic pathway. Known bacteria associated with methylation are Clostridium and Pseudomonas. (1, 12, 14)

1.3.1.2. Abiotic methylation (1):

This methylation process is non enzymatic and involves a reagent which is produced biotically. The two main abiotic processes are transmethylation and by photochemical reaction. In transmethylation, one methyl group is transferred from one metal to another (reaction 1).

$$\text{Reaction 1: } (CH_3)_n Sn^{(4-n)+} + Hg^{+} \rightarrow (CH_3)_{n-1} Sn^{(5-n)+} + CH_3Hg^{+}$$

In the photochemical process, mainly occurring in surface waters, Methyl is produced photochemically e.g. by exposure of acetate, methanol, ethanol or aliphatic α-amino acids to intense UV light. The methylating factors are usually associated with humic and fulvic fractions.

1.3.2. Factors influencing methylation and demethylation:

As mentioned before, the difficulty of studying mercury biogeochemical cycle and the role of methylation is due to the number of factors influencing methylating processes and other parameters such as bacteria population.

1.3.2.1. Effect of oxygen:

In sediments, the best conditions seem to be anaerobic. The oxygenation of sediments inhibits methylation activity (11). However previous work showed an increase in released methyl mercury under aerobic conditions (12, 16). This could be explained by a better release of mercury in disturbed sediments rather than an increase in methyl mercury production.

It is also believed that in aerobic conditions, sulphides react to give sulphate ions rather than link with mercury which is then available for methylation (1) but Callister (11) suspected that micro-organisms are also able to methylate sediment bound mercury or enhance desorption of mercury, allowing methylation to take place.

1.3.2.2. Effect of temperature:

All studies agree that methylation increases with temperature (1, 11, 12). Furthermore some studies have noted different methylating rates following the seasons. These two observations could be linked by the effect of temperature on bacterial population.

1.3.2.3. Effect of pH:

The influence of pH on methylation is difficult to determine because of its effects on bacterial population, and other factors such as redox conditions. However it has been reported that high levels of methyl mercury were reported in fish living in acidic lakes. It has been shown that methylation is more important under slightly acidic conditions (pH= 5.5 - 6.5) and that demethylation is increased by any changes of pH. However different studies came up with different results: such as methylation occurs only at natural pH (5.5)(15), or mainly at a pH between 5.5 and 6.5 (10) or even that the optimum pH for methylation is 7 (13).

Those differences could probably be explained by the differences between the environment. However it has not been proved that the acidity of a lake has any influence on methylation in sediments.

1.3.2.4. Effect of other factors:

Mercury concentration: The formation of methyl mercury increases quicker when mercury is added to a sediment sample. However the increase of methyl mercury is not linear (10). Other studies have shown that it is linear. This could be explained by the differences between samples (soil composition, pH). Furthermore, the rate of demethylation seems to increase with methyl mercury concentration. This would support the theory that in sediments, as in soils, organisms adapt to the levels of methyl mercury to keep levels constant (13).

Sulphides concentration: At constant mercury concentration, methyl mercury production increases with sulphide content. At even higher sulphides concentration, methyl mercury production is inhibited (18). A possible reaction when sulphide levels are greater than 160 $\mu g.g^{-1}$ is shown by (reaction 2) (1):

Reaction 2: $2\ CH_3Hg^+ + S^{2-} \rightarrow (CH_3Hg)_2S \rightarrow (CH_3)_2Hg + HgS$

This is controversial because when sulphide ions are present, a strong bond will form between mercury and sulphides rather than methylation. Furthermore, the influence of sulphides appears to be dependent upon redox conditions (1, 18).

Depth: Depth distribution of mercury and methyl mercury correlate with depth distribution of overall microbial activity. Methylation occurs mainly on sediment surface. In deeper layers, mercury is often bound to particles or precipitates, and demethylation seems to take place (11).

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CHAPTER 2: Determination of Total Mercury and Speciation in Soils and Sediments.

2.1. Total mercury analysis

A summary of the various analytical techniques for the determination of mercury is given in Table 4.

Table 4: Characteristics of widely used analytical techniques for the determination of mercury:

Techniques	Characteristics:	References:	Comments
Flameless atomic absorption spectrometry (AAS)	*Detection limit*: 0.02 $mg.kg^{-1}$ *Sensitivity:* 0.10 $mg.kg^{-1}$ *Range of application:*0.01-0.50 $mg.kg^{-1}$ dry sample.	19-24	Details on sampling and sample preparation are given in 16,18 and 29, and on acid digestion procedure (21)
Flame AAS	*Detection limit*: 300 $\mu g.l^{-1}$	25	Decomposition under pressure with aqua regia
Graphite Furnace AAS	*Detection limit:* <10 $\mu g.l^{-1}$	25	
Cold Vapour AAS	*Detection limit*: <0.01µg Hg *Linear dynamic range/* 0.01-1µg Hg	25-34	A comparison of instrumental performances is given in 24 and of different types of digestion (33-34). A pre-concentration step using cellulose-hyphan is described (38)

Atomic Fluorescence Spectrometry (AFS)	*Detection limit:* 10 $ng.l^{-1}$ (has been reported at 10$pg.l^{-1}$ in water sample) *Precision:* 3.5% at 2 ng, 8.7% at 200 pg *Linear Range:*10$ng.l^{-1}$-100$ng.ml^{-1}$	27, 35-37	
Atomic Emission Spectrometry (AES)	*Detection limit:* 0.1 $\mu g.l^{-1}$	27	
Neutron Activation Analysis (NAA)	*Detection limit:* 0.03 $\mu g.l^{-1}$	31	
Inductively Coupled Plasma-Mass Spectrometry	*Detection limit:* 2 $ng.g^{-1}$ for 0.5g sample	38, 39	

2.1.1. Atomic Absorption Spectrometry:

Mercury and all its forms can be determined by atomic absorption spectrometry after adequate sample pre-treatment. Most of the methods are based on the measurement of the absorption of the mercury resonance line at 253.7 nm by ground state atoms. Mercury must be in the elemental form Hg(0) in order for it to be determined by AAS. This form is extremely volatile and can easily be transported by a carrier gas to the spectrometer.

Graphite furnace, flame and cold vapour cells are the most widely used. In the latter mercury is converted to atomic mercury by chemical reduction (Reaction 3).

Reaction 3: $Sn^{2+} + Hg^{2+} \rightarrow Sn^{4+} + Hg(0)$

This method of vaporisation is also used with techniques such as AFS, ICP-OES and ICP-MS. Factors affecting the detection limit of CV-AAS includes sample size, the aeration flow rate, the shape of the gas cell, the instrumental noise level, the sample matrix, and the contamination from the laboratory environment. In geological materials, sample matrix contains elements such as gold or selenium, the absorbance signal is depressed by up to 50% because mercury is adsorbed by these elements.

The disadvantages of AAS are mainly its limited linear calibration range and the spectral interferences which result from non specific background absorption of volatile organics. At lower concentration, a preconcentration step can be used. The most widely used technique is gold trapping. Elemental mercury is trapped onto gold wool and released by rapid heating of the column.

2.1.2 Atomic Fluorescence Spectrometry:

A typical atomic fluorescence arrangement is shown in Figure 2. Atoms produced in the flame are excited to higher energy levels by an high intensity mercury lamp. The excited atoms are then deactivated partly by collisional quenching and partly by emission of fluorescence radiations in all directions which pass to a detector positioned at right angles to the incident light measures the fluorescence emissions. The wavelength of the emitted radiation is characteristic of the absorbing atoms.

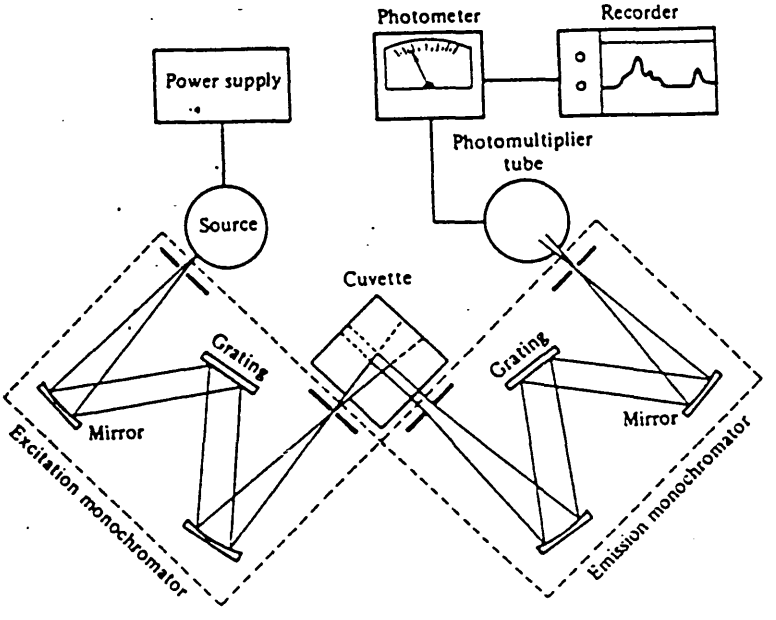

Figure 2: Schematic diagram of AFS principle.

Data source: Instrumental methods of Analysis (40)

At room temperature, atomic mercury absorbs and fluoresces at the same wavelength. Compared to AAS, the spectral matrix interferences are considerably less for AFS (23). Furthermore, the electronic amplification of the atomic fluorescence detector signal is simpler and produces less noise than in AAS mainly because fluorescence emissions are detected only at a certain angle. The linear calibration range of AFS is at least one order of magnitude better than in AAS and the detection limit is lower (AAS detection limit: 0.2 $ng.mL^{-1}$ and AFS detection limit: $0.001ng.mL^{-1}$).

However, for samples with high levels of mercury, carryover effects and self absorption problems can occur. This can be partly corrected by using a flow injection system.

Table 5: Atomic Fluorescence Spectrometry, Sensitivity characteristics:

Detection limit	1 $ng.kg^{-1}$ for real samples
Linear dynamic range	from $ng.kg^{-1}$ to $ng.mg^{-1}$ levels

2.1.3. Atomic Emission Spectrometry.

Plasma sources used for atomic emission spectrometry measurements include Inductively Coupled Plasma (ICP), Direct Current Plasma (DCP), and Microwave Induced Plasma (MIP).

One of the main advantages of AES is the multielement character of the technique. Furthermore, it allows nearly chemical interference free measurement, and it is possible to control the physical interferences. This technique has a high level of accuracy and precision as well as specificity and uses low samples volumes. However, it is not always commercially available (ie atmospheric pressure helium-MIP-AES) and the cost can be very high.

