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Speciation of mercury in different environmental compartments. Design, development and optimization of analytical methods and procedures

Makiese, Julien Lusilao

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CHAPTER 1

INTRODUCTION

1.1. General Background

"Mercurial, the metaphor for volatile unpredictable behaviour, aptly reflects the complexities of one of the most insidiously interesting and scientifically challenging biochemical cycles at the Earth's surface" (Fitzgerald and Lamborg, 2004b). Mercury is the 67th most abundant element of the naturally occurring elements in the Earth's continental crust (Krauskopf and Bird, 1995) and it is highly concentrated by factors of 10^4 and 10^5 in low–high grade sulphide ore deposits (Perelman, 1977). The Earth's surface consists of more than 25 mercury containing minerals (Schroeder and Munthe, 1998).
Elemental mercury (Hg) occurs as a silvery liquid at room temperature but its gas phase is important, since elemental mercury and, to a lesser extent, some of its compounds have relatively high vapour pressures.

Mercury is a chalcophile and significant deposits are found in mineralised regions characterised by relatively recent subduction associated with magmatism and deep focus earthquakes and continent–continent collision (Schülter, 2000). The traditional applications of mercury and its ore cinnabar (HgS) like in silvering mirrors, preserving wood and in medicine have been well known in some cases for thousands of years. Mercury products include thermostats, catalysts, electrodes, batteries, switches, fluorescent lighting and pesticides and its applications range from chlor-alkali production, gold and silver refining and dentistry to pharmaceuticals including as preservatives in vaccines.

Mercury enters the atmosphere as a result of natural events such as volcanic eruptions, or anthropogenic activities, such as mining, chlor-alkali production and the combustion of fossil fuels, especially coal. The most commonly used model for the Hg cycle in the environment (Mason et al., 1994) suggests that the natural cycle of mercury has been seriously perturbed by human–related emissions and that most of the mercury in the atmosphere and surface of the oceans is now anthropogenic.

Mercury is persistent, bio-accumulative, and toxic especially its organic species (monomethylmercury (CH_3Hg^+) and dimethylmercury ($(CH_3)_2Hg$). Because of these properties, it poses potential human health risks, especially for pregnant women, developing foetuses, and young children. Mercury is also toxic to wildlife, especially fish, birds, and far-bearing mammals that consume organisms contaminated with mercury. Human exposure to mercury occurs almost exclusively through fish consumption (USEPA, 1997). Today mercury is considered as a chronic pollutant problem in our environment (Anderson et al., 2006).

Although mercury pollution has probably peaked or even diminished in many developed countries, mercury pollution from developing nations, particularly in Asia has probably increased (Pacyna and Pacyna, 2002).

1.2. Problem statement

In international monitoring programmes, ongoing measurement of heavy metals is becoming important in the production of basic data. The data are used for validation of transport models which estimate the atmospheric input of heavy metals to the environment.

Mercury pollution is a world-wide problem requiring attention at global, regional and national levels. In recent years, there has been growing interest in studying the environmental turnover of mercury species, and analytical methods have been developed for determination of inorganic and organic mercury forms in different environmental matrices (Haraldsson et al., 1994).
In order to evaluate environmental contamination, impact and constraints on new regulations are on mercury and other metals, there is a great and urgent requirement to develop mercury analysis and speciation methods.

While there appears to be substantial sources of mercury input in Southern Africa, the amount of mercury emissions in the region is unknown (Crouch, 2006, SAMA). In addition, the fate of mercury pollution in ecosystems (biological, geological and atmospheric), and its consequences in terms of ecosystem services and human health appear to be highly underestimated in Southern Africa.

South Africa is one of the world's largest producers of coal, mining more than 200 Mt of coal per annum (Wagner and Hlatshwayo, 2005). Combustion of fossil fuels and the incineration of waste materials are estimated to account for 70% of the total mercury in the atmosphere from global human activities. Average mercury levels in South African coals are equivalent to the global average (~0.2 mg kg^{-1}). With 75% of South Africa's primary energy needs being satisfied by coal and more than 90% of the electricity derived from coal-fired power stations, coal combustion may be a significant source of mercury emissions. Measurements to assess the extent to which fossil fuel combustion or waste incineration contributes to mercury releases and impacts to the environment are needed throughout South Africa (Leaner, 2007).
A significant amount of coal is also burned for cooking and heating in poorer households in South Africa. This increases in winter with concomitant increases in

atmospheric particulates (and associated mercury). This has also been observed in other developing countries although it has not been extensively studied.

The formation of methylmercury, the most toxic form of mercury in the environment, can also be enhanced when large dams are constructed (known as the "reservoir effect").
The effects can remain for up to 25 years in, for example, fish with high methylmercury levels. Large dams in South Africa provide most of the water for drinking and for energy generation. Many are also used for recreational and subsistence fishing. High mercury levels have been found in catfish cultured with water from the Krugersdrift Dam in Bloemfontein (Leaner, 2007).

Small-scale artisanal gold mining in southern Africa (and in the rest of Africa) is also of concern. Gold extraction relies on amalgamating the gold with liquid mercury. This process results in much mercury being released into the environment. This technique is still illegally used in certain areas in South Africa, the extent of which, and their associated impacts, are unknown.

In 2006 it was reported that mercury emissions in South Africa were second after China (Pacyna et al., 2005), contributing more than 10% of global mercury emissions. This was reported mainly from coal combustion (releasing the mercury that occurs naturally in coal) and gold mining. Mercury emission estimates were based on the total amount of coal burned in all power plants per year (112.3 Mt y^{-1}), the mercury content of South African coals and the emission control devices used in each power plant. Some doubt exists about the validity of these figures, and a more detailed national study is required to clarify this.
A recent study done by Dabrowski et al. (2008) indicates that mercury emissions arising from South Africa's coal-fired power plants (ranging between 2.6 and 17.6

tonnes y^{-1}, with an estimated average emission of 9.8 tonnes y^{-1}) are significantly lower than suggested in the literature (approximately 50 tonnes y^{-1}).

Mercury research in South Africa is practically non-existent and only a few fragmented measurements present total mercury concentration among other elements (Leaner, SAMA 2005). Most studies have been in reaction to emergency incidents relating to mercury. The effluent spill into the Mngceweni River in KwaZulu-Natal during the late 1990's is one example (Leaner, 2007). A detailed study needs to be done on a larger scale. Unfortunately, there is no laboratory well developed for mercury speciation in South Africa. Previous works on mercury speciation in South Africa were done in overseas laboratory (CNRS-LCABIE-France or Mason research group, Connecticut, USA) (Nsengimana, 2007; Mason, 2007).

When it was implied that South Africa was the world's 2nd worst polluter in terms of mercury emissions, local scientists could not confirm or deny the claims since the nature and extent of mercury pollution and its impacts in South Africa have not been extensively studied (CSIR e-News, 2007).

Based on the limited understanding of the biochemical and physical associations, the standard analytical procedures are not sufficient for complete and reliable monitoring of mercury in complex matrices, such as coal ash or biological samples (hair, fish, etc.). In order to assess the environmental impact of mercury pollution, adequate information concerning its speciation is thus required.

CHAPTER 2

LITERATURE REVIEW

2.1. Chemistry of mercury

Mercury can exist in three oxidation states: Hg^0 (metallic), Hg_2^{2+} (mercurous), and Hg^{2+} (mercuric- Hg(II)). The properties and chemical behavior of mercury strongly depend on the oxidation state. Hg_2^{2+} and Hg^{2+} can form numerous inorganic and organic chemical compounds; however, Hg_2^{2+} is rarely stable under ordinary environmental conditions. Mercury is unusual among metals because it tends to form covalent rather than ionic bonds. Most of the mercury encountered in water, soil, sediments and biota is in the form of inorganic mercuric salts and organomercury compounds. Organomercury compounds are defined by the presence of a covalent C-Hg bond. The presence of a covalent C-Hg bond differentiates organomercury from inorganic mercury compounds that merely associate with the organic material in the environment but do not have the C-Hg bond. The compounds most likely to be found under environmental conditions are: the mercuric salts ($HgCl_2$, $Hg(OH)_2$ and HgS); the methylmercury compounds (CH_3HgCl, CH_3HgOH) and, in small fractions, other organomercury compounds (i.e., dimethylmercury and phenylmercury).

Mercury compounds in the aqueous phase often remain as undisassociated molecules, and the reported solubility values reflect this (Table 2.1). Most organomercurics are not soluble and do not react with weak acids or bases due to the low affinity of the mercury for oxygen bonded to carbon. CH_3HgOH, however, is highly soluble due to the strong hydrogen bonding capability of the hydroxide group (USEPA, 1997).

The mercuric salts vary widely in solubility. For example $HgCl_2$ is readily soluble in water, and HgS is unreactive due to the high affinity of mercury for sulfur (USEPA, 1997).

Table 2.1: Physical/ Chemical Properties of Mercury and Some of its Compounds (Schroeder and Munthe, 1998)

Property	**Hg**	**$HgCl_2$**	**HgO**	**HgS**	**CH_3HgCl**	**$(CH_3)_2Hg$**
Melting Point (°C)	-39	277	Decomposes > 500	584 (sublimes)	167 (sublimes)	–
Boiling Point (°C) at 1 atm	357	303	–	–	–	96
Vapour Pressure (Pa)	0.180^{a}	$8.99 \times 10^{-3\ a}$	$9.20 \times 10^{-12\ b}$	n.d.	1.76^{b}	8.30×10^{3b}
Water Solubility (g/ζ)	$49.4 \times 10^{-6\ a}$	66^{a}	$5.3 \times 10^{-2\ b}$	$\sim 2 \times 10^{-24\ b}$	~ 5–6^{b}	2.95^{c}
Henry's Law Coefficient (Pa m^3 mol^{-1})	729^{a} $0.32^{b\ g}$ $0.18^{f\ g}$	$3.69 \times 10^{-5\ a}$	$3.76 \times 10^{-11\ b}$	n.d.	$1.6 \times 10^{-5\ e}$ pH = 5.2	646^{b} $0.31^{b\ g}$ $0.15^{d\ g}$
Octanol-Water Partition Coefficient	4.2^{g}	0.5^{g}	–	n.d.	2.5^{g}	180^{g}

Notes

[a] at 20°C; [b] at 25°C; [c] at 24°C; [d] at 0°C; [e] at 15°C; [f] at 5°C; [g] Dimensionless units.

Mercury has seven stable isotopes which occur in the following proportions: ^{196}Hg, 0.15%; ^{198}Hg, 10.0%; ^{199}Hg, 16.7%; ^{200}Hg, 23.2%; ^{201}Hg, 13.2%; ^{202}Hg, 29.8%; ^{204}Hg, 6.8% (Friedlander et al., 1981). The isotopic distribution of mercury in terrestrial and extraterrestrial material is very similar (e.g. Allende meteorite: 0.03–0.3ppm; Lauretta et al., 2001) but to date there have been relatively little success in using mercury isotopes as an indication of magmatic or biological fractionation.