When coupled with Atomic Fluorescence detection, Inductively Coupled Plasma combines the multi-element character of AES and the specificity of AFS.

Table 6: Comparison of AAS and AES:

Factors	CV-AAS	ND-AAS	He-MIP-AES
Precision (%) at 2 ng	2.0	3.5	1.8
at 200 pg	4.0	8.7	6.5
Absolute blank (pg)	76 ± 3	92 ± 16	88 ± 5
Detection limits (pg)	8.7	10	15
Recoveries: $[Hg]_{expected}$=6.8 +/-1.3	5.5 ± 0.5	6.0 ± 0.3	6.0 ± 0.3

2.1.4. Neutron activation analysis:

In this technique, stable isotopes of mercury (and many other elements) are converted into radioactive daughter isotopes by irradiation with thermal neutrons. The radioactive daughter isotopes are identified and quantified by high resolution gamma spectrometry (no speciation study possible). Its analytical sensitivity depends on the isotope used.

This method is very precise, sensitive and specific. However, it is time consuming and the sample throughput is very low. Furthermore, it is expensive, and it requires the use of a nuclear reactor.

2.1.5. Other methods:

The other methods used for mercury determination include mass spectrometry, X-ray fluorescence, radiometric and voltammetric methods.

2.2 Methyl mercury analysis:

Methyl mercury can be extracted from sediments using different methods such as solvent extraction or water vapour distillation extraction. These two methods are quite popular and largely documented (see table 7). Either benzene, toluene, or chloroform are used for solvent extraction. Other methods have been developed using hydrochloric acid or sulphydryl cotton. However, these are not very popular.

The separation step is usually achieved by using chromatographic techniques.

When atomic detection is used, a decomposition step is often necessary, this is done by using either UV irradiation, acid digestion or an oxidation step.

Finally the detection methods most widely used are atomic absorption spectrometry and electron capture detections. However, when studying the rates of methylation or demethylation, scintillation counting is often used as it allows tracing of the mercury through its different forms, the isotope used is $^{203}Hg^{2+}$.

Table 7: Methyl mercury analysis in soils and sediments samples:

Extraction	Separation	Degradation	Analytical method	Comments	References
Chloroform	back extraction in Sodium Thiosulphate		CV-AAS		41
Solvent extraction			Electron capture gas chromatography		42
Water vapour distillation	ion exchange chromatography	UV irradiation	CV-AAS	Detection limit: $0.2\mu g.kg^{-1}$	43, 44

Copper Sulphate and Sodium Bromate Extraction			Scintillation counting		45
Water vapour distillation		UV irradiation	CV-AAS	Recoveries: 95 %	46
Benzene extraction	Gas Chromato-graphy		Microwave Induced Plasma emission	Detection limit: 90 $ng.L^{-1}$	47
Methyl mercury isolated as MeHgCl	Gas Chromato-graphy		Atmospheric pressure active nitrogen	Detection limit: 50pg. In 10g sediment: 50$ng.g^{-1}$	48

2.3. Mercury speciation:

A summary of the methods for mercury speciation is presented in Table 8.

Table 8: Methods for speciation studies in sediments and soil:

Separation	Detection	Comments	References
Chromatography	AFS or AAS	Detection limit using HPLC-AFS: 10 $\mu g.L^{-1}$	49-51

High Performance Liquid Chromatography	UV	9 organo mercury species were separated. Detection limits: 7.0-95.1 $\mu g.L^{-1}$	52, 53
Isothermal Gas Chromatography	CV-AFS	Detection limit: 0.001ng MeHg.g^{-1} as Hg for 0.1g sample	54
High Performance Liquid Chromatography	AFS	Detection limit: 0.8 $\mu g.L^{-1}$ Calibration linear range: 0.05-10 ng	55

The methods presented in Table 8 achieve low detection limits. In this thesis, it is reported the development of a simple and effective method for quick speciation of mercury in environmental samples such as soil and sediments. For this purpose, we have combined the separation power of HPLC with the specificity of Atomic Fluorescence detection.

Aim of the present investigation:

In this work, methods for the determination of total mercury in sediments, using Cold Vapour Atomic Fluorescence Spectrometry and for the speciation of mercury in sediments, coupling an HPLC column on-line with CV-AFS were investigated and applied.

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3.1. Instrumentation

3.1.1. Open Microwave Digestion System:

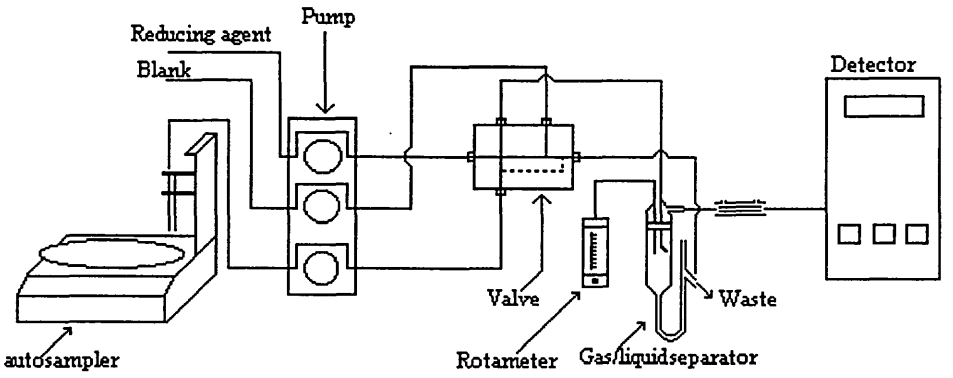
The system used was supplied by Prolabo (France). It consists of a control console, a digestion unit, and an extraction unit. The latter was not used in this investigation. As no autosampler or carousel was fitted on this instrument, only one sample was digested at a time. The digestion flasks were made of quartz and fitted with a glass condenser head in order to reduce vapour losses. The times and powers to be used during the digestion were programmed via the control unit.

3.1.2. Closed Microwave Digestion System:

This system was a MIS 2000 MEGA, Milestone (USA). It consisted of a closed oven, an extraction and a control units. As before, the control unit allowed the experimenter to program times and powers to be used during digestion. Up to 10 digestion flasks made of teflon could be held in a carousel.

3.1.3. The Cold Vapour-Atomic Fluorescence system:

A Merlin Mercury Plus System (PS.Analytical Ltd, England), which consisted of a 386 SX computer, a printer, a random access autosampler, a peristaltic pump and a Merlin Plus Mercury detector, was used throughout (see Figure 3). PSA TouchStone software controlled the whole system. The instrumental settings are shown in Table 9.



igure 3: Merlin Mercury Plus system:

Table 9: Settings of the CV-AFS system.

Parameter	*Setting*
Drying gas flow	3.5 L/min
Reaction gas	300 mL/min
Shield gas	300 mL/min
Detector range	Determined by the highest standard used

For the speciation of mercury, the printer was replaced by an integrator (DP700 Carlo Erba Instruments), and the autosampler was by-passed. A HPLC pump and a column (S5μ ODS2, Hichrom, USA) were placed on-line with the detector and another peristaltic pump was added to the system (see Figure 4). The settings for the HPLC system are shown in Table 10.

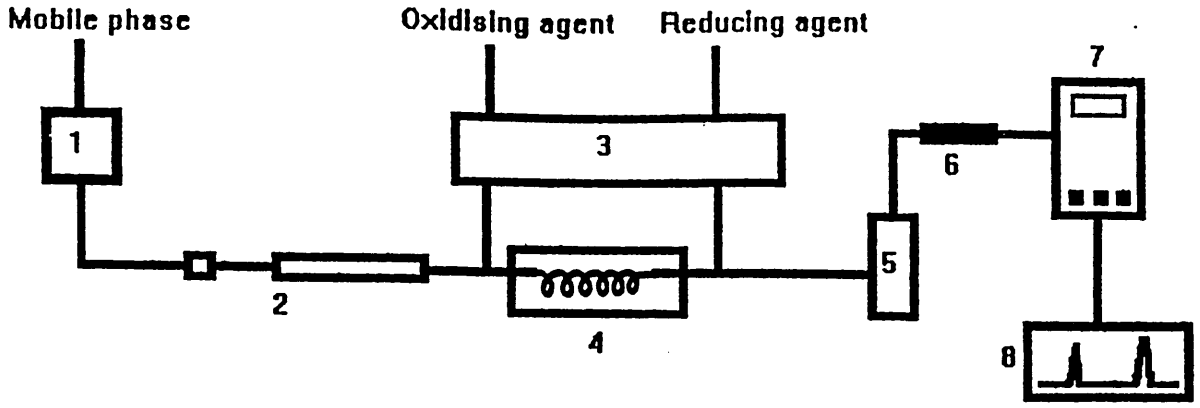


Figure 4: Speciation system:

1:HPLC pump, 2:Injection valve and column, 3:Peristaltic pump, 4:Hot water bath/ reaction coil, 5:Gas/liquid separator, 6:Drying cell, 7:Atomic Fluorescence detector, 8:Integrator

Table 10: settings for the HPLC system.

Parameter	*Setting*
HPLC pump rate	2.5 mL/min
Oxidising agent pump rate	1.66 mL/min
Reducing agent pump rate	4.46 mL/min
Range and fine control	Determined by the highest standard used

3.2. Reagents:

3.2.1. Reagents and chemicals:

Nitric, hydrochloric and sulphuric acids (Merck, England) used were of Aristar grade. Stannous chloride, hydroxyammonium chloride, sodium hydroxide, potassium persulfate (Merck, England) and hydrogen peroxide (Aldrich, England) were "low in mercury" and of Aristar grade. For speciation, the solvents used (methanol, chloroform) were of HPLC grade. All the other reagents were of Analar grade. Deionised water, obtained from a Millipore Milli-Q50 still was used throughout.

3.2.2. Preparation of the standard and reagents:

3.2.2.1. Total mercury analysis:

Standards: The commercially available mercury standard was 1000 $mg.L^{-1}$. It was used to prepare a 10 $mg.L^{-1}$ and a 100 $\mu g.L^{-1}$ standard by dilution, and the working standards were prepared by dilution from 100 $\mu g.g^{-1}$ standard, with 1 % potassium dichromate as stabiliser solution and the same acid matrix as the sample analysed. All the standards were prepared fresh daily.

Stabiliser solution: 0.5 g $K_2Cr_2O_7$ was dissolved with 50 mL nitric acid and diluted to 100 mL with water in a graduated flask.

Reducing agent: 10 g of $SnCl_2$ was dissolved in a 500 mL flask with 50 mL hydrochloric acid and the volume made up with water.

Hydroxyammonium chloride: 10 g of $H_2NOH.HCl$ was dissolved in a 1000 mL flask with water. This solution was used as the blank (see Figure 4) to reduce any excess stannous chloride.

Certified Reference Material: the Soil Certified Reference Material C74-05 used was from the Laboratory of the Government Chemist and its concentration in mercury was 294 $ng.g^{-1}$.

3.2.2.2. Speciation analysis:

Standards: 0.0125 g of commercially available methyl mercury chloride was dissolved in 10 mL of methanol to give a 1000 $mg.L^{-1}$ standard, which was stocked for 3 weeks in the dark at 4° C. This standard was used to prepare 10 $mg.L^{-1}$ and 100 $\mu g.L^{-1}$ standard by dilution. The working standard was obtained by successive dilution of the 100 $\mu g.g^{-1}$ standard in water. These standards were prepared daily prior to analysis.

Reducing agent: 15 g of $SnCl_2$ and 48 g of NaOH were diluted in a 1000 mL flask with water.

Oxidising agent: 25 g of potassium persulfate and 1.62 g of copper sulfate were diluted with 20 mL of sulphuric acid and the volume made up to 1000 mL.

Mobile phase: a solution of 4% (v/v) methanol and 0.01 % (v/v) of 2-mercapto-ethanol was used.

Solutions used for extraction of organo compounds from a sample:

Citrate buffer: It consisted of citric acid ($21 g.L^{-1}$) and sodium hydroxide ($8 g.L^{-1}$) adjusted to pH 2 with hydrochloric acid ($0.1 mol.L^{-1}$).

Dithizone extractant: $0.25 mmol.L^{-1}$ was prepared in chloroform.

Nitrite/acid solution: This is a 1:1 (v/v) mixture of sodium nitrite solution (5% w/v) and of an acidic solution containing $0.01 mol.L^{-1}$ HCl, $0.01 mol.L^{-1}$ H_2SO_4 and $0.1 mol.L^{-1}$ NaCl.

Sodium thiosulfate: $1 mmol.L^{-1}$ buffered with ammonium acetate ($0.05 mol.L^{-1}$)

3.3. Sample collection:

3.3.1. Sampling technique:

The samples were collected from 50 different places on the River Rother, between Chesterfield and Rotherham (Derbyshire and South Yorkshire). The sample sites are shown on the site maps page 44 to 47. The samples were taken using an auger driven through a metal tube into the river bed. This enabled the removal of a core sediment through the tube from a depth of approximately 30 cm, protected from the often sizeable wash of the river, and thus preserving the smaller, lighter particles. However, this technique was not always usable as, in some areas, the substrate consisted of hard-packed rocks and stones. In this case, the auger was driven through the stones to a similar depth and an effort was made to preserve as much of the core as possible.

Three to four samples were taken from the same site (1 m^2), and immediately placed into a labelled plastic bag and sealed. The sampler was carefully washed between each sample to minimise inter-sample contamination.

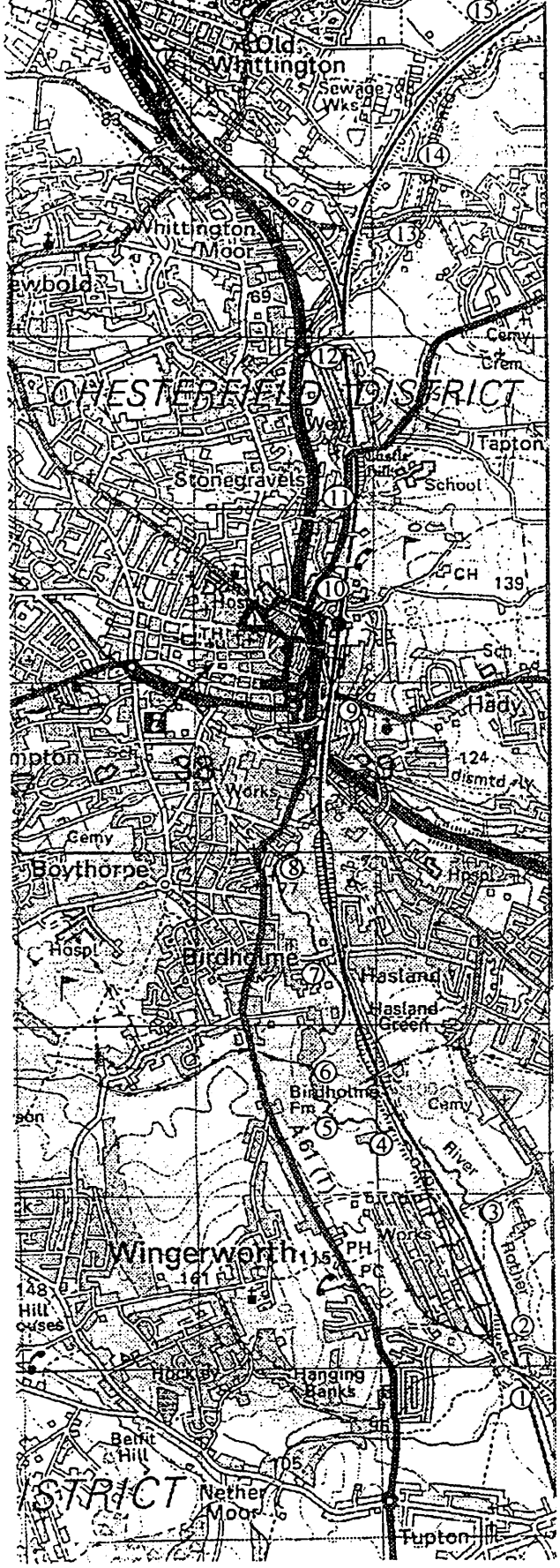
When it was thought to be too dangerous to enter the water, the sample was taken to the best of the sampler's ability without compromising safety.

3.3.2. Sample preparation:

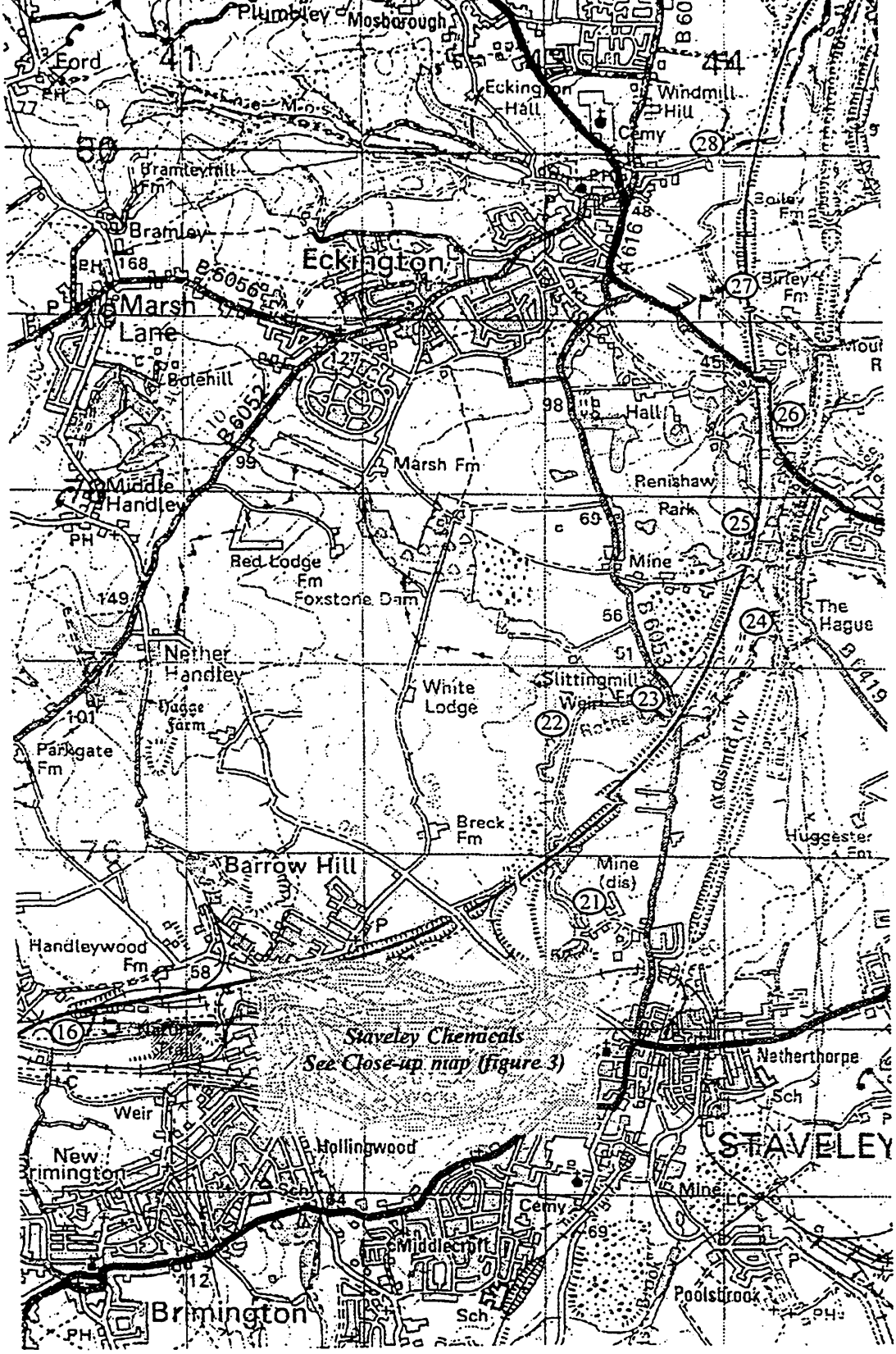
Samples were stored in a cool, dry and dark room until analysis.
They were then transferred to clean petri dishes for drying in an oven at 65 °C. When transferred to the drying dishes, spatula-sized subsamples were taken from different positions in the bag to ensure that the sample was representative.
The dried samples were fine-ground by hand using a mortar and pestle, sieved to 2 mm, and placed in a small, labelled air-tight bag. Care was taken to ensure that every accessory had been thoroughly washed and cleaned before used for the next sample.