2.2. Mineralogy

There are more than 25 mercury bearing minerals (Schroeder and Munthe, 1998); some of the commonest are listed in Table 2.2. The most common mercury bearing mineral is cinnabar (HgS). Under typical surface water conditions HgS is extremely insoluble (Martell et al., 1998), so that mercury transport from mineral rich environments at the Earth's surface generally involves sediment transport (Ganguli et al., 2000). Concentration of mercury to form ore bodies involves hydrothermal systems in which HgS solubility is controlled strongly by fluid pH, temperature, chloride, sulphide, and organic carbon (White, 1967). High concentrations of mercury also occur in other sulphide ore minerals such as sphalerite ((Zn, Fe)S).

2.3. Abundance and distribution in the environment

2.3.1. Rocks, soils and sediments

A summary of mercury data from the report of Turekian and Wedepohl (1961) is shown in Table 2.3. Due to the chalcophilic nature of its associations, mercury is found in higher abundances in intrusive magmatic rocks and locations of subaerial and submarine volcanism. Peak concentrations in these rocks may be as high as several percent in ore-grade minerals (e.g., 35% mercury in sphalerite; Ozerova, 1996). Also as a result of this association, the distribution of highest mercury concentrations in rocks at the surface and near surface mirrors regions of current and past tectonic activity and has been described as the "global mercury belt" (Fitzgerald and Lamborg, 2004b).

Table 2.2: Mercury-bearing minerals (Jasinski, 1995)

Mineral	Formulae
Arquerite	$AgHg_3$, Ag_5Hg, Ag_2Hg_5
Barcenite	Antimonate of Hg
Bordosite	AgHgI, AgCl·2HgCl
Calomel	HgCl
Cinnabar	HgS
Coccinite	Hg_2OCl
Coccinite (variation)	HgI_2
Coloradoite	HgTe
Corderoite	$Hg_3S_2Cl_2$
Eglestonite	$Hg_2Cl_3O_2H$
Gold amalgam	(Au,Ag)Hg
Guadalcazarite	Hg_5·Zn·Se
Hermesite	Tetrahedrite + Hg
Kalgoorlite	$Ag_2Au_2HgTe_4$
Kleinite	$Hg_2N(Cl,SO_4)\cdot nH_2O$
Kongsbergite	Mercurian Ag
Lehrbrachite	HgSe + PbSe
Leviglianite	HgS + Zn
Livingstonite	$HgSb_4S_7$
Magnolite	Hg_2TeO_4
Metacinnabar	HgS
Montroydite	HgO
Moschellandsbergite	Ag_2Hg_3
Mosesite	$Hg_2N(Cl,SO_4,MoO_4,CO_3)\cdot H_2O$
Onofrite	Selenina metacinnabar
Potarite	PdHg
Schwatzite	Mercurian tetrahedrite
Terlinguaite	Hg_2ClO
Tiemannite	HgSe
Tocornalite	(Ag,Hg)I

Table 2.3: Distribution of mercury in the Earth's crust.

(Fitzgerald and Lamborg, 2004b)

Rock Type	Hg content (mg Kg^{-1})
Ultrabasic igneous rocks	0.0X*
Basaltic rocks	0.09
High-and low-Ca granites	0.08
Syenites	0.0X
Shales	0.4
Sandstones	0.03
Carbonates	0.04
Deep-Sea carbonates	0.0X
Clays	0.X

*: the X notation indicates order of magnitude estimate

New data on mercury distribution in sediments, soils and humus (Table 2.4) has been obtained as a result of work by the Forum of the European Geological Surveys (FOREGS, 2005) group to prepare a geochemical atlas of Europe.

Table 2.4: Statistical data of analytical results from FOREGS* (FOREGS, 2005)

Media	Min.	Max.	Mean	Median	Standard Deviation
Stream sediment	0.003	13.6	0.085	0.038	0.492
Floodplain sediment	0.002	4.39	0.105	0.045	0.28
Subsoil	0.002	0.93	0.035	0.022	0.057
Topsoil	0.005	1.35	0.061	0.037	0.10
Humus	0.022	3.75	0.226	0.202	0.213

* Values are in mg Kg^{-1}

Note: Mercury levels generally increase with decreasing size fraction. Therefore the values for different media cannot be compared directly.

2.3.2 Minable deposits

As mentioned, higher mercury concentrations in rock and soil are associated with the global mercury belt. However, the occurrence of minable deposits is not continuous along this belt. Very high concentrations of mercury have been reported to be associated with oceanic hydrothermal sulfide chimneys and their weathered remains (Fitzgerald and Lamborg, 2004b).

In all of these cases, mercury occurs almost exclusively as cinnabar (red HgS), with smaller amounts of metacinnabar (black HgS) and elemental mercury (often as inclusion with HgS in minerals such as sphalerite and chalcopyrite).

2.3.3. Fossil fuels

Even with the stated stability of cinnabar, mercury is mobile in the surface environment. This is indicated by the relatively high concentration of mercury in organic-rich deposits, such as fossil fuels and shales. As mentioned above, mercury has high affinities for organic carbon as well as sulfide.

With notable exceptions, concentrations in coal appear higher than those in oil, suggesting preferential burial of mercury in terrestrial and coastal systems rather than in pelagic marine environments. Though mercury concentrations are not as high in various refined petroleum materials as in coal, the oil and gas recovery process often liberates large amounts of mercury leading to localized contamination of marine sediments (Grieb et al., 2001). The concentration of mercury is sufficiently high in some crude petroleum materials to also pose a corrosion hazard to the drilling and transportation apparatus and represents a significant engineering problem (Wilhelm, 1999; Bloom, 2000).

2.3.4. Natural water

Mercury distributions in surface waters reflect the magnitude of the atmospheric deposition source and the strength of local removal processes (scavenging and gas evasion) superimposed on water circulation. Thus, concentrations are likely to change seasonally depending on the seasonal extent of atmospheric deposition, evasion and removal of mercury by particulate sinking. Also, concentrations vary between ocean basins due to differences in the relative amount of mercury in deposition, and other factors. Overall, it appears that surface water concentrations are higher in the Atlantic Ocean than in the Pacific Ocean surface waters (Gill and Fitzgerald,1987; Cossa and Fileman, 1991; Cossa et al., 1996, Mason and Fitzgerald, 1993; Mason et al., 1998; Mason and Sullivan, 1999; Mason et al., 2001).

Overall, there is data from only a few cruises on which to base these generalizations. In addition, in earlier studies, measurements were made only for "reactive mercury", which represents the fraction of the mercury which is reducible by tin chloride ($SnCl_2$).

Deep ocean water concentrations tend to decrease along deep water flow paths between the Atlantic and Pacific (See figure 2.1). Hence, their distributional features are often dominated by their point of entry and removal in the ocean. For example, surface water concentrations of mercury are typically elevated compared to deep waters (Fitzgerald and Mason, 1997), reflecting the atmosphere as a dominant input source of mercury to the ocean. The distribution of mercury differs from that of other particle-reactive metals because of its propensity to be reduced in surface waters to Hg^0, with resultant loss to the atmosphere by evasion. This re-emission to the atmosphere tends to reduce the imprint of the atmosphere in some locations and decreases the overall amount of mercury transported to deeper ocean waters (Mason and Gill, 2005) as it is shown in table 2.5.

Table 2.5: Range of mean concentrations of Hg and MeHg in subsurface water at different stations of the world ocean (Source: Mason and Gill, 2005)

Location	**Hg_T (pM)**	**MeHg (fM)**	**% MeHg**
South and Equatorial Atlantic	0.8-2.4	25-200	5-10
North Atlantic	2.4 ± 1.6	29-160	2-7
North Pacific	1.2 ± 0.9	<50	<4
Equatorial Pacific	0.5-4.0	35-670	2-15
Mediterranean	0.5-4.0	20-460	1-35

Note: Hg_T: Total Hg; MeHg: methylated Hg including MeHg and Me_2Hg

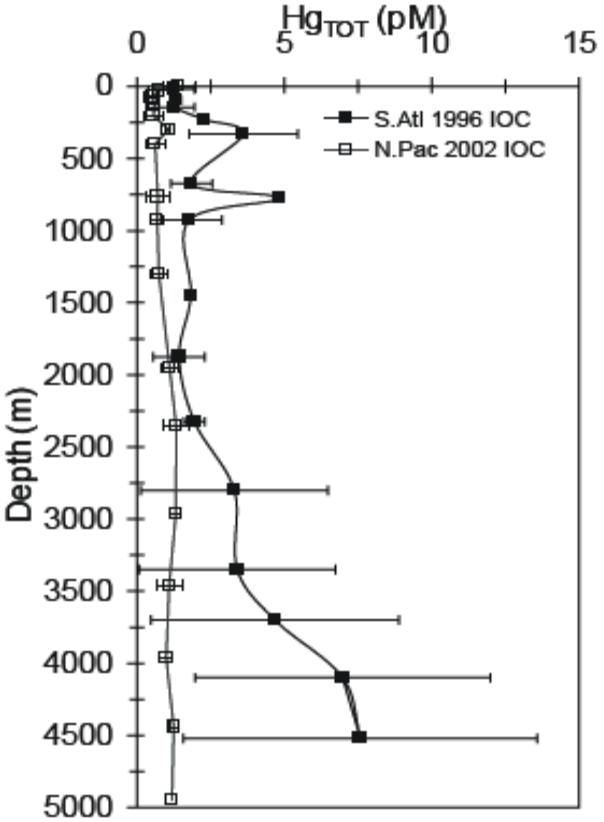


Figure 2.1: A comparison of the mercury concentrations measured in the South Atlantic with those measured in 2002 in the North Pacific, after Laurier et al., 2004.

2.4. Sources

Sources of mercury emissions to the atmosphere can be broadly classified as re-emitted mercury, natural mercury emissions, and anthropogenic mercury emissions. Re-emitted mercury is mercury that was previously deposited on the Earth's surface following either anthropogenic or natural releases and is re-emitted to the atmosphere by natural, biologic or geologic processes.

2.4.1. Natural mercury emissions

Natural mercury emissions occur when geologically-bound mercury is released during natural processes, such as volcanic eruptions, geothermal releases, and from naturally enriched substances. Volcanic eruptions are estimated to contribute approximately 700 Mg yr^{-1} of mercury to the atmosphere (Pyle and Mather, 2003), while geothermal emissions are estimated to contribute another 60 Mg yr^{-1} (Varenkamp and Busek, 1988). Emissions from naturally enriched substances have been estimated at more than 1500 Mg yr^{-1} (EUEC, 2005).
However, it should be noted that all estimates of natural mercury emissions are highly uncertain as is the relative proportion of emissions from various sources.

2.4.2. Anthropogenic sources

The mobilization and release of mercury by human activities is referred to as anthropogenic mercury emissions.
Sources of mercury to the environment from human activity are many and widespread. In developed countries, most point sources of mercury in aquatic systems have been eliminated or reduced, although inputs of mercury via the atmosphere remain of concern. These, coupled with the long-distance transport of elemental mercury, have lead to elevated concentrations of mercury occurring in

fish from the open-ocean as well as in semi-remote regions in the United States, Canada and Scandinavia (Wiener et al., 2002). A summary of mercury fluxes to the atmosphere from major sources for 1995 is given in Table 2.6.

Anthropogenic emissions can be classified as point or area sources. The largest single combustion source is coal burning. During the combustion of pulverised coals, most of the mercury volatilizes and variable amounts are captured by fly ash (0.01–12 mg kg^{-1}) and with negligible amounts in bottom ash (0.01–4 mg kg^{-1}) (Yudovich and Ketris, 2005).