3.3.3. Cleaning of the glassware:

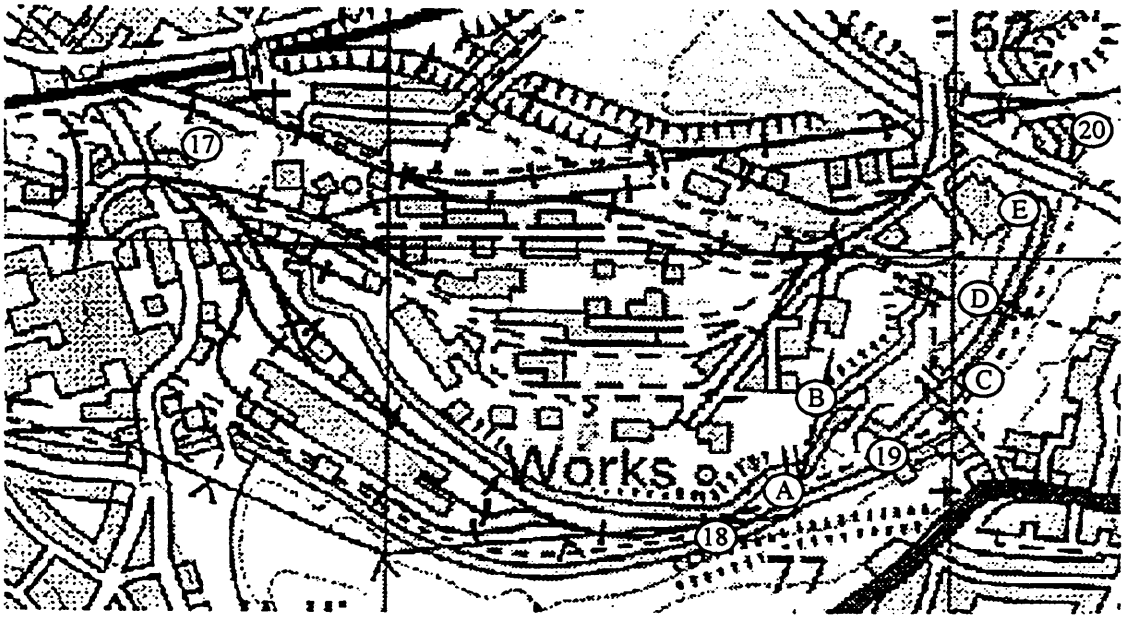
All glassware was cleaned by soaking overnight in a nitric acid bath and rinsed thoroughly with water before use. They were also rinsed before returning to the acid bath after use. The Teflon digestion flask was cleaned following the manufacturer's recommended procedure: 5 mL of nitric acid was poured into the flasks and they were placed in the microwave for 10 min at 500 W, they were then thoroughly rinsed with water.



Site map: Chesterfield



Site map: Staveley and Renishaw



Site map: Staveley (close up)

Site map: Rotherham

3.4. Digestion procedure:

Three types of digestion methods were investigated, a hot water bath (method A), an open microwave system (method B) and a closed microwave system(method C). Certified sediments were used to validate the three methods. Method A and C were also used for the analysis of environmental samples.

Method	*Type of digestion*	*Procedure*	*Microwave program*
A	open digestion	5 mL of aqua regia (1:3 HNO_3/HCl) was added to 0.5g of sample The samples were heated at 70° C for 3 hours in a hot water bath at constant temperature	none
B	microwave open digestion	A mixture of 1.5 mL of HNO_3 and 1.5 mL of H_2SO_4 were added to 0.5g of sample	5 min: 150 W Cooled in ice for 10 min 5 min: 180 W Cooled in ice for 15 min
C	microwave closed digestion	A mixture of 3 mL of HNO_3 and 1 mL of H_2O_2 were added to 0.5g of sample	1 min: 250 W 2 min: 0 W 5 min: 250 W 5 min: 400 W 5 min: 600 W and 10 min of ventilation

After digestion methods A or B, 0.5-1 mL of potassium persulfate (60 $g.L^{-1}$) was added to the digest for at least 4 hours. The excess of $KMnO_4$ was then reduced with

hydroxyammonium chloride (20 $g.L^{-1}$). The samples were then diluted to an appropriate volume (usually 50 mL).

Modified method C includes this oxidation step after microwave digestion.

3.5. Extraction procedures:

3.5.1 Organic extraction:

To about 5 g of sample, 5 mL of citrate buffer and 10 of dithizone extractant were added. The mixture was shaken for 15 min and transferred to a centrifuge tube, and centrifuged at 3000 rpm for 30 min. As the separation of the three phases (aqueous, organic and solid) was not complete, aliquots from the aqueous and the organic phases were taken to be analysed. The aqueous phase was analysed as such and the organic phase was evaporated to dryness and the organo compounds re-dissolved into water.

3.5.2 Acid leaching

To 1 g of sample, 10 mL of 6 M HCl was added. The sample was shaken for at least 15 min before being centrifuged. 5 mL aliquot was pipetted from the aqueous phase and transferred into a 50 mL graduated flask. About 22 mL of a alkaline solution (NaOH, 10 $g.L^{-1}$) was added to obtain a pH > 4.0. The volume was made up to 50 mL.

4.1. Total mercury determination

4.1.1. Comparison of digestion procedures.

Soil Certified Reference Materials (CRM) are materials for which one or more property values are certified by a technically valid procedure, traceable to a certificate or document and allow to demonstrate the quality and validity of a method.

The results of the determination of mercury in soil certified reference material (C74-05) are presented in Table 11.

Table 11: Recovery of mercury in Certified Reference Material.

Method	Number of Samples	Expected Concentration ($ng.g^{-1}$)	Measured concentration $\pm$ SD ($ng.g^{-1}$)
A	4	2.94	1.94 ± 0.08
B	6	2.94	2.67 ± 0.16
C	11	2.94	2.95 ± 0.12

Lower results for methods A and B suggest the loss of some mercury probably through volatilisation as elemental mercury is volatile at relatively low temperature. Mercury vapour at room temperature is almost all monoatomic. Methods A and B are gentle digestions compared to method C, an incomplete release of mercury from the matrix could explain the lower recoveries obtained. Furthermore both methods are time consuming and although method B gives acceptable results, we will only consider method C for further development.

4.1.2. Methylmercury recovery:

Methylmercury represents about 1-1.5 % of the total mercury present in soil or sediments (56). Therefore, we need to ensure that method C is reliable and oxidises effectively all organic mercury present.

Method C was further investigated, adding an oxidation step to the procedure and the results are summarised in Table 12.

Table 12: Recovery of methylmercury from standards and spiked CRMs.

		Method C	Modified Method C
Methyl Mercury Standards	Number of samples	4	5
	Expected concentration	3 $ng.mL^{-1}$	3 $ng.mL^{-1}$
	Mean measured level ± SD	2.11± 0.11	3.18 ± 0.19
Spiked CRMs	Number of samples	3	4
	Expected concentration	7.94 $ng.g^{-1}$	7.94 $ng.g^{-1}$
	Mean measured level ± SD	8.33 ± 0.21 $ng.g^{-1}$	8.75 ± $ng.g^{-1}$

The results show that methyl mercury in standards is recovered only after an oxidation step (modified method C). But this step does not seem necessary for the complete oxidation of methyl mercury when in soil. Some more experiments were carried out to investigate the extent to which an extra oxidation step is important.

4.1.3. Optimisation of the method:

4.1.3.1. Optimisation of the oxidation step:

A sediment sample (sample 25, see section 3.3.1) was digested both using method C and its modified version. The oxidising step was optimised, varying reaction times and added amount of potassium permanganate.

The results are presented in Table 13 and Figure 5.

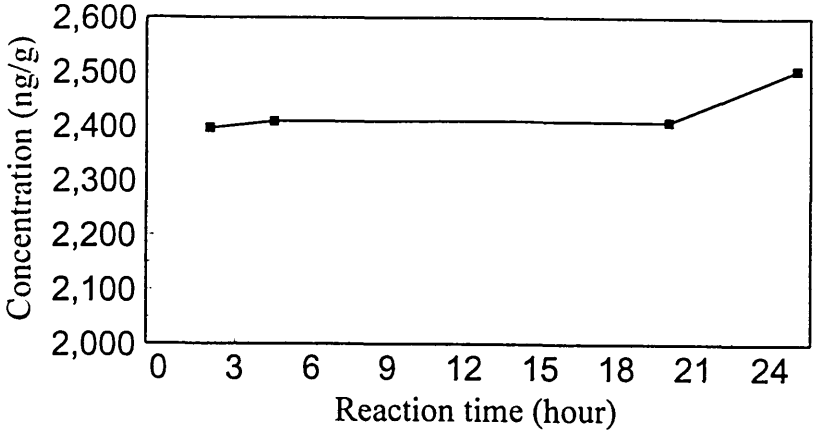
Table 13: Effect of added amount of potassium permanganate ($KMnO_4$) on oxidation

Added amount of $KMnO_4$ solution (mL)	Concentration ($ng.g^{-1}$)
0	1556.1
0.5	2691.7
1	2505.0

Reaction time: 4 hours.

These results show that the optimum amount of potassium permanganate to be used is 0.5 mL. The latter applications suggest that an oxidation step is particularly important when the sample studied has either a high organic matter or a high total mercury content. Indeed sediments with a higher organic matter content show higher methylation rate. This would explain why an oxidation step is necessary in a sample containing organic matter and less important when the organic content is lower such as in a soil based reference material as opposed say to a sewage sludge.

As can be seen from Figure 5 the reaction time is not critical. However, when time is not critical when obtaining results, a reaction time of about 2 hours will be used when digesting samples. But when the time of analysis is important, the reaction time may be limited to a few minutes.



Amount of potassium permanganate added: 0.5 mL

Figure 5: Optimisation of reaction time

4.1.3.2. Other considerations:

As the concentration in this sample is greater than the concentration of mercury in the CRM, a larger volume of nitric acid (5 mL instead of the 3 mL used in method C) was used for the digestion to ensure the acid solution was not exhausted. The results obtained for both digests were similar.

Finally, some experiments were carried out to determine how long the samples could be stored after digestion and before analysis. One batch of 8 samples was analysed immediately after digestion and after storage for 7 days at 4°C. During which time, the samples were stored in a fridge (4° C). The results showed that after 7 days, only 64.5% of the mercury was recovered. Volatilisation as well as adsorption on the sample bottle wall could partly explain this loss, although the latter is the most likely.

4.1.4. Factors influencing instrumental performance:

4.1.4.1. Effect of moisture carry-over.

It is known that the presence of moisture in the mercury vapour is a problem. Condensation occurs on the transfer tube walls and eventually enters the detection system which can lead to a gradual loss of sensitivity and baseline drift (56). This problem was identified on the first day of this project as very bad reproducibility was obtained as well as a rapid diminution in the signal after approximately 30 samples (Figure 6).