Other major contributing point combustion sources include municipal waste incinerator, medical waste incinerators, and hazardous waste combustion. Manufacturing sources must be taken in account for mercury emissions with the majority of these point sources resulting from chlor-alkali production.

Table 2.6: Major classes of anthropogenic emissions of mercury to the atmosphere in 1995. (Pacyna and Pacyna, 2002)

Source type [a]	**1995 Flux (Mmol yr^{-1})**
Stationary combustion	7.4
Non-ferrous metal production	0.8
Cement production	0.7
Waste disposal	0.6
Pig iron and steel production	0.1
Total	9.6

[a] Stationary combustion includes fossil fuel burning power stations, while waste disposal includes municipal waste combustion.

Seigneur et al., (2004) estimated that 50% of mercury emissions in the United States are in the form of elemental mercury (Hg^0), 46% are gaseous divalent mercury (Hg^{2+}), while only 4% occurs as particulate emissions (Hg_p).

Total mercury emissions from southern Canada (14.7 Mg yr^{-1}) and Northern Mexico (33.5 Mg yr^{-1}) bring the total 2004 anthropogenic mercury emissions in North America to approximately 200 Mg yr^{-1} (Seigneur et al., 2004).

On a global scale, the amount of mercury released annually from natural sources has been estimated to be between 2000 tons per year (Nriagu and Pacyna, 1988) and 3000 tons per year (Jackson, 1997). Global anthropogenic emissions have been estimated to be between 3560 tons per year (Nriagu and Pacyna, 1988) and 4000 tons per year (Jackson, 1997).

The uncertainties associated with these estimates can be significant and are discussed by Schroeder and Munthe (1998).

More recent global mercury balances estimate new anthropogenic emissions at 2160 Mg yr^{-1} (Bergan et al., 1999), 2400 Mg yr^{-1} (Mason and Sheu, 2002) and 2143 Mg yr^{-1} (Seigneur et al., 2003). Re-emitted anthropogenic emissions are estimated to be 2000 Mg yr^{-1} by Bergan et al. (1999), 2090 Mg yr^{-1} by Mason and Sheu (2002), 4800 Mg yr^{-1} by Lamborg et al. (2002) and 2134 Mg yr^{-1} (range 1067 Mg yr^{-1} to 2670 Mg yr^{-1}) by Seigneur et al. (2003). Natural emissions from land and oceans ranged from 1600 Mg yr^{-1} (1000 Mg yr^{-1} land, 600 Mg yr^{-1} oceans) (Lamborg et al., 2002) to 2134 Mg yr^{-1} (1180 Mg yr^{-1} land, 954 Mg yr^{-1} oceans) (Seigneur et al., 2003).

Clearly, considerable uncertainty in mercury emission estimates by source exists within the scientific community.

Although the United States is a major source of anthropogenic mercury emissions (151.9 Mg yr^{-1}) (Seigneur et al., 2004), its contribution on a global scale is relatively small. Annual (2003) anthropogenic emissions from Asia (1118 Mg), Europe (508 Mg), Africa (246 Mg) and South/Central America (176 Mg) play a much larger role on a global scale. Across the Northern Hemisphere the largest source of emissions occurs in China (EUEC, 2005).

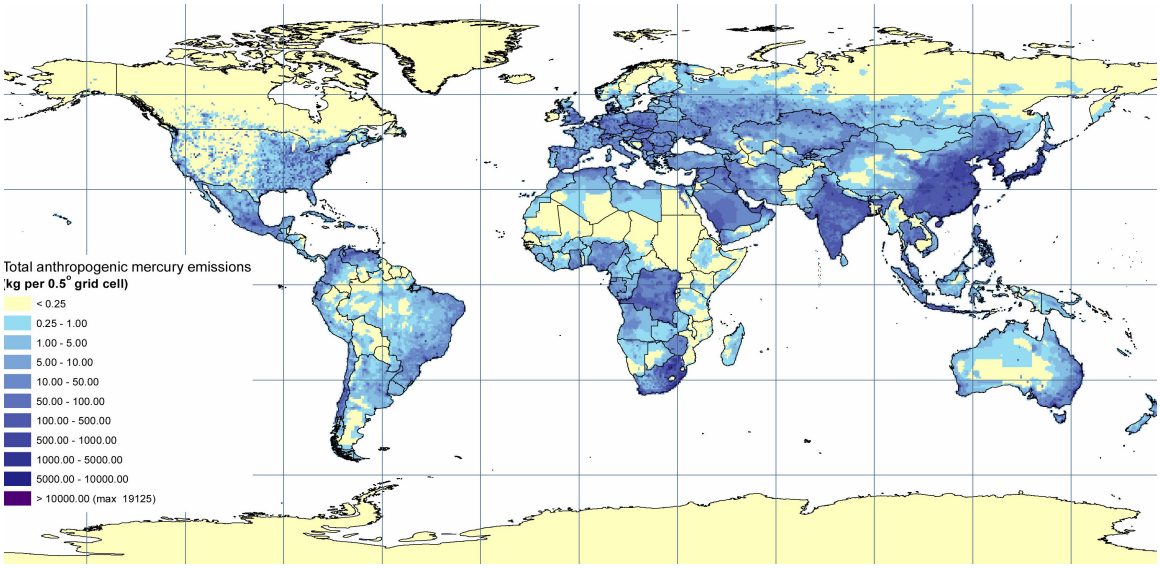


Figure 2.2: Spatially distributed inventory of global anthropogenic emissions of mercury to the atmosphere, 2000 (From Pacyna et al., 2005)

2.4.3. Mineral Working

Mineral working has long been a source of mercury contamination. Trace amounts of gold and silver are still concentrated using large amounts of liquid mercury in some enrichment processes. The dense amalgam formed is separated from crushed, parent rock or placer and alluvial deposits, often with a considerable loss of mercury. The gold or silver is recovered by heating the amalgam and vaporizing the mercury. This technology has been employed since its introduction by Barolome de Medina in 1557 (Nriagu, 1979).

Uses of mercury in gold and silver mining were especially significant in the Americas from the mid-1500s until the beginning of the twentieth century. The damaging practice continues today, and on a large scale in many countries including Brazil, China, Kenya, Philippines, and Tanzania.

Lacerda and Salomons (1998) reported that environmental losses of mercury from this process are large at about 1– 1.7 Kg per 1 Kg gold recovered (Lacerda and Salomons, 1998). Much of the mercury accumulates in watersheds, waterways, and mine tailings, giving rise to local and regional environmental and human-health concerns. The mercury lost to the atmosphere is also of concern globally (Porcella et al., 1997).

It has to be noticed that, although in South Africa the gold industry has phased out the use of mercury, this latest is still used by small-scale illegal miners in artisanal gold mining to form an amalgam, after which it is burnt off over an open flame. Obviously such practices pose a threat to their health, as well as to people who consume mercury-contaminated fish from freshwater ecosystems nearby (Leaner, 2007).

Recent media reports indicate that the practice is even carried out in hostels housing illegal miners, popularly known as "gold pirates" or "*zama-zamas*". The impact of these activities remains unclear (CSIR e-News, 2007).

Mercury mines remain a significant source of mercury to terrestrial and coastal marine ecosystems. These include historical sites such as Idrija and Clear Lake, California where mercury continues to leach from abandoned tailings (Lewis and Plant, 2005).

2.5. Pathways and Behaviour of Mercury in the Environment

As mentioned above, mercury exists in the environment in three oxidation states – Hg(0), Hg(I), and Hg(II) – and for each valence many chemical forms can occur in solid, aqueous, and gaseous phases. The environmental chemistry of mercury is very complex, and subtle changes in chemical, physical, biological, and hydrologic conditions can cause substantial shifts in its physical form and valence state over time scales ranging from hourly to seasonal. Here we briefly summarize selective aspects of mercury speciation in the atmospheric, terrestrial, and aquatic

environments, focusing on linkages between the observed speciation, sources and transport processes.

2.5.1. Mercury in the atmosphere

The atmosphere is the most important pathway for the worldwide dispersion and transport of mercury (Fitzgerald et al., 1998; Mason et al., 1994).

Understanding the global transport and atmospheric transformations of mercury is important because of the ability of mercury deposited to aquatic systems, to be converted to methylmercury (MeHg) and to bioaccumulate through all levels of the food chain. Most of the mercury in the atmosphere is elemental mercury (Hg^0), which is relatively unreactive with the net average atmospheric residence time of around one year (Figure 2.4). In addition to Hg^0, two other atmospheric mercury fractions have been operationally defined based on physicochemical properties – the gaseous ionic mercury (II) fraction, which has been termed reactive gaseous mercury (RGHg), and particulate-bound mercury (Hg-P). The speciation of RGHg is not known in detail but based on laboratory studies and the methods of its collection (Landis et al., 2002; Sheu and Mason, 2001; Ariya et al., 2002; Sheu and Mason, 2004), it is assumed to consist of gaseous neutral mercury (II) complexes such as $HgCl_2$, $HgBr_2$, and HgOBr (Balabanov and Peterson, 2003). Such compounds are highly surface-reactive and substantially more water-soluble than Hg^0. Estimates of dry deposition velocities for RGHg for the open ocean range from 0.5-4 cm s^{-1} (Laurier et al., 2003), estimated using the formulation of Shahin et al. (2002) and are much higher than those for Hg-P (0.1-0.5 cm s^{-1}), which being mostly derived from high combustion sources, is associated with the fine particulate fraction (Bullock et al., 2000). In many locations, dry deposition could be as important as wet deposition in terms of being a mercury source to the Earth's surface. Global mercury models have identified wet and dry particle deposition and evasion of dissolved gaseous mercury from the ocean (Figure 2.3)

as critical pathways for global mercury cycling (Mason et al., 1994; Hudson et al., 1995; Lamborg et al., 1999; Shia et al., 1999).

Natural sources of mercury to the atmosphere are mainly in the form of Hg^0 although emissions of Hg-P also occur (e.g., volcanoes, dust) while anthropogenic sources contribute all forms of mercury to the atmosphere (Ebinghaus et al., 1999). In addition, *in situ* oxidation of atmospheric Hg^0 in the gas phase could be a source of RGHg as Hg^0 can be oxidized by hydrogen peroxide (H_2O_2), albeit slowly (Tokos et al., 1998), the nitrate (NO_3) radical (Sommar et al., 1997) and other reactive nitrogen intermediates, ozone (O_3) (Hall, 1995), and the hydroxyl (OH) radical (Sommar et al., 2001). Given the typical atmospheric mercury concentrations of these oxidants, it is unlikely that these homogeneous reactions dominate the Hg^0 oxidation in general, based on estimates of the rate that is required for its removal via wet and dry deposition. However, the gas phase oxidation of Hg^0 by halogen atoms and molecules (Cl, Br, Br_2, Cl_2; Sliger et al., 2000; Ariya et al., 2002), and potentially by other halogen compounds (e.g., BrCl, BrO), has been recently demonstrated (Figure 2.5). The reactions with atomic Cl and Br have the larger rate constants (Ariya et al., 2002) and therefore oxidation by these mechanisms may proceed much faster than oxidation by O_3 and OH, in certain locations such as the polar region, the marine boundary layer, and at high altitudes (Laurier et al., 2003; Lindberg et al., 2002).