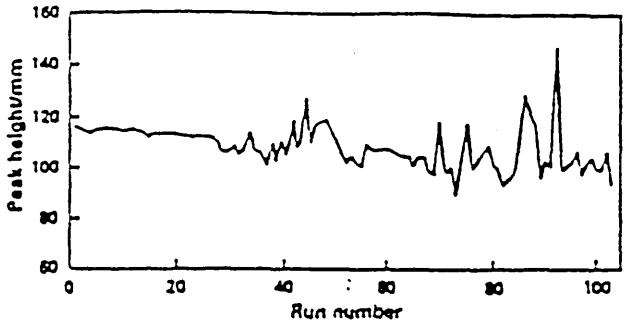


Figure 6: Variation of peak height with run number with no moisture trap.

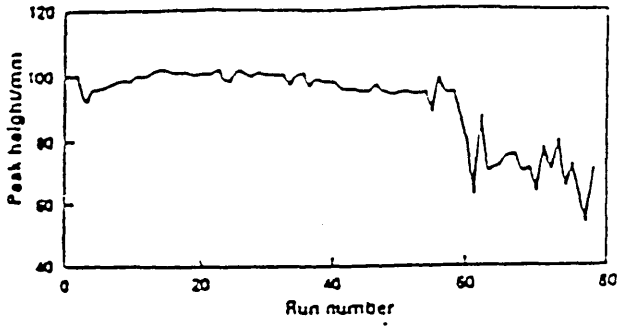


Figure 7: Variation of peak height with run number with a physical moisture trap.

To overcome this problem, the gas carrier needs drying before reaching the detector. A few methods have been used previously using mainly physical moisture traps such as silica gel. However this method is not very satisfactory as silica gel tends to saturate very quickly, after about an hour i.e. at about sample number 58 (Figure 7).

The dryer (Perma Pure drying cell) used was placed between the gas/liquid separator and the detector and consists of two concentric tubes, the outer made of PTFE, the inner one a hygroscopic Nafion membrane. The carrier gas (wet gas) passes through the inner tube, the moisture is then removed into the outer tube where it is dried by a drying gas blowing in the opposite direction to the wet gas (Figure 8). The effect of this drying cell is shown on Figure 9.

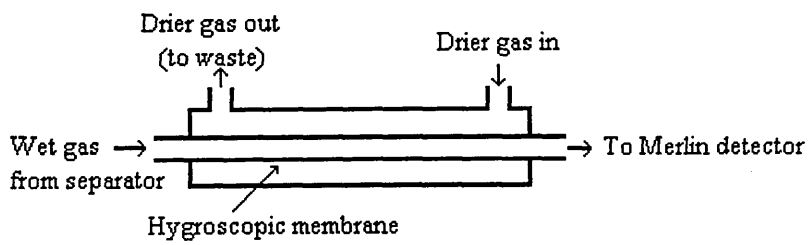


Figure 8: The Perma Pure drying cell.

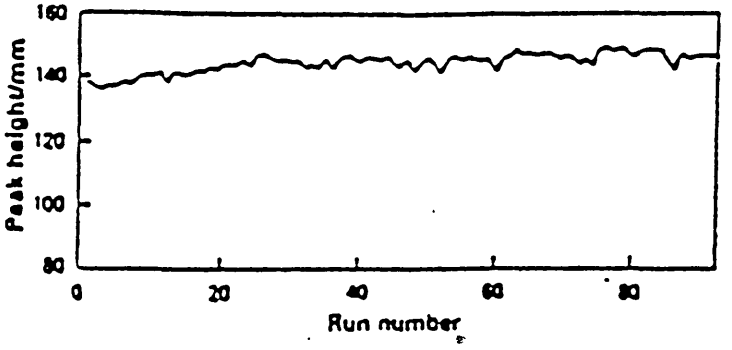


Figure 9: Variation of peak height with run number with the perma pure drying cell.

This method offers excellent stability and in the long term, enhances the reproducibility of the method.

4.1.4.2. Drift of the instrument

During the course of these experiments, it was observed that the instrument had a tendency to drift with time (Figure 10). The nature of the drift has since been studied (57), and it is recommended that the measurements are carried out 3-4 hours after the instrument is first switched on in order to obtain a stable baseline.

Figure 10 shows the effect of the drift on a 5 ng/mL standard analysed 25 times.

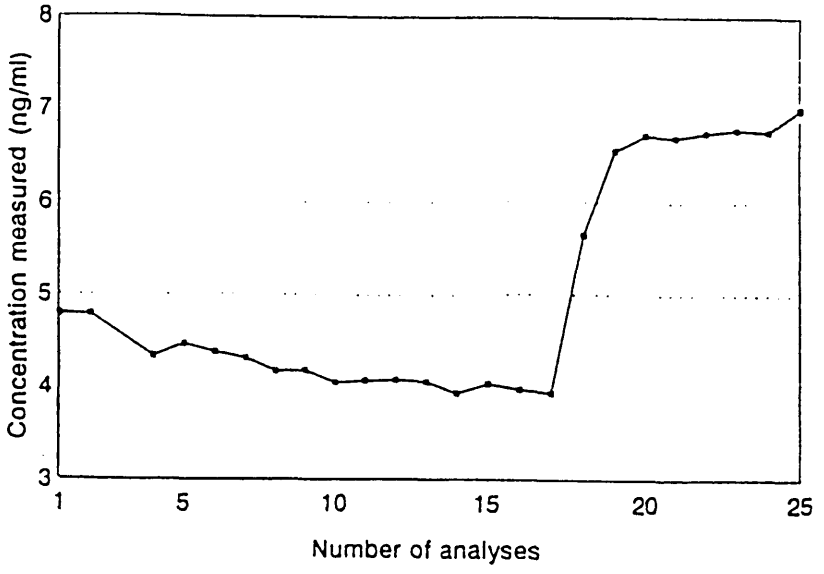


Figure 10: Variation of the instrumental response with time.

The cause was believed to be due to instability in the mercury discharge lamp.

4.1.4.3. Accuracy and precision of the instrument:

The detection limit of this instrument is 10 $ng.L^{-1}$ and the coefficient of variation is only 2% at $ng.mL^{-1}$, at levels which this work was performed. These values were calculated from the standard deviation on 10 measurements of a 50 $ng.L^{-1}$ solution.

Recoveries for a soil certified reference material (C74-05) of 101.6 ± 4.4 % were obtained for soil certified reference material containing 294 ng/g mercury, on 11 samples analysed on the same day (Table 14).

Table 14: Results obtained for modified digestion C

Sample	Expected Concentration ($ng.g^{-1}$)	Measured concentration ($ng.g^{-1}$)
1	2.951	3.138
2	3.535	3.820
3	2.94	3.093
4	2.948	2.960
5	3.098	3.151
6	2.993	3.044
7	3.108	2.949
8	3.125	3.040
9	3.047	3.098
10	3.088	2.995
11	3.086	2.971

Concentration (µg/L)

4.1.5. Applications:

The results obtained for 50 samples collected upstream and downstream of a chemical plant (Staveley chemicals) are presented in Figure 11. This company used an chloroalkali process until recently. Maps, sampling method, storage conditions are detailed in section 3.3.1.

Normal levels of mercury in clean sediments are 0.05, 0.1-1 and 0.4-2.7 pg.g"1 (dry weight) in lake, oceans and river respectively (58). Samples 1 to 18 contain between 5 and 100 pg.g'1 total mercury. This indicates higher levels of mercury than usually found in clean sediments but these values are still acceptable as levels as high as 290 pg.g'1 can be found in some sediments (59). We can conclude that there is no evidence of pollution upstream from the chemical plant. However, if we refer to the site maps (pages 44-47), we can see that higher levels of mercury are present at the level of the plant (Samples 19-20) as 66 % of the samples have a concentration higher than 500 pg.g"1 with sample A as high as 3405 pg.g"1. But the highest levels of mercury are found downstream (samples 23-45). This could indicate a shift ot the contaminated sediments along the river.

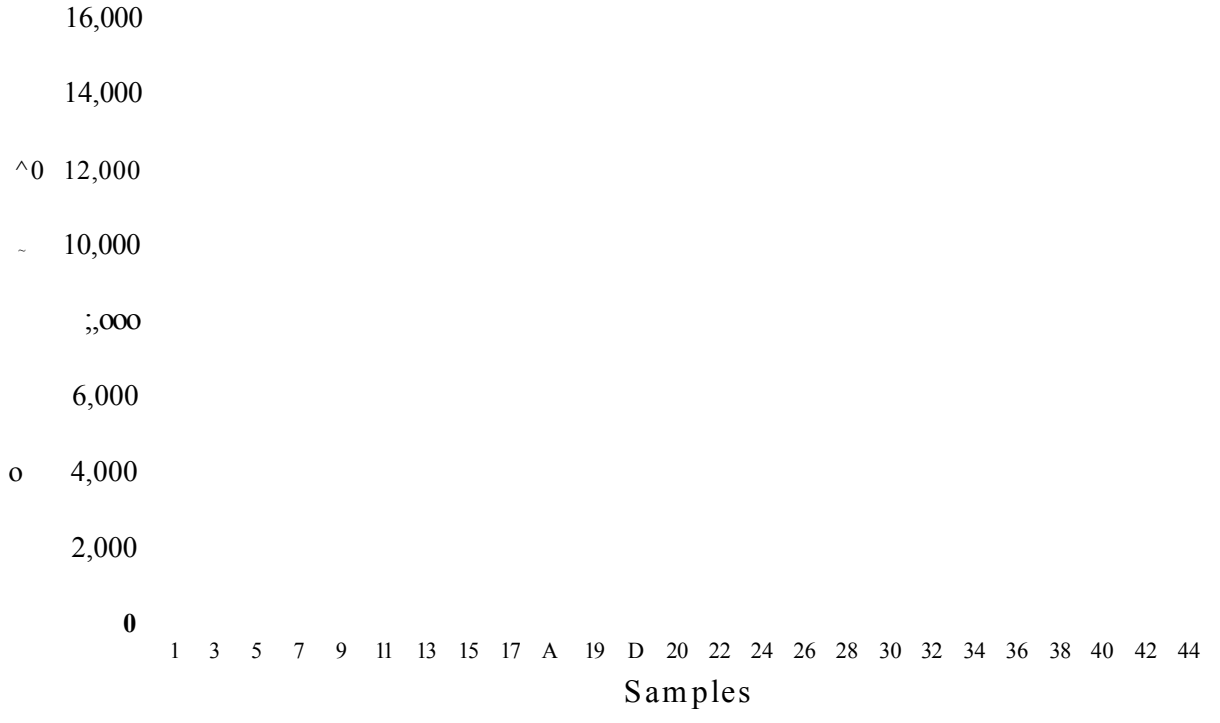


Figure 11: Analysis of 50 samples from River Rother (Chesterfield-Rotherham)
(Curve l(blue line): data obtained in 1995, Curve 2 (red line): data obtained in 1988) (60)
These results are obtained after digestion of the sediments by modified method C.