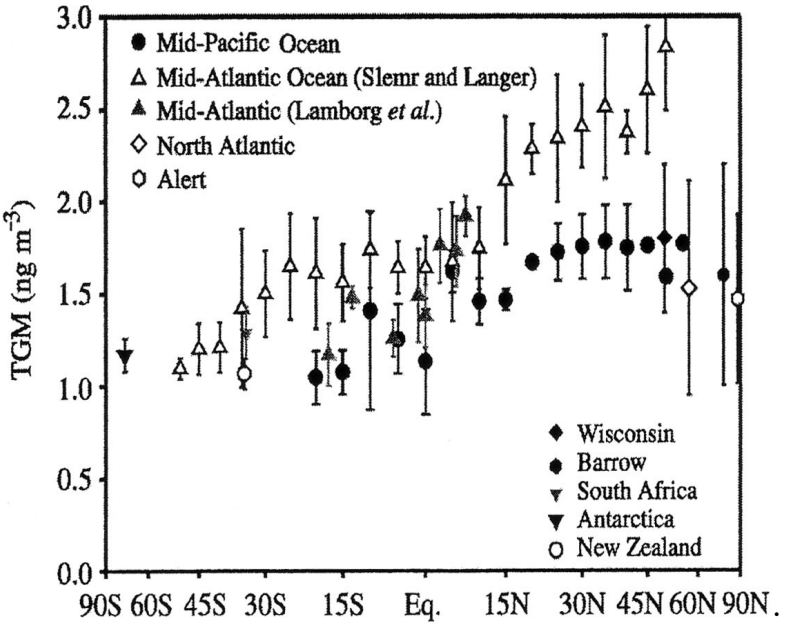


Figure 2.3: TGM in the atmosphere at several locations. (Lamborg et al., 2002)

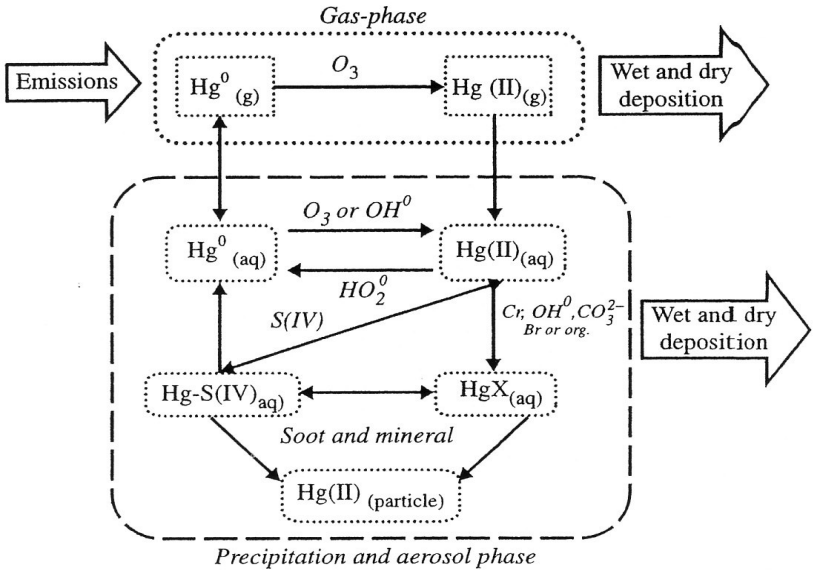


Figure 2.4: Summary of some of the important physical and chemical transformations of mercury in the atmosphere. (Fitzgerald and Lamborg, 2004b)

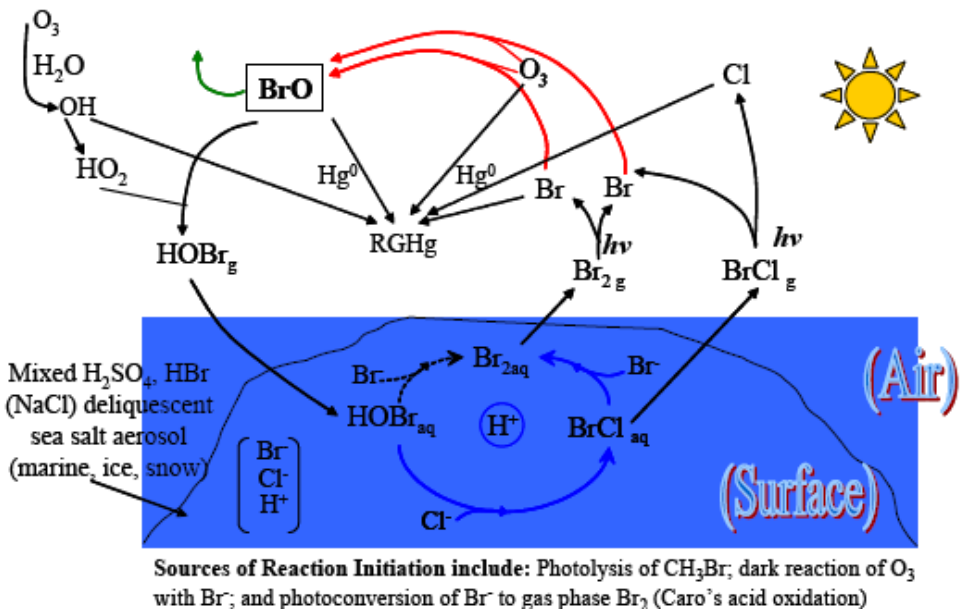


Figure 2.5: Proposed mechanism for elemental oxidation in the marine boundary layer, or in other regimes where there is the presence of halogen-containing aerosol (Source Mason, 2004).

2.5.2. Terrestrial Components

In the terrestrial environment, heterogeneity of the distribution of mercury in natural materials makes estimates of mass and generalizations about speciation and concentrations uncertain. Current research shows that gaseous mercury emissions from mercury-enriched soils and parent rock may make important contributions to the regional and global atmospheric inventories (Gustin et al., 2003), but that environmental impacts from mercuriferous deposits are almost always associated with physical mining activities and secondary processing (Rytuba, 2003) and thus should be viewed as anthropogenic point sources.

Terrestrial soils represent a very large pool of mercury (Mason et al., 1994), and the speciation of this mercury is dominated by Hg(II). Once deposited, mercury is

quickly sorbed by living plants and the soil humus layer (Hintelmann *et al*. 2002), and secondarily to mineral constituents. Hintelmann *et al*. (2002) found strongly decreasing concentration gradients from the surface organic layer (>0.200 mg kg^{-1}) down to only 15 cm below the surface (<0.025 mg kg^{-1}) in multiple cores from upland soils in northwestern Ontario. Schwesig *et al.* (1999) found a similar pattern in forest soils in Bavaria, Germany, with maximum concentrations of >0.400 mg kg^{-1} total mercury found in the organic humus layer, decreasing to <0.025 mg kg^{-1} in the mineral horizons which is approaching the geogenic background concentration for the region. These surface enrichment patterns are clearly the consequence of a widely distributed atmospheric deposition of natural and anthropogenic mercury firstly to vegetation, and secondarily directly to the forest floor (Grigal, 2003).

Total mercury concentrations found in wetland sediments tend to be similar to those found in the humus layers of forest soils (Schwesig *et al*. 1999, Heyes *et al*. 2000, Grigal 2003). Unlike forest soils which generally have maximum methylmercury concentrations in humus of <0.0005 mg kg^{-1} and negligible concentrations in the mineral fraction, wetland sediments may have substantially elevated concentrations of methylmercury, in some cases exceeding 0.040 mg kg^{-1} (Schwesig *et al*. 1999, Heyes *et al*. 2000) as a consequence of high rates of *in situ* biomethylation. Thus, given that in many watersheds the majority of water that falls as precipitation and flows through terrestrial components and to down-gradient aquatic systems will have come in contact with the forest floor and wetlands, these pools of reactive mercury are critically important compartments in the watershed-scale mercury cycle.

In soil and sediment, mercury likely associates with organic matter and iron oxides under oxidizing conditions, and organic matter and sulfides under more reducing conditions (Skyllberg *et al*. 2000, Heyes *et al*. 2004). The strong association of mercury with organic matter is in part because of the overwhelming amount of organic matter in soils and sediments. The most likely bonding pairs for mercury

in oxidizing soils and sediments is thiol groups on soil organic matter. Organic matter interacts with iron oxides and sulfides, thus oxidation-reduction cycles of iron that impact organic matter will also impact associated mercury (Krabbenhoft et al., 2005)..

2.5.3. Aquatic components

The speciation of mercury in water is dependent on a number of factors. First, the dissolved solid phase partition coefficient (K_D) for mercury in water is on the order of 10^{-4} to 10^{-5}, demonstrating the strong affinity of mercury for suspended natural particles in aquatic ecosystems (Hurley *et al.* 1994, Babiarz *et al.* 2001). As with all metals, the pH, pE and composition of the water matrix will determine the thermodynamically favored species both in the dissolved and particulate phases. For the dissolved phase, current analytical procedures only allow for the measurement total Hg, MeHg and Hg^0. The latter two mercury species generally account for up to a few percent of the dissolved total mercury in water (Wiener *et al.* 2003). Therefore, most researchers currently rely on thermodynamic modeling in order to provide insights into the speciation of the majority of the mercury in aquatic environments, and to relate speciation to important considerations like cycling and bioavailability (Benoit *et al.* 2003).

Early mercury speciation modeling focused mostly on inorganic speciation; however, even in the absence of much data on possible organic ligands, researchers concluded that mercury complexation by possible organic ligands was critically important (Hurley *et al.* 1994). In aerobic natural waters, the most important inorganic ligands are Cl^- and OH^- while dissolved organic carbon (DOC) is the most important organic ligand. In freshwater, where concentrations from 3 to 30 mg L^{-1} are common, DOC is the most important ligand for mercury. Simple organic ligands such as carboxylic acid have weak binding constants for mercury, whereas thiols have very strong binding constants and will dominate the

inorganic mercury species distribution under most environmental conditions (Haitzer *et al*. 2002). A simplified description of Hg–DOC interactions is that of a mixture of strong and weak binding sites. Although there are many potential ligands for mercury on DOC, mercury concentrations in natural waters are much lower than the amount of the strongest binding sites for mercury, thus the assumption is made that mercury would bind at these sites which are thiols, or thiol-like groups (Drexel *et al*. 2002, Haitzer *et al*. 2002).

The predominance of DOC regulating inorganic mercury speciation in aerobic freshwaters is now widely accepted; however, under brackish to marine conditions where Cl^- ion concentrations are high, mercury speciation is less certain. Results presently available suggest that at marine-to-brackish Cl^- concentrations, HgClx species could predominate over Hg–DOC binding at the generally low DOC levels (2–4 mg L^{-1}) observed in seawater (Stumm and Morgan 1981).

In anoxic waters, reduced sulfur species (most notably sulfide) are the strongest inorganic ligands for mercury. As sulfide concentrations increase, HgS is favored followed by $HgHS^{2-}$ then HgS_2^{2-} and finally $Hg(HS)_2$ (Benoit *et al*. 1999a).

The formation of a neutral HgS complex, which has been suggested to be a critical step for the uptake of mercury by methylating bacteria, is dominant at low concentrations of sulfide (Benoit *et al.* 1999b). However, at low sulfide levels, thiols may then become competitive for mercury binding, and thus the precise nature of mercury speciation at the site of methylation remains in question. The role of DOC at low pE has not been fully explored, as experiments require manipulation of the natural DOC, and have not been conducted under anoxic conditions.