The hypothesis that the sediments have translocated along the river can be verified by using previous results, obtained in 1988 by Murfin (curve 2, Figure 11)(60). Samples from the same points had been analysed after a similar oxidative open digestion as method A, by CV-AAS. As shown previously, this method does not allow complete recovery of mercury and the results obtained in 1988 are lower than the results obtained after digestion following modified method C. However, even if the levels of mercury cannot be directly compared, the results can be used as a reference to evaluate if the levels of mercury are at the same geographic points in 1995 as in 1988. If translocation of sediment has taken place, the process is very slow as only the last samples present a significant difference (Samples 40-45) (figure 11).

The reproducibility of the method was illustrated by the analysis of 3 samples (1, 25, and 42) at 4 consecutive times over 2 weeks (Table 15).

Table 15: Reproducibility of the results.

Sample	Concentration ($ng.g^{-1}$)	Mean Concentration ($ng.g^{-1}$)	Relative Standard Deviation (%)
1	109.64 110.54 113.00 110.2	110.84	1.3
25	2156.42 2410.50 2373.95 2290.88	2307.93	4.8
42	1198.51 1204.65 1206.45 1202.92	1203.13	0.28

Modified digestion C offers a quick, efficient and reliable method for the determination of total mercury in soil and sediments. Coupled with Atomic Fluorescence detection, it allows simple and effective routine analysis in a laboratory environment. The method is reliable, reproducible and achieves a very low detection limit.

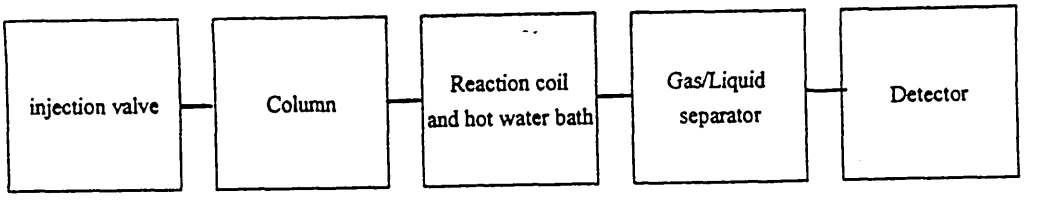
4.2. Mercury speciation

The second part of this work looks at the speciation of mercury and more particularly at the separation of methylmercury and mercury in sediments. The aim was to be able to develop a qualitative and quantitative analysis of mercury and its organo compounds. Firstly, we looked at developing a reliable method of analysis for the determination of methylmercury and mercury, together or separately. Then a different extraction method was followed allowing the quantitative analysis of these two compounds in sediments.

4.2.1. Method development

4.2.1.1. Equipment

The HPLC system is made of different stages, all having an important impact on the final step which is detection. The High Performance Liquid Chromatography - Atomic Fluorescence Spectrometry (HPLC-AFS) kit includes:



Each of these steps have been studied in order to obtain the best signal possible.

The Column: The choice of a column is very important as it is the key to a good separation. Methyl mercury and mercury have atomic weight lower than 300, they are water soluble, and have a low polarity. These characteristics can help us to determine the type of column to use. The separation mechanism which seems the most suitable is reverse phase chromatography and the packing used in the column was a C_{18} loaded silica (5 μm)

Three different columns were tested (Table 16). The main considerations were the time of elution of mercury and methylmercury peaks and the separation.

Table 16: Retention times and resolution obtained for three different columns.

Column Type	Mobile phase	Retention time	Separation time
S5 ODS1 (low carbon load)	5% Methanol	MeHg: 10 min Hg: 12.22 min	2.22 min
S5 ODS2 (high carbon load)	5% Methanol	MeHg: 13.66 min Hg: 16.57 min	2.91 min
Hypersil (medium carbon load)	4% Methanol	MeHg: 19.77 min Hg: 24.85 min	5.08 min

The valve: The valve used was a Rheodyne (USA) type 7000 and allows the injection of different volumes by changing the injection loop size.

The reaction coil: Its role is to allow methyl mercury to be degraded into Hg(II) in order to be detected by atomic detection. By varying the length of the coil, we vary the time of the reaction taking place in it. The longer the coil, the longer the time taken by the compound to go through. However, the length of the coil could also affect the resolution of the separation and the presence of two compounds at the same time in the mixing coil is to be avoided. Figure 12 shows the effect of coil length on signal. The optimum length was of the order of 50 cm.

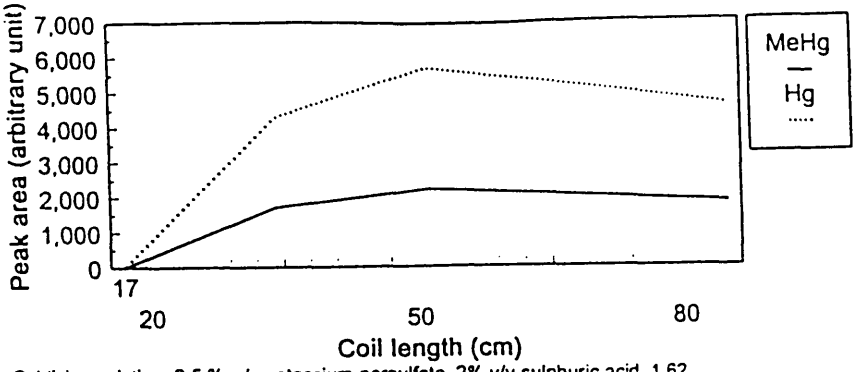


Figure 12: Effect of the reaction coil length on mercury and methyl mercury signals

Hot water bath: The temperature of the reaction coil was also found to have a significant effect on signal intensity. As a consequence, the reaction coil was placed in a hot water bath. The temperatures tested did not exceed 88° C as this was the maximum attainable temperature for this bath. To see the effect of temperature on the signal, a solution containing mercury and methylmercury was injected at different temperature. The results are presented in Figure 13.

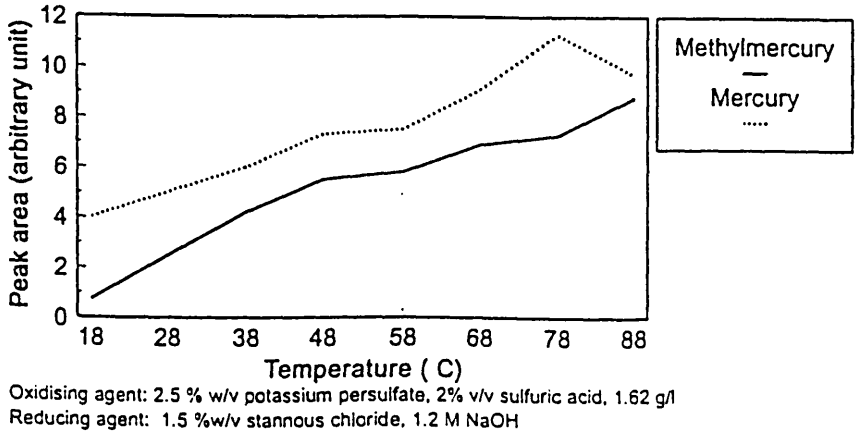


Figure 13: Effect of temperature on mercury and methyl mercury signals

From this graph, we can see that mercury signal is also effected by temperature as it increases in the same way as methylmercury signal with temperature. This is believed to be due to solution being warm when it arrives in the gas/liquid separator. Hence this may increase the efficiency of the reduction reaction and affect mercury detection.

A temperature of 88 °C was adopted for use.

Flow rates: When the oxidising and reducing agents were mixed together, a precipitate appeared in the gas/liquid separator, blocking tubes prior to it and reducing the efficiency of the reduction reaction. It was found that by varying the flow rates of the peristaltic pumps, this precipitation reaction could be significantly reduced. This determined the set-up for the pumps. This was adjusted on a day to day basis but it was found that the ideal ratio reducing agent/oxidising agent was of about 10 to 1. The optimal conditions were determined once the solutions were optimised.

4.2.1.2. Solution testing:

The solutions used at first were solutions used by Hintelmann and Wilken (55), his system being similar to ours.

Oxidising agent: The effects of the concentration of potassium persulphate as well as concentration of the copper sulphate on the signal were studied (Figure 14).

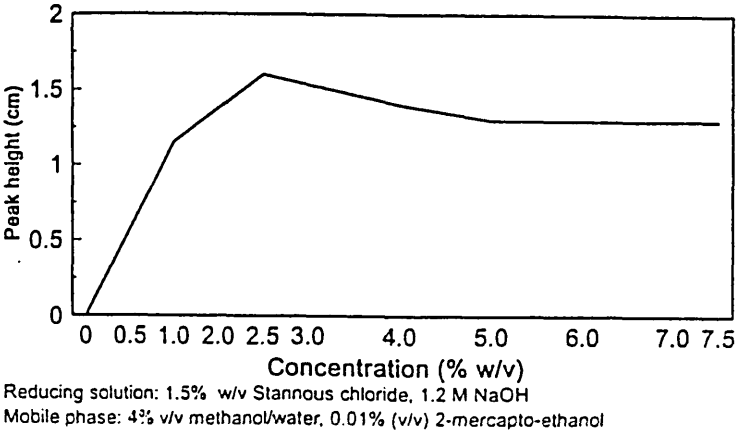
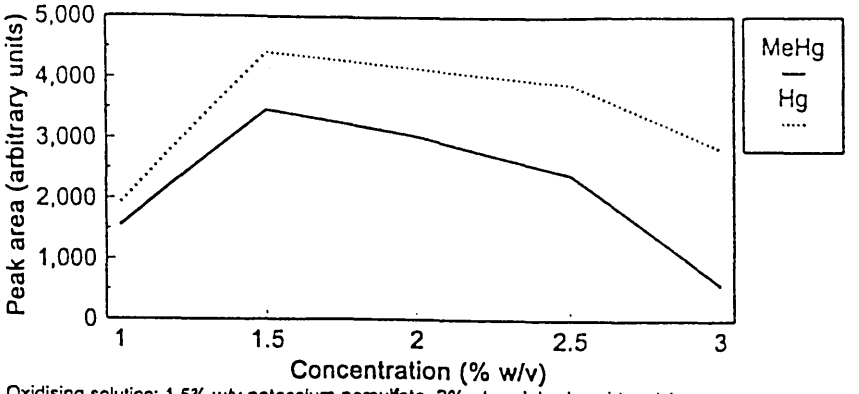


Figure 14: Effect of potassium persulphate on methyl mercury signal

The sulphuric acid was found to be necessary mainly to help the dilution of potassium persulphate in water. It has little effect on the signal intensity. The optimal concentration for oxidising agent in these conditions was 2.5 %w/v.