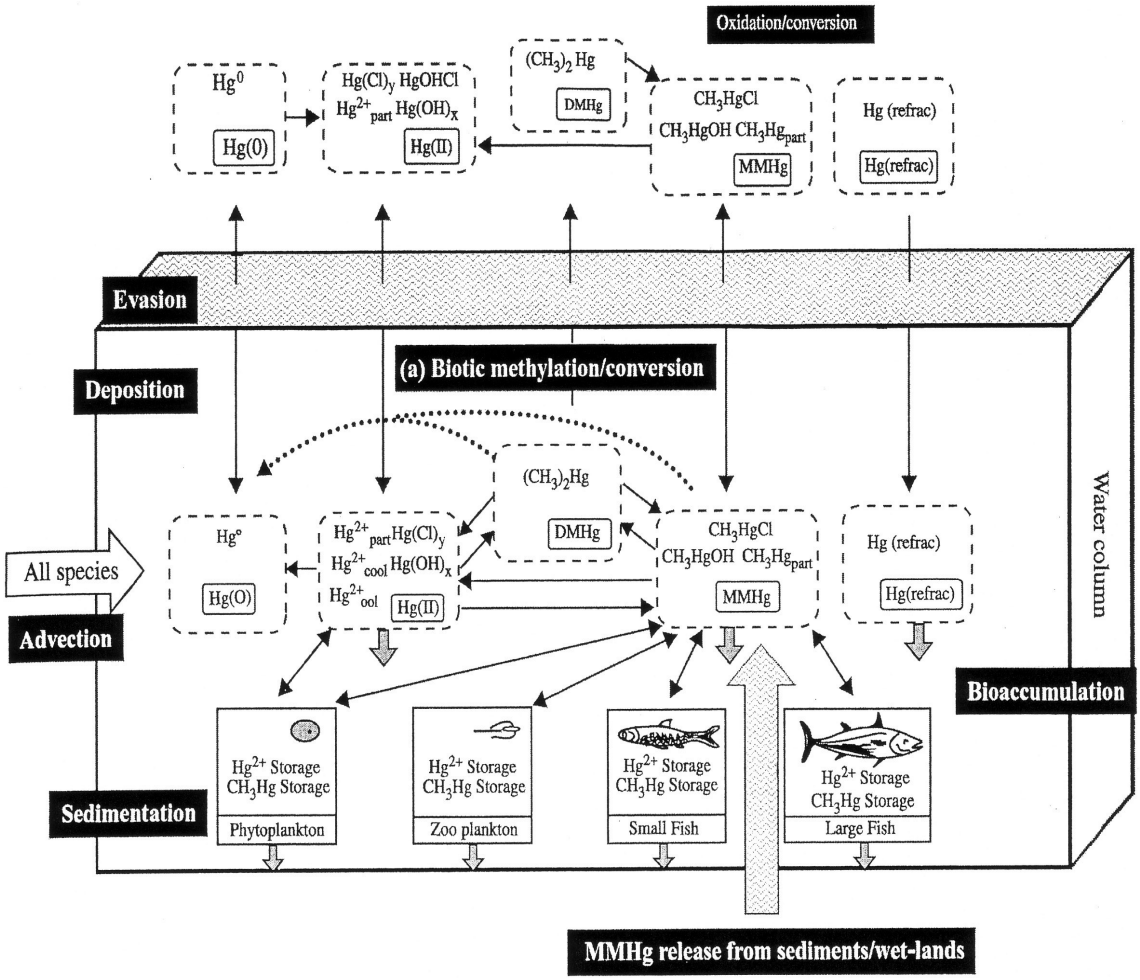


Figure 2.6: Generalised view of mercury biogeochemistry in the aquatic environment. Prominent processes are labelled.
(Source R. Lewis and J. Plant, 2005)

At this point it has to be recalled that other factors should be considered that play important roles in controlling aqueous mercury speciation such as the effect of pH and pE. For example, pH has a dramatic impact on the Hg–DOC binding constant, with the Log K decreasing from 24 to 18 as pH decreases from 8 to 4 (Haitzer *et al*. 2002). It also must be noticed that due to the comparatively low mercury concentrations in water, competition reactions with other metals can be very important. Therefore, kinetic and other factors are likely very important in

controlling mercury speciation in the environment, possibly have significant implications regarding the formation of methylmercury, and merit further research (Krabbenhoft et al., 2005).

In discussing the various reactions of mercury, another important process that needs to be addressed is the issue of photochemistry.
Once deposited in natural waters, mercury undergoes an aquatic redox cycling between oxidized Hg(II), dissolved gaseous Hg° (DGM), and methylmercury.
Diel changes of DGM that track solar radiation (typically, highest DGM levels around noon and lowest during night) have been observed in natural freshwaters. These findings implicate an intrinsic role of sunlight in controlling aquatic DGM dynamics. Recent field sunlight incubations of northern lake waters in Teflon bottles further suggest natural production of DGM through sunlight-induced photochemical reduction of Hg(II) in freshwater. This is of significance for aquatic mercury biogeochemical cycling and its environmental impacts because the competition of this sunlight-driven pathway for Hg(II) substrate with the methylation pathway would reduce mercury toxic hazards in local aquatic ecosystems through removal of Hg(II) as a result of its reduction to Hg(0) and its subsequent evasion. Yet, despite increasing numbers of observations of sunlight-induced aquatic production of DGM in both northern and southern aquatic systems, mechanistic understanding of the phenomena has remained limited (Zhang and Lindberg, 2001).

2.5.4. Transport and exchange processes

Mercury exchange between environmental compartments is largely governed by speciation. Details of the chemical speciation of mercury have been described above, but are mentioned here in the context of the mediation of the movement of mercury between environmental compartments. In brief, the transfer of mercury

between the atmosphere and Earth's surface is controlled by the oxidation of Hg^0 and reduction of Hg (II) species. Although Hg^0 dominates the atmospheric pool, it is the oxidation of a very small proportion of this pool that supports the overwhelming majority of mercury deposition to the Earth's surface. Similarly, it is the reduction of Hg (II) to gaseous Hg^0 that governs the flux of mercury from the earth's surface back to air. The complex interplay between mercury methylation and demethylation governs the uptake of mercury into the biotic compartment. Finally, exchanges within the watershed are governed by hydrologic fluxes and the degree to which the watershed compartments are connected by those fluxes.

2.6. Organomercury compounds in the environment

Interest in the impact of mercury released into the environment, from both natural but primarily anthropogenic sources, has been driven by the human health concerns associated with mercury ingestion, mainly through the consumption of seafood. More recently, similar concerns have been raised in terms of wildlife, especially with regard to piscivorus mammals and birds. Two large-scale poisonings, in Minamata, Japan and in Iraq, between 1950 and 1975, focused attention on the health risks associated with mercury consumption, especially in its methylated form. In Minamata, methylmercury from a vinyl chloride-acetic acid plant was the source of contamination. Inorganic mercury, used as a catalyst, was primarily methylated to methylmercury within the plant and subsequently discharged in the wastewater. Historically, fish levels as high as 20 mg kg^{-1} and sediment levels above 500 mg kg^{-1} were reported (Fujiki and Tajima, 1992; Kudo et al., 1998).

Patient hair concentrations were similarly elevated - as high as 700 mg kg^{-1}. More than 1000 people have died, and about 2250 patients have been recognized, (Harada, 1995) and mercury poisoning is now often referred to as Minamata

disease. Dredging began in 1971, and since the removal of contamination, fish levels have decreased to values below 0.5 mg kg^{-1}. Human hair levels have also decreased to values below 10 mg kg^{-1}, which is the currently considered as a "safe" level (Grandjean et al., 1998). However, a recent investigation of fishermen from the region found neurological effects even though the patients current hair concentrations were <10 mg kg^{-1} (Harada et al., 1998).

The poisoning incident in Iraq was the result of the consumption of seed grain preserved with methylmercury, a commonly used preservative at the time (Clarkson et al., 1976).

In many direct discharge incidents, contrary to Minamata, elevated levels of mercury in fish, as methylmercury, have resulted from methylation of the mercury in the aquatic environment.

2.6.1. Methylmercury in water

Concentrations of methylmercury in surface waters are typically higher in freshwater environments compared to the open ocean. The amount of methylmercury in a system is to a degree a function of size (sediment surface area/water volume) because of the importance of methylation in sediments, but other factors also control methylmercury formation. The role of sulfate in stimulating methylmercury formation by sulfate reducing bacteria at low sulfate concentrations has been documented (Gilmour and Henry, 1991), but there is also inhibition of methylation at higher sulfate concentrations through the complex interaction between mercury and sulfide, and inorganic mercury complexation appears to control the bioavailability of mercury to the methylating bacteria (Benoit et al., 1999; Benoit et al., 1999b**).** Wetlands are important sources of methylmercury both in temperate lakes and in the coastal zone, and seasonal anoxia plays a role in increasing methylmercury concentrations in the water

column and biota by enhancing the release of methylmercury from sediments (Gill et al., 1999; Bloom et al., 1991).

Monomethylmercury appears to be the principal methylated mercury species in uncontaminated terrestrial systems (Gill et al., 1999; Bloom et al., 1991) while dimethylmercury is more prevalent, and at times it is the dominant species, in open ocean waters (Mason et al., 1995).

Methylation of methylmercury to form dimethylmercury can also be biologically-mediated, but it is not known whether sulfate-reducing bacteria, or other organisms, are the primary methylators in open ocean sub-thermocline waters where the highest concentrations of methylated mercury occur. While methylmercury is likely produced directly, it is also the product of dimethylmercury decomposition, and the stability of dimethylmercury in ocean waters, even in the absence of light, is such that its decomposition accounts for a large fraction of the methylmercury (Mason and Sullivan, 1999).

The sunlight-induced decomposition of monomethylmercury also was observed and this reduces its availability for accumulation in aquatic food webs.

Hammerschmidt and Fitzgerald (2006) examined methylmercury degradation in epilimnetic waters of Toolik Lake (68° 38' N, 149° 36' W) in arctic Alaska (USA), a region illuminated by sunlight almost continuously during the summer. Methylmercury decomposition in surface water of Toolik Lake was found to be exclusively abiotic and mediated by sunlight. The estimated annual loss of methylmercury to photodecomposition in Toolik Lake accounted for about 80% of the methylmercury mobilized annually from in situ sedimentary production which is the primary source in Toolik Lake. These results suggested that greater light attenuation in lacustrine surface waters may result in less photodecomposition and subsequently greater availability of methylmercury for bioaccumulation.

In general, concentrations of dimethylmercury and methylmercury in the ocean are low in surface waters (at or below detection limits; $<2 \times 10^{-9}$ mg L^{-1} (0.01 pM) for

dimethylmercury; $<10^{-8}$ mg L^{-1} for methylmercury), and are a maximum in sub-thermocline environments below productive regions (e.g. equatorial upwelling zones; up to 14 x 10^{-8} mg L^{-1} (0.7 pM) (Mason and Fitzgerald, 1990).

In deep ocean waters, overall decomposition is greater than formation and concentrations are low in these environments.

The speciation of methylmercury in oxic natural waters is a function of pH, chloride concentration and dissolved organic carbon. In the open ocean, where organic matter concentrations are low, chloride complexation dominates (i.e. CH_3HgCl is >90% of the total) but organic ligands are likely more dominant in the coastal zone and in many freshwaters, out-competing the chloride and hydroxide ligands.

Clearly, the extent of organic complexation will impact the bioavailability of the methylmercury to microorganisms, especially for uptake via passive diffusion processes (Hudson et al., 1994). Similarly, complexation of inorganic mercury plays a crucial role in the accumulation, and thus methylation, of mercury by microorganisms.

For sediments, absorption of methylmercury to solid oxide phases may be important in both oxic freshwater and estuarine systems. The presence of organic matter enhances adsorption at low organic content via the formation of tertiary complexes (oxide-organic matter-methylmercury) but at high dissolved organic matter concentrations, adsorption is decreased (Mason and Lawrence, 1999).

Understanding the binding of methylmercury in sediments is crucial if the fate and mobility of methylmercury in sediments is to be properly determined.

While methylmercury concentrations are often a relatively small fraction of the total mercury in oxic waters, in seasonally or permanently anoxic environments, methylmercury concentrations can be significantly higher (Verta and Matalianen, 1995).

The other major source to the ocean is riverine input. Assuming 3% of the total mercury as methylmercury in river water entering the ocean (Cossa et al., 1996), the

flux is 3 x10^4 mol yr^{-1}. Similarly, considering deep ocean sediment to contain about 0.3% methylmercury on average, the burial flux for the deep ocean is around 3 x 10^3 mol yr^{-1}. The major sink for methylmercury is accumulation into fish, and Rolfhus and Fitzgerald (Rolfhus and Fitzgerald, 1995) estimated the annual net accumulation of methylmercury into fish to be 22 x 10^4 mol yr^{-1}.

Another sink is the potential loss of dimethylmercury from the ocean. The concentrations in the surface ocean are mostly at the detection limit (<10 fM), except in regions of deep water mixing or upwelling (Cossa et al., 1996). Given the rapid decomposition of dimethylmercury in the Atmosphere (Nikki et al., 1983), any dimethylmercury evaded, except in coastal areas, is likely redeposited to the ocean as methylmercury (Bloom et al., 1996). Thus the maximum flux from this source is probably less than that of atmospheric deposition.

In lakes, a number of investigators have similarly concluded that *in situ* production is the main source of methylmercury (Fitzgerald et al., 1991). However, when considering the mass balance approaches above in freshwater systems, it is clear that, because of the lack of dimethylmercury, there is no mechanism for significant exchange of methylated mercury from the water to the atmosphere, except if methylmercury is being lost from the lake surface.

2.6.2. Methylated mercury in the atmosphere

Concentrations of methylmercury in the atmosphere are small, typically a few percent of the total mercury in the gas and particulate phase, as they are in wet deposition. Methylmercury in wet deposition varies from typically <1 % of the total mercury for open ocean rain to a few percent (<1-7 %) for terrestrial precipitation (Mason and Fitzgerald, 1996).

Measurements of methylmercury in atmospheric particulate and in the gas phase are essentially non-existent, and this is a function to a large degree of the difficulty of measuring the low concentrations present.

While incineration is not a likely source of methylmercury, there are other potential anthropogenic inputs to the atmosphere. Loss of methylmercury from municipal sludge applied to soil has been suggested as an important source (Carpi and Lindberg, 1997). Other sources such as losses via gas exchange from landfills and from sewage treatment plants, and other waters of high methylmercury concentration, need further investigation.

Given the relatively high solubility of methylmercury, and its correspondingly low Henry's Law coefficient ($H = 2\text{-}4 \times 10^{-5}$) (Iverfeldt and Lindqvist, 1982), dry deposition of methylmercury in the gas phase to surface waters is a potential sink for the atmosphere.

2.6.3. Methylmercury Production and Decomposition

Biological methylation of mercury in aquatic sediments was first demonstrated by Jensen and Jernelov (1969) and it is nowadays generally accepted that mercury methylation in nature is principally a biological process. A number of organisms in pure culture can produce methylmercury from added Hg(II) including: *Neurospora*, *Clostridium*, *Pseudomonas*, *Bacillus*, and *Escherichia coli*, to name only a few (Robinson and Tuovinen, 1984). Early evidence of mercury methylation by extract of methanogens (Wood et al., 1968) pointed to this group as the important methylmercury producers in sediments. However several lines of evidence now specifically identify sulfate reducing bacteria as the most important mercury methylators in the environment (Chen et al., 1997; Choi and Bartha, 1994).

The ability of methylcobalamin to spontaneously methylate mercury *in vitro* (Compeau and Bartha, 1983), prompted investigations into the methyl carrier in sulfate reducers. Berman et al (1990) suggested, based on the selective inhibition of mercury methylation in *D. desulfuricans* LS, that methylation was mediated by a cobalt porphyrin compound in this organism. Their continued work (Choi and

Bartha, 1993) identified this corrinoid to be cobalamin (vitamin B-12), and they suggested that mercury methylation is an enzymatically catalyzed process *in vivo*. The following sequence and reaction (Equations 2.1 and 2.2) have been proposed (Rodriguez Martin-Doimeadios, 2004) to explain the mercury methylation:

$$Hg^0 \rightarrow Hg^{2+} \rightarrow CH_3Hg^+ \rightarrow (CH_3)_2Hg \qquad (2.1)$$

$$HgCl_2 + CH_3CoB_{12} \rightarrow CH_3HgCl + CoB_{12}Cl \qquad (2.2)$$

Cobalamin is not unique to sulfate reducing bacteria, however, and has been identified in some acetogens and methanogens (Stupperich et al., 1990), so not all organisms that contain cobalamin are capable of methylating mercury. The acetyl-CoA synthase pathway was shown to be a significant metabolic pathway of mercury methylation in *D. desulfuricans* LS. In view of the presence of cobalamin and high levels of acetyl-CoA enzymes in methanogens and acetogens that do not methylate mercury, it has been suggested that some sulfate reducers possess a distinct or highly specific enzyme to catalyze this step (Choi et al., 1994).

Bacteria with broad-spectrum resistence to mercury are able to degrade a variety of organomercury compounds including methyl- and ethylmercury chloride (Foster, 1987). Microbial degradation of methylmercury occurs through the cleavage of the carbon-mercury bond by the enzyme organomercurial lyase followed by reduction of Hg(II) by mercuric reductase to yield methane and Hg^0 (Robinson and Tuovinen, 1984).

In an early study using lake sediments, Spangler et al (Spangler, 1973) found methane and Hg^0 as the products of bacterially-mediated mercury demethylation, which prompted the conclusion that methylmercury demethylation in nature, occurs primarily through the *mer* pathway (Winfrey and Rudd, 1990). However, CO_2 has also been observed as a major methylmercury demethylation product by

Oremland and co-workers (Oremland et al., 1991). These authors suggested that methylmercury degradation can occur through biochemical pathways used to derive energy from single carbon substrates, and they termed this process "oxidative demethylation". Marvin-DiPasquale and Oremland (1998) further proposed a reaction (Equation 2.3) analogous to microbial monomethylamine degradation:

$$4CH_3Hg^+ + 2H_2O + 4H^+ \rightarrow 3CH_4 + CO_2 + 4Hg^{2+} + 4H_2 \qquad (2.3)$$

Environmental factors affecting mercury methylation and demethylation fall into two categories: 1) those that control the overall metabolic activity of the methylating organsims, and 2) those that control the chemical form of the mercury in the matrix where methylation occurs. To date, the focus of most research has been on the chemical and physical characteristics that stimulate mercury methylation by affecting microbial activity, rather than on chemical forms of mercury that are available for uptake and methylation.

Since sulfate reducing bacteria are the important methylators of mercury, a general relationship between %methylmercury in sediments and sulfate-reduction rate is expected. At relatively low sulfate concentrations, sulfate stimulates both sulfate reduction and mercury methylation, (Gilmour et al., 1992) and increased sulfate deposition associated with acid rain may explain increased methylmercury bioaccumulation in aquatic systems undergoing acidification (Gilmour and Henry, 1991).

To summarize, acidification of lakes may increase methylmercury production in the sediments due to sulfate fertilization and possibly due to lowered pH, which may stimulate methylmercury production near the sediment-water interface. Although decreased pH may decrease methylation in deeper sediments, in many aquatic ecosystems the bulk of mercury methylation occurs near the sediment

surface (Winfrey and Rudd. 1990). The effects of organic matter are less straight-forward, and the relationship between dissolved organic carbon content and fish mercury concentrations differs depending on lake type. These parameters are positively correlated in drainage lakes (Wren et al., 1991) and negatively correlated in seepage lakes (Grieb et al., 1990). A possible explanation for this difference is that in drainage lakes the bulk of the organic matter is supplied from the watershed, which may bring in exogenous mercury and methylmercury, or provide labile carbon for increased microbial activity and methylmercury production (Lee and Hultberg, 1990). In seepage lakes, where organic carbon is mainly produced in situ, its presence may inhibit in-lake methylmercury production (Miskimmin et al., 1992). Furthermore, low pH and high dissolved organic carbon tend to favor Hg(II) methylation relative to reduction and evasion (Schindler et al., 1980). All of these studies on pH, organic carbon, and mercury methylation suggest that the effects of these water chemistry parameters on mercury concentrations in fish may only be partially explained by increased methylmercury production within aquatic ecosystems.

2.6.4. Bioaccumulation

As previously mentioned, chemical and physical characteristics of aquatic ecosystems including acidity, dissolved organic content, sediment organic matter content, and temperature may indirectly effect mercury accumulation in biota by influencing methylmercury production by resident bacteria.

While mercury is different from other metals in that it biomagnifies through all levels of the food web, the largest bioconcentration of mercury occurs between water and phytoplankton (Lindqvist et al., 1991). Therefore, uptake at the base of the food chain may exert a primary control on mercury reaching higher trophic levels. Mason et al. (1996) demonstrated passive uptake of neutral species of both inorganic mercury and methylmercury by the diatom *T. weisflogii*. In these

experiments, uptake was most efficient for the complex CH_3HgCl. At a given chloride concentration, the fraction of the methylmercury present as this complex decreases with increasing pH as it is replaced by CH_3HgOH.

On the other hand, in several lakes in the USA (Watras et al., 1994) lake water dissolved organic carbon concentration was positively correlated with dissolved methylmercury concentration but negatively correlated with bioaccumulation factors (BAF) for seston (phytoplankton).

This finding suggests that organic matter complexation makes methylmercury less bioavailable, so that positive relationships between lake organic carbon content and mercury in fish cannot be explained by enhanced uptake of methylmercury at the base of the food chain. Therefore, the effects of dissolved organic carbon on bioaccumulation are controlled by the conflicting effects of: 1) increased methylmercury loading and/or bacterial production with increased supply of organic carbon from the watershed; 2) decreased bioavailability of Hg(II) to methylating organisms; 3) decreased bioavailability of organically-complexed methylmercury to phytoplankton; and 4) changes in the food chain structure.