Reducing agent: The effects of stannous chloride concentration on methyl mercury signal was tested (Figure 15). The optimal concentration for the reducing agent in these conditions was 1.5 %w/v.

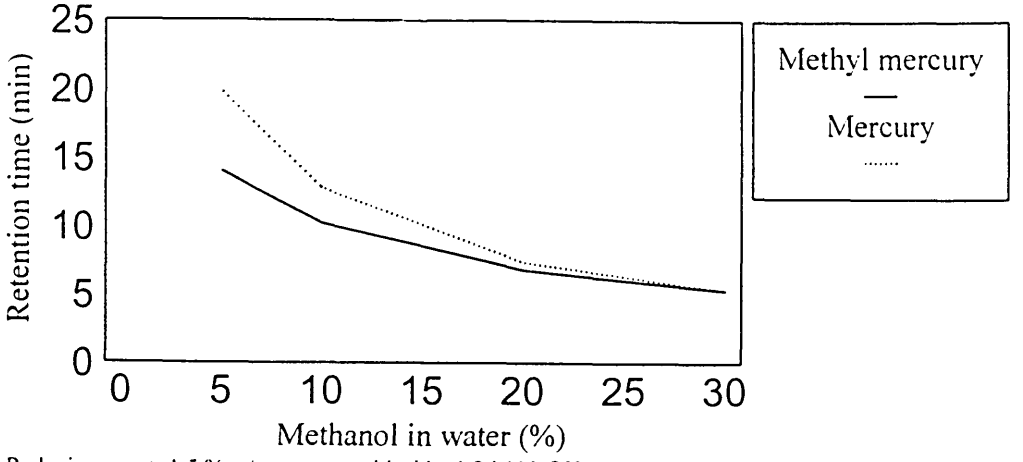


Oxidising solution: 1.5% w/v potassium persulfate, 2% v/v sulphuric acid and 1.
Mobile phase: 4% v/v methanol/water, 0.01 % 2-mercapto-ethanol

Figure 15: Effect of stannous chloride on mercury and methyl mercury signals

Mobile phase: Methanol proportion in water was tested to obtain the best separation as possible (Figure 16), methanol had an effect on the retention time whereas 2-mercapto-ethanol had an effect on peak intensity. When no 2-mercapto-ethanol was present in the mobile phase none of the peaks for mercury or methyl mercury appeared.

To obtain these results, mercury and methylmercury were injected separately and the retention times compared. When methanol concentration was of about 30% v/v, the retention times measured were exactly the same. For lower levels of methanol (10-20%v/v), the retention times were different but when both compounds were injected at the same time, the peaks could not be resolved. The optimal proportion of methanol in the mobile phase was between 2.0 and 10% v/v.



Reducing agent: 1.5 % w/v stannous chloride, 1.2 M NaOH
Oxidising agent: 2.5 % w/v potassium persulfate, 2 % v/v sulfuric acid and 1.62 g/l copper sulfate

Figure 16: Effect of methanol on retention times

4.2.2. Performance of the instrument:

4.2.2.1. Resolution of the column:

The resolving power and sensitivity of the column were calculated using the general equations [1] and [2]. The resolution can be affected by the mobile phase composition. It is recommended to decrease the amount of methanol in the mobile phase when carrying out quantitative experiments as the time of elution is increased. However when time is more important than resolution, the amount of methanol should be increased.

Equation [1]: $R = 2\,[(t_{Rb}-t_{Ra})/(w_b-w_a)]$

Equation [2]: $N = 16\,(t_R/W_b)^2$

Where t_R = Retention time

w = Peak width

R = Resolution

N = Efficiency (Number of theoretical plates)

Column Type	Resolution power, R	Efficiency, N
S5-ODS1	25	132
S5-ODS2	28	146
Hypersil	67	183

4.2.2.2. Calibration:

Calibration curves are needed for both methyl mercury and mercury. To get the best separation possible, a mobile phase containing only 4 % of methanol was used. The analysis time was also increased but it avoided any overlapping of the peaks at high concentrations. Figures 16a, b and c show the curves obtained.

The calibration curves obtained were for standard solutions containing between 5 and 50 ng/mL. Over this range, the calibration is linear and the slope 0.99876 (+/-0.0001).

Figure 16a: Calibration curve 0-50 ng.g-1

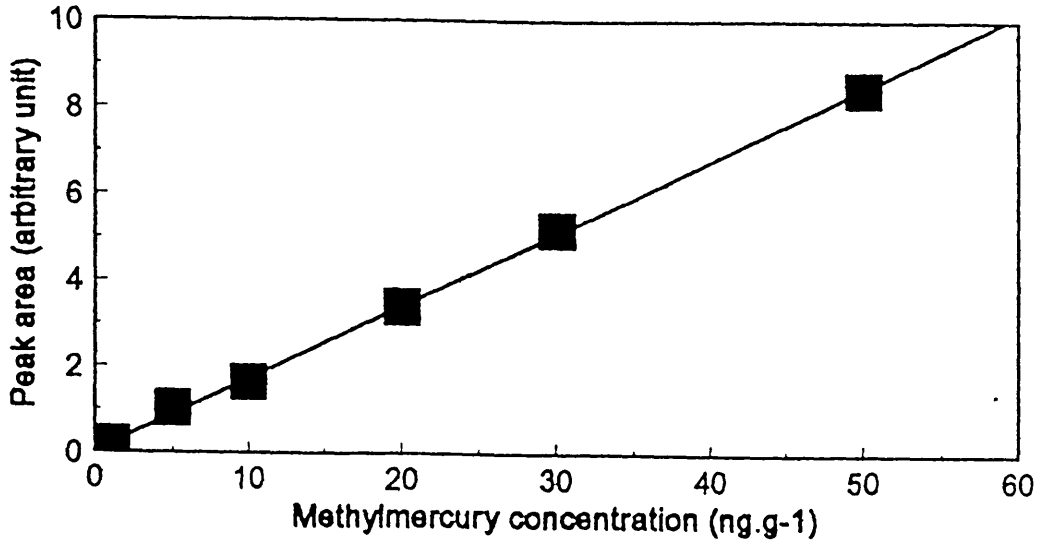


Figure 16b: Calibration curve 0-10 ng.g-1

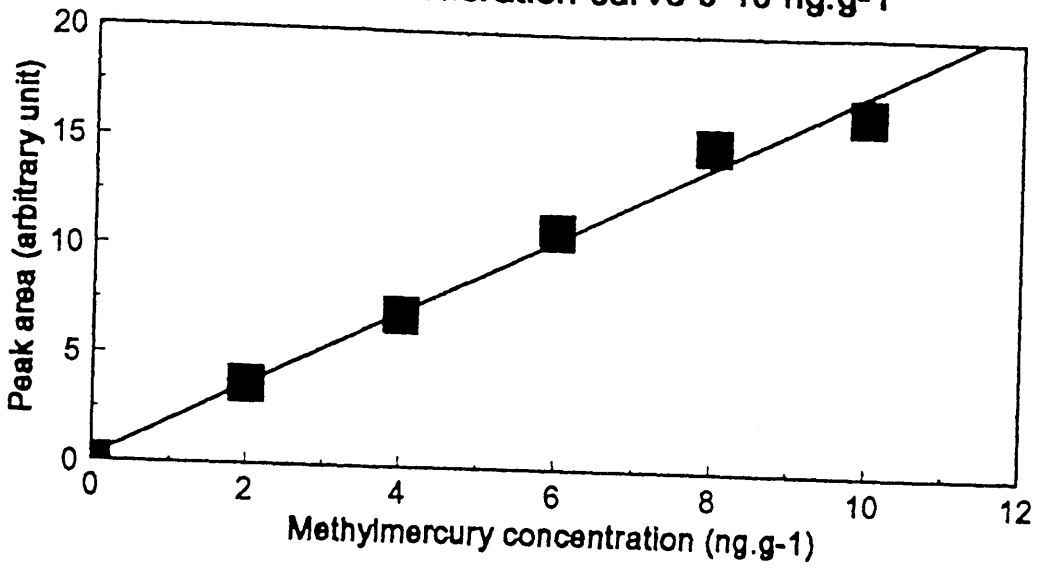
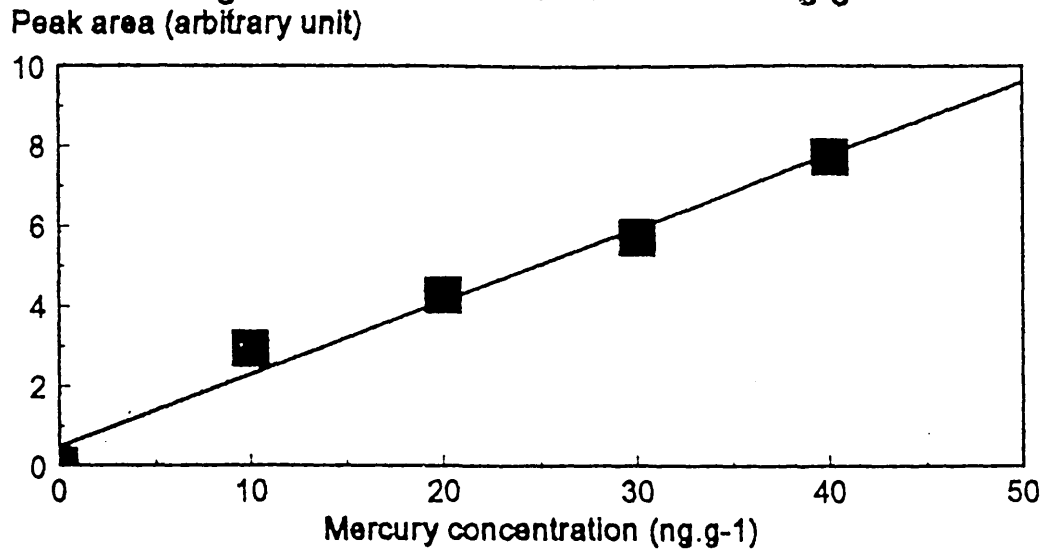


Figure 16c: Calibration curve 0-40 ng.g-1



4.2.2.3 Reproducibility:

The same standard was injected 5 times and the standard deviation was calculated from the results obtained (Table 17).

Table 17: Reproducibility

	Standard Deviation	(%)
Standard (ng/mL)	Methyl mercury	Mercury
10	9.8	2.3
30	8.0	4.4
50	4.9	4.6

4.2.3. Sample Extraction:

All methyl mercury must be extracted without being oxidised to Hg(II) and if possible, all the inorganic mercury must also be extracted. Two methods were tested, one using solvent extraction (Method 1) and the other using acid leaching (Method 2).

4.2.3.1 Solvent extraction:

The main problem encountered was that the mercury must be in an aqueous phase to be injected onto the column and to react with the aqueous reagent. The extractant used was dithizone in chloroform and the organic phase was not soluble in water. As a consequence there was a need for a further step allowing mercury to be in an aqueous phase. For this, the organic phase was separated and evaporated in a gentle stream of nitrogen gas. The mercury was then re-dissolved into water.

The results obtained showed that methyl mercury was extracted and separated from mercury (Figure 17).

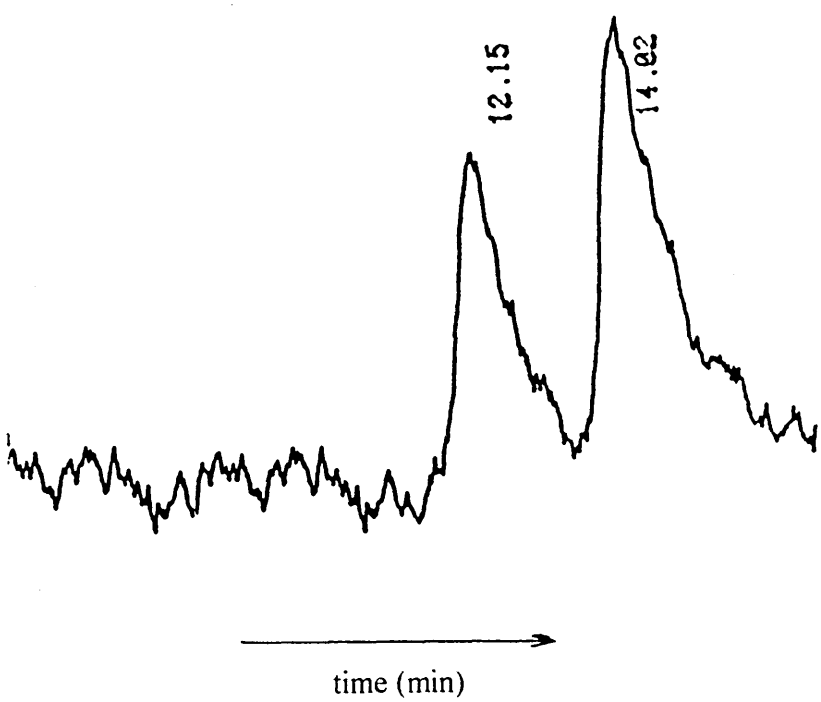


Figure 17: Chromatogram obtained for a sediment sample.

4.2.3.2. Acid leaching:

This extraction method is very simple and allows the organo-mercury compounds to be obtained directly in an aqueous solution. However as the pH is very low, the sample can be injected onto the column only after being diluted in a basic solution to bring the pH to a value > 4.0.

Different aspects of this method have been studied to improve extraction efficiency, the final objective being the quantitative analysis of organo-mercury compounds.

Firstly the time of reaction was investigated. The shaking time during the extraction procedure was varied between 10 and 75 min. All samples were of about 1g and extracted with 5 mL of extracting solution.

Table 18: Effect of shaking time on the recovery of methyl mercury.

Number of Aliquots	Time (min)	Recovery (%)
5	10	60
3	15	49
3	30	41
3	45	51
3	75	50

The results do not show any consistency and the time of reaction did not seem to have a very important effect on the recovery of methyl mercury. The next step was to study the amount of sample and the amount of extractant needed.

Firstly, it was found that the optimum conditions for the recovery of methyl mercury from fish muscle are 0.5 g of material and 10 mL of extractant, shaken for 15 min. The recoveries obtained were consistently of about 80 % for 5 samples. For a sample weight of 1g, the recovery was only of 28 %. This can be explained by the extracting solution being saturated before the extraction is complete.

However, as these results were not reproducible in sediment samples, a slightly different approach was taken. To make sure that as much as possible of the mercury was extracted, the samples were extracted several times (between 1 to 3 times) and the amount of mercury left in the slurry was analysed after they had been digested using modified method C (see determination of total mercury).

The lowest content indicates the best extraction method as we measure the amount of total mercury left in the sediment after extraction and it means that the lower the levels found in the extracted sediment, the higher the levels in the extractant.

Table 19: Mercury content of the slurry after extraction.

Number of extraction	Mercury content ($ng.g^{-1}$)
1	3757
2	2727
3	4087

Finally the method proved to be of poor reproducibility. Mercury and methyl mercury were actually separated (Figure 18) and the proportions observed between the mercury and methyl mercury peaks are closer to the theory (MeHg= 1.5 % of total mercury present in sediment) than for those obtained by method 1.

Although this method does not allow quantitation at this stage of development, it offers a quick and rapid alternative for qualitative analysis of organo mercury compounds in sediments and hence might be suitable as a screening method to identify sediments of interest.

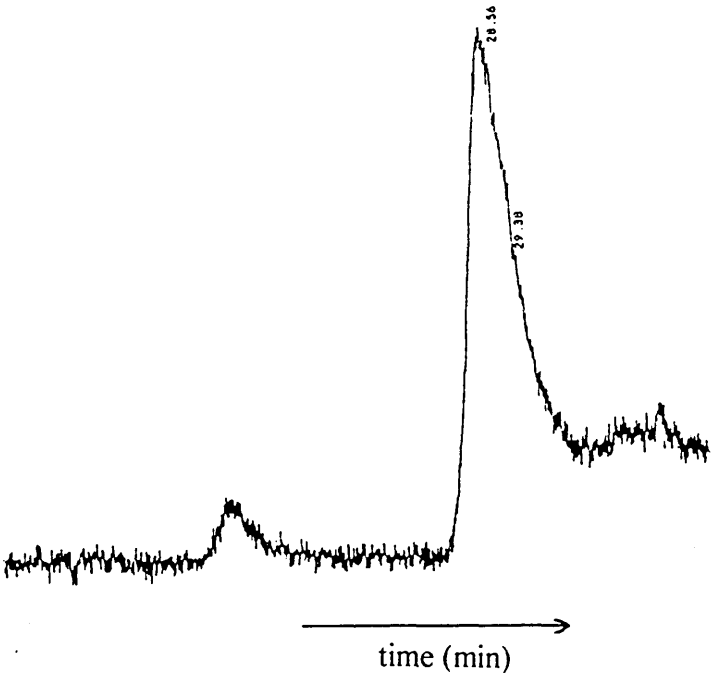


Figure 18: Chromatogram obtained after extraction method 2 for a sediment sample.

To conclude, in this case, the acid leaching method is better than solvent extraction as it is easier, simpler and faster. However none of the methods allow quantitation at this stage of development.

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60. R.N. Murfin. An investigation into the effectiveness of environmental legislation with reference to the persistence of mercury in the River Rother. 1988 (Unpublished SCP dissertation).

In this study, A method based on microwave digestion of soils and sediments followed by the determination of total mercury by atomic fluorescence spectrometry has been developed and optimised. The method is fast, accurate, simple and sensitive. The microwave digestion procedure requires two manipulation steps: the microwave digestion and an oxidation step for organically rich sediments. The microwave digestion step is completed in 27 minutes and the oxidation step up to 2 hours. This means that up to 30 samples can be prepared and analysed in one working day. Atomic fluorescence spectrometry is a very sensitive mercury detection technique and no preconcentration step was required. As the instrument is fully automated, work can be carried overnight, and the analyst is not bound to the instrument.

50 sediments samples were analysed using this method. They were taken from a depth of approximately 30 cm. Three to four samples were taken from the same sampling site (1 m^2) in order to insure that the sample was representative of the area sampled. Each sample was dried, fine ground by hand, sieved and placed in an air tight bag. They were stored in a cool, dry and dark room until analysis. Once digested, the samples could be stored for up to one week in air tight glass containers at 4°C.

In the second part of this study, a speciation method using chromatographic separation and atomic fluorescence detection was developed for the analysis of mercury in sediments. High performance liquid chromatography was chosen for its resolving power and also because it allowed easy interface with the atomic fluorescence detector. The chosen column was C_{18} loaded silica (5 μ). The pH of the solutions (mobile phase and samples) going through the column was of 5.5. After separation, the sample was oxidised in a 50 cm long mixing coil placed in a hot water bath at 88°C. The solutions were optimised in order to obtain the best separation and the best signal as possible. This system allowed the separation and quantification of methyl mercury and mercury from standard solutions. It also allowed the qualitative analysis of sediment samples. Mercury species had to be extracted from the sediment matrix and two methods were investigated: solvent extraction, using dithizone and chloroform, and acid leaching. None of them allowed quantitative analysis but acid leaching method seemed more promising. It was less time consuming, more reproducible, and the results obtained agreed better with theory.

This work covered the separation and detection aspects of the analysis of organo mercury compounds by high performance liquid chromatography - atomic fluorescence spectrometry. The main area of work to be further developed is the preparation of sediment samples in order to obtain quantitative as well as qualitative results. It would also be interesting to determine which factors affect methyl mercury determination such as organic enrichment and sediment composition.

Further work could also cover the investigation of dimethyl mercury presence in both fresh samples and in the same samples after a few weeks storage.

1. Conferences:

I have attended to the following conferences and symposiums:

The Royal Society of Chemistry Annual Chemical Congress, University of Liverpool, 12-15 April 1994

International Conference on Environmental and Biological Aspects of Main-Group Organometals, ICEBAMO 94. University of Bordeaux (France), 6-9 September 1994.

Research and Development Topics in the Analytical Chemistry Meeting, The Royal Society of Chemistry, Analytical Division. University of Hull, 10-11 July 1995 where I presented a poster: "Chemical Speciation of Mercury in Sediments by HPLC-AFS" in collaboration with Dr.Gardiner and Prof.Cooke.

Pre-Doctoral Chemistry Symposium, Autumn Meeting of The Royal Society of Chemistry, University of Sheffield, 5 September 1995 where my poster was presented.

2. Training:

I have attended a course on word processing (Word) and use of spreadsheet (Excel) as part of my degree.

I have helped to supervise laboratory classes in Physical Chemistry and general chemistry.