It was also observed that trophic transfer is more efficient for methylmercury than inorganic mercury (Boudou and Ribeyre, 1981) so that the percentage as methylmercury tends to increase with increasing trophic levels until the bulk of mercury (>90%) in predatory fish tissues is in the form of methylmercury (Spry and Wiener, 1991). However, this is not true for herbivorous/detritivorous fish. For example, freshwater fish such as brown bullhead (*Ameierus nebulosus*) have only 40-70% of the total mercury as methylmercury. It should be noted that many measurements of mercury in fish tissue focus only on muscle as this is the tissue fraction consumed by humans. In general, the %methylmercury in muscle tissue is much greater than that of other tissues, and thus measurements of muscle tissue alone do not reflect the overall fraction of the mercury as methylmercury for the whole organism. For example, in freshwater crayfish, muscle %methylmercury is

>80% but the fraction was <60% for the hepatopancreas, gut and gonad, and <20% for the gill and carapace (Mason, R.P. et al., 2000). Overall, the % methylmercury of the whole tissue was only 50-75 % of the total mercury.

The dominant uptake pathway, food versus water, of methylmercury by zooplankton is not well known, but the methylmercury content of these organisms may reflect water column methylmercury concentrations (Watras and Bloom, 1992).

Typical biomagnification factors (BMF=[Hg]predator/[Hg]prey) for methylmercury are 3-5 per trophic level (Watras and Bloom, 1992), but BMFs of less than one have been observed. A lack of relationship between pH and bioconcentration factors (BCF = [Hg]organism/[Hg]water) in phytoplankton, zooplankton, and fish (Watras et al., 1994) supports the idea that the lake acidification influences mercury bioaccumulation primarily through enhanced bacterial methylmercury production and uptake at the level of phytoplankton, rather than through effects at higher trophic levels.

Concerns about human exposure to mercury via fish consumption have prompted a great deal of investigation into mercury bioaccumulation and toxicity in fish (Wiener and Spry, 1996). In general, piscivorus fishes contain higher concentrations of mercury than fishes of lower trophic level from the same body of water (MacCrimmon et al., 1983) and the mercury concentration in fish is positively correlated with trophic level (Kidd et al., 1995). Additionally, longer food chains can lead to higher mercury tissue concentrations in the top predators (Futter, 1994). Finally, mercury concentration in fish tends to increase with body size (Rask et al., 1994) as a fish's prey typically changes with age towards higher trophic levels (MacCrimmon et al., 1983).

Table 2.7: Percent of mercury present as MeHg in tissues of invertebrae aquatic (Source: Mason and Benoit, 2007)

Organism	Percent methylmercury
Plants-phytoplankton & macroalgae	<40
Deposit Feeders	
Scobicularia plana (Bivalve)	20
Nereis diversicolor (Polychaete)	18
Diporeia (amphipods)	35 ± 12
Isopods (*Cyathura*)	30-55
Snail- *Littorina*	3-10
Annelids, Echinoderms	4-36
Polychaetes, Echinoderms	25-50
Polychaetes, Lavaca Bay	13 ± 13
Primary Consumer/ herbivores	
Cardium spp. (Cockles)	30-90
Caddisfly, Stonefly, Mayfly, Cranefly Larvae	5-55
Zooplankton	18 ± 2
Suspension Feeders	
Mytilus edulis (Mussel)	15
Clams-*Rangia*, *Macoma*	5-25
Various bivalve mollusks, contaminated Marsh	<1-35
Bivalves	18-30
Bivalves, Lavaca Bay, Texas	62 ± 43
Omnivores	
Dipteran & Trichpteran larvae	30-60
Freshwater Crayfish	50-75
Various Crustaceans	65-90
Grass Shrimp, Lavaca Bay, USA	87 ± 12
Blue crab, Lavaca Bay, USA	88 ± 16
Predators	
Stonefly, Dragonfly, Dobsonfly Larvae	60-100
Crangon crangon (Shrimp)	75
Mysis (Zooplankton)	103 ± 36

Overall, the bioaccumulation studies show the importance of the transfer of methylmercury from water to microorganisms (phytoplankton/bacteria) and the accumulation from sediment into benthic invertebrates in controlling the net accumulation at higher trophic levels (Mason, 2001). For example, BAFs can range over two orders of magnitude, depending on sediment characteristics, and accumulation for the water can also be significantly influenced by water quality parameters. In contrast, transfer factors between trophic levels do not vary by more than an order of magnitude.

Food chain length is also an important factor given the bioaccumulative nature of methylmercury. While more studies are clearly warranted, current knowledge is sufficient that the bioaccumulation of methylmercury into food chains can be adequately modeled. This is not so for the processes of formation and destruction of methylmercury.

While substantial advances have been made, there are still important gaps in our knowledge of the principal factors controlling the formation, fate and transport of methylmercury in the environment.

2.7. Health Effects

Mercury, particularly as methylmercury, is a neurotoxin. Exposure of humans to elemental and ionic mercury (Hg(II)) has occurred throughout history because of the use of mercury compounds in various application such as in hat making (hence the term “mad as a hatter”), in medicine, and in industrial activities including a recent widespread use in the chlor-alkali industry.

In these cases, exposure was primarily related to the inhalation of elemental mercury. Elemental mercury is taken up via gas exchange in the lungs and is oxidized within the blood to ionic mercury in the presence of catalase. Severe poisoning incidents from inorganic mercury are now rare (Clarkson, 1998).

The same is not true for methylmercury as there is a continuing and widespread exposure of the human population to methylmercury, primarily from consumption of fish and other aquatic organisms (Clarkson, 1998).

Methylmercury selectively damages the brain as it readily transverse the blood-brain barrier. Methylmercury can also transverse the placenta and disrupt the normal development of the fetal brain. Therefore fetal exposure is a major concern as this represents the most susceptible stage in the life cycle (Clarkson, 1998). Estimations of toxicity related to consumption of fish have lead to consumption advisories in many countries, especially in Europe and North America. For example, in the USA, more than 40 states have issued fish consumption advisories based on mercury (EPA, 1997). Long-range atmospheric transport and deposition of mercury is the dominant source of this contamination in remote environments in the Northern Hemisphere (Mason et al., 1994).

Table 2.8: Levels of Total Mercury in Seafood. (U.S. FDA, 2001)

Fish species	**Mean (mg kg^{-1} wet weight)**	**Range (mg kg^{-1} wet weight)**	**Number of samples analyzed**
Tilefish	1.45	0.65–3.73	60
Swordfish	1.00	0.10–3.22	598
King Mackerel	0.73	0.30–1.67	213
Shark	0.96	0.05–4.54	324
Tuna (fresh or frozen)	0.32	n.d.–1.30	191
Tuna (canned)	0.17	n.d.–0.75	248
Atlantic cod	0.19	n.d.–0.33	11
Pollock	0.20	n.d.–0.78	107
Mahi mahi	0.19	0.12–0.25	15
American lobster	0.31	0.05–1.31	88

n.d. denotes that the Hg level was not detectable.

Most (>95%) of the total Hg in edible fish tissue is MeHg. In contrast, health effects and high fish levels in South America, Africa and other regions have resulted from the use of mercury in gold mining, and from other sources such as biomass burning and forest clearing (Bruhn et al., 1997; Kehrig et al., 1998).

The widespread occurrence of elevated methylmercury levels in fish has lead to a reevaluation of the toxic dose (USEPA, 1995) especially as the current advisories are based primarily on the older data sets, such as the Iraq poisoning incident. Clarkson (1990) concludes that at-risk populations are those that consume large quantities of freshwater fish, and pregnant mothers and their offspring. For example, while most of the population has exposure below the World Health Organization (WHO) limit, about 0.1% of the population is at risk based on the WHO (2005a) guidelines (0.47×10^{-3} mg MeHg kg^{-1} body weight/d).

2.8. Risk Assessment and Exposure Pathways

The risk of a pollutant causing harm to receptors such as human beings or the environment occurs only when a hazardous source is linked to the receptor via a pathway. There are many ways of assessing risk, and different methods are presented in the following sections.

2.8.1. Exposure Assessment

A very large number of people are exposed to mercury throughout the world causing concerns about human health. The main source of exposure is diet, through ingestion of Hg contaminated food and water.

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2.8.2. Risk Characterisation

A reference dose (RfD) is defined as an estimate of a daily exposure to the human population (including sensitive subpopulations) that is likely to be without an appreciable risk of deleterious effects during lifetime (Risher and DeWoskin, 1999). The RfD for methylmercury has been determined by the EPA as 10^{-4} mg per Kg of body weight per day. A recent study mandated by the U. S. Congress, including an evaluation of three large epidemiological studies in the Seychelles Islands, Faroe Islands and New Zealand by the National Academy of Sciences, endorsed EPA's RfD for methylmercury and found it to be scientifically justifiable for the protection of public health (NCR, 2000).

2.8.3. Risk Management

Due to its high potential to cause adverse health effects in exposed people, several regulations and guidelines have been established for various inorganic and organic forms of mercury. The permissible limit for mercury in drinking water (maximum contaminant level – MCL) has been set at 6×10^{-3} mg kg^{-1}. The World Health Organization's tolerable daily intake for inorganic Hg is 0.002 mg per Kg of body weight (WHO, 2005b).

2.8.4 Exposure Pathways

Humans are exposed to Hg^0 primarily by inhalation and to methylmercury by ingestion (Gochfeld, 2003). An exposure matrix summarizing the pathways of exposure to various mercury compounds is presented in

Table 2.9. Primary exposure pathways (Sipes and Badger, 2001) of toxins and pathogens such as mercury include the gastrointestinal tract (GI), the respiratory tract (lungs), and the skin (percutaneous absorption).

Table 2.9: Pathways of exposure to various species of mercury. (Gochfeld, 2003)

Route/ medium	Air	Soil	Water	Food	Other
Inhalation	Mainly Hg^0	Mainly Hg_i in dust	Hg^0 in cooking	N/A	Amalgam form oral cavities (often in 200ng m^{-3} range)
Ingestion	Airborne deposition on food. Mainly occupational	*Geophagia* in toddlers	Contaminated water supply	Mainly MMHg in fish	
Skin	N/A	Some organic compounds	Some organic compounds	N/A	DMHg from historical medicines
Injection	N/A	N/A	N/A	N/A	Thimerosal in vaccines

Hg_i - ionic species; MMHg - monomethylmercury; DMHg - dimethylmercury

Most organic mercurial compounds are readily absorbed through lungs and the GI tract, and some are readily absorbed trough the skin. The GI tract and the lungs differ in their absorptive properties for each species of mercury, and absorption may vary by age, and dietary variables. Elemental mercury vapour is readily absorbed through the lungs (50–100%), but that absorption of liquid elemental mercury from the GI tract is <1% (Table 2.10). However, methylmercury is readily absorbed from the GI tract (close to 100%) and from the lungs. Once in the bloodstream, mercury is subject to very complex and inadequately understood processes involving binding and redox cycling, both extracellularly and intracellularly (Gochfeld, 2003).

Table 2.10: Absorption of mercury species by routes. (Gochfeld, 2003)

	Elemental (Hg^0)	**Ionic/ salts (Hg_i)**	**Organic**
Lungs	Almost complete	Variable	Almost complete
GI tract	Negligible	Variable	Almost complete
Dermal	Negligible	Negligible	Moderate to high

About 80% of inhaled Hg is retained in the body; however, approximately 7–14% is exhaled within a week after exposure. The half-time of the process is about 2 days (Clarkson, 2002).

2.9. Legislation and Guidelines

2.9.1. Water

Almost all mercury in uncontaminated drinking-water is thought to be in the form of Hg^{2+}. Thus, it is unlikely that there is any direct risk of the intake of organic mercury compounds, and especially of alkylmercurials, as a result of the ingestion of drinking water. However, there is a real possibility that methylmercury will be converted into inorganic mercury (WHO, 2005b).

An International Programme on Chemical Safety (IPCS) Working Group (IPCS, 2003) recommended a tolerable daily intake (TDI) of 2 ppb of body weight for inorganic mercury based on the NOAEL (no-adverse-affect-effect-level) of 0.23 mg Kg^{-1} of body weight per day for kidney effects in the NTP 26–week study in rats and applying an uncertainty factor of 100 (for inter– and intraspecies variation) after adjusting for 5 days per week dosing. A similar TDI was obtained by applying an uncertainty factor of 1000 (an additional uncertainty factor of 10 for adjustment from a lowest-adverse-affect-effect-level (LOAEL) to a NOAEL)

to the LOAEL for renal effects of 1.9 mg $Kg^{-1}$of body weight per day in the 2–year NTP study in rats (WHO, 2005b).

Assuming a 60–kg adult drinking 2 litres of water per day and allocating 10% of the TDI to drinking-water, since the major sources of exposure are through food, the guideline value for inorganic mercury is 0.006 mg kg^{-1} (WHO, 2005b).
In South Africa the guidelines for water quality are as follows (Leaner, 2005):
- Aquatic ecosystem: 0.001 mg Hg L^{-1} (SAWQG, 1996)
- Aquaculture: 0.001 mg Hg L^{-1} (SAWQG, 1996)
- Drinking water: 0.005 mg Hg L^{-1}(SABS, 1984)
- Agricultural soils: 0.5 mg Hg Kg^{-1}.

2.9.2. Food

The U.S.E.P.A. established a reference dose for methylmercury of 10^{-4} mg Kg^{-1} Day^{-1}, and the World Health Organization (WHO,2005b) has lowered the provisional tolerable weekly dose from 0.0033 mg Kg^{-1} $week^{-1}$ to 0.0016 mg Kg^{-1} $week^{-1}$.

2.9.3. Air

The World Health Organization has set a limit of 10^{-6} mg L^{-1} for total mercury in air, with an averaging time of 1 year. In the U.K. levels of mercury alkyls (as Hg) over short term exposure (15 minute reference period) is 3 x 10^{-5} mg L^{-1}, and longer term exposure the level is 10^{-5} mg L^{-1} (8 hour time–weighted average reference period) (Health and Safety Executive, 2000).

CHAPTER 3

AN OVERVIEW OF ANALYTICAL METHODS USED IN MERCURY SPECIATION

Though numerous questions about the biogeochemical cycle of metals in the environment have been answered, many still remain unresolved. Sensitive, specific and precise analytical methods are needed to perform studies at ambient levels. Accurate determinations of metal species are critical to understand the biogeochemical cycling of the contaminant and estimating associated potential exposures of aquatic food webs.

In particular, accurate determinations of the organometallic species monomethylmercury (MeHg) pose a challenge to the analytical chemist (Monperrus et al., 2004).

Speciation analysis (i.e. the determination of specific chemical forms of an element) may be achieved in a number of ways. Most commonly, the analyte species are extracted from the sample matrix, ensuring that there are no losses of, or alterations to, the different chemical forms. This is followed by separation of these chemical forms using a chromatographic technique, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFE), or capillary zone electrophoresis (CZE). A number of detection methods are available for the speciation analysis of mercury (Monperrus et al., 2004; Sanchez Uria and Sanz-Medel, 1998): atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), microwave-induced plasma atomic emission spectrometry (MIP-AES), and inductively coupled plasma mass spectrometry (ICP-MS).

These techniques are often hyphenated with chemical vapor generation (CVG) also referred as cold vapor (CV) or cold vapor technique (CVT) for separation of Hg^0, MeHg, Et_2Hg, and other volatile vapors from solution (Tsalev, 1999).

The best performance is obtained with ICP-MS because it is sensitive and specific for mercury isotopes. It is capable to correct the artifacts during analysis. The main inconveniences are the high instrumental and operational costs. The AFS detector is also very sensitive for mercury, simple, and much lower priced. Coupled systems with AFS could be used in situ to overcome the sample storage problems. The limitations are scatter and background levels of impurities. Therefore, some spectral interferences may disturb the mercury species determination (B. Bouyssiere et al., 2000).

In a summary, Stoichev et al. (2006) presented the following steps to consider for mercury speciation in environmental samples:

- Sample collection with special attention to clean procedures.
- Sample pretreatment/ preservation/ storage to preserve mercury species concentration and distribution.
- Extraction from the environmental sample (i.e., from sediment or biota) securing the integrity of species.
- Clean-up/ preconcentration to achieve a final concentration matching the limits of detection (LODs) accessible with the detection technique selected.
- Separation of mercury species of interest.
- Detection and quantification.

Since it is difficult to compile all the methods used for mercury speciation, this chapter will give a brief overview with a particular emphasis on GC-ICP-MS.

3.1. Sampling and Storage

Sampling, the first step in trace element analysis, must ensure the representativity of the sample in the context studied. For this reason also, it represents the most potential source of errors. If the sampling is not based on the use of appropriate tools with particular cautions, both systematic and random errors may occur, attaining in some cases several orders of magnitude (M. Hoenig, 2001). That is why in this section special attention is given to the sampling and appropriate storage of the samples. It also has to be noticed that laboratory conditions such as working in a clean room are necessary throughout the analytical steps.

The choice of material is critical to preserve the speciation of mercury in water samples. Several studies investigated the stability of mercury species in standard solutions depending on the storage material (Krivan and Haas, 1988) and it was found that the solutions of MeHg are stable in glass bottles at 4^0C but the best storage material is polytetrafluoroethylene (PTFE) (P. Lansens et al., 1990). It is giving the lowest contamination. This fact is very important at extremely low concentrations found in real samples.

Rigorous cleaning procedures must be used for all laboratory ware and other equipment that comes into contact with samples. There are different cleaning procedures, but usually cleaning for several days in acid baths is included (T.A. Jackson, 1988).

After such treatments, the material is usually rinsed with mercury free deionized water or double distilled water and stored in mercury free area, preferably sealed in clean plastic bags. Some authors recommend storage of laboratory ware in dilute nitric or hydrochloric acids until use (Stojchev et al., 2006; Yu and Yan, 2003).

3.1.1. Water Samples

To adopt a sampling strategy it is necessary to consider the nature of the studied sites and the heterogeneity due to mixing of different water masses (Quevauviller, 2001).

The sampling and storage bottles have to be rinsed with the site water immediately before sampling.

Surface waters can be sampled with pumps using PTFE tubes but in many cases surface waters are taken "by hand" directly in the sampling bottle using long polyethylene gloves. The bottle has to be opened and closed under water to avoid mixing with the surface microlayer or oxidation sample (Stojchev et al., 2006).

The porewaters are collected by direct filtration of the wet sediments or centrifugation of the samples followed by filtration (Gilmour et al., 1998; Bloom et al., 1999). After sampling, the porewaters are treated in the same manner as the surface waters (Baeyens, 1992).

For the analysis of volatile mercury forms (Hg^0 and Me_2Hg), large volume of samples are recommended and the samples should not be acidified to avoid the oxidation of the volatile species (Parker and Bloom, 2005). If samples cannot be purged and trapped in the field, they should be collected in completely full glass bottles with Teflon-lined caps, as those species are lost rapidly from Teflon and polyethylene bottles (Parker and Bloom, 2005).

For the determination of mercury forms the best choice is "on-site" analysis to preserve the speciation. If it is not possible to analyze the sample immediately it has to be stored at special conditions (Stojchev et al., 2006).

The water samples are stored normally at 4^0C in the dark after the addition of a small volume ultra pure concentrated acid (0.1 to 1% v/v). The losses depend on the sample matrix and need further investigation (Yu and Yan, 2003).

Different factors like concentration of mercury species, matrix decomposition, container material, pH, temperature, and light have been considered, and ways of decreasing the loss and the transformation of the mercury species are proposed (Varekamp et al., 2000; Stojchev et al., 2004).

3.1.2. Solid sample

The sampling procedure for suspended particles is described previously, dealing with waters (water and suspended particles are simultaneously collected).
The contamination risk for sediments is less important than for waters but the technical problems can be very complicated. The sampling strategy depends on the objective of the study. For the investigation of the temporal variations it is necessary to collect the samples from the same site using Global Positioning System (GPS). For stratigraphic studies it is obligatory not to mix sedimentary layers (Stojchev et al., 2006).
The sediments can be sieved with Nylon® sieves to eliminate stones and other gross particles. They are transferred in acid-cleaned vials and immediately frozen (-20 ^{0}C) to increase the stability of MeHg (Parker and Bloom, 2005). Later in the laboratory the sediments can be dried either with clean air flux (Rodriguez Martin-Doimeadios et al., 2000) or freeze-dried (Varekamp et al., 2000).

3.1.3. Biological samples

To adopt a sampling strategy for biological samples it is necessary to consider the trophic level of the studied organisms (Zooplankton, plants, benthic organisms, macroorganisms, etc.) (Horvat et al., 1999; Heller and Weber, 1998).

In general, the samples are frozen immediately after the preliminary treatment. Later in the laboratory they are analyzed directly or after freeze-drying

(T.Stojchev et al., 2006). The biotissues should be stored in the dark to avoid photodegradation (Yu and Yan, 2003).

3.2. Preparation of solid samples

Sample preparation is one of the most crucial steps in trace element analysis and frequently controls the quality of the final result results obtained (Hoenig, 2001). Environmental solid samples are generally made into a solution with wet digestion methods and analyzed by compatible instrumental techniques.

Most of the conventional digestion procedures are not only laborious and time-consuming, but also lack sufficient efficiency and reliability (Tseng et al., 1998). Other extraction methods, such as sonication, distillation or soxhlet extraction, also have the above drawbacks, even though reliable results are usually achieved (Tseng et al., 1998).

Innovative techniques such as supercritical fluid extraction (SFE) and microwave-assisted extraction (MAE) (Lorenzo et al., 1999) have been developed and are a substantial advance. However, SFE potentially has technological limitations and shows insufficient extraction efficiency, usually depending on sample matrix and analyte polarity (Tseng et al., 1998). Besides, the expensive equipment required increases the cost of the analysis.

Two different approaches in microwave extraction procedures are the use of a closed system (pressurized with a closed vessel) (Vazquez et al., 1997) or an open system (non-pressurized with an open vessel) (Tseng et al., 1997). They have different characteristics and application. The main advantages of the MAE technique are absence of inertia, rapidity of heating, reduction of extraction time, better reproducibility and reliability, ease of automation, and good ability for selective leaching and total digestion in a wide array of sample matrices. Thus, the application of this technique to sample preparation has been widely investigated in various fields of the environmental and analytical chemistry (Tseng et al., 1998).

The extraction of total mercury (Hg_{TOT}) from sediments is performed with concentrated HNO_3 (Mikac et al., 1999) or acid mixtures (Gilmour et al., 1998) under efficient reflux or bomb decomposition. Sometimes additional oxidants are added, such as H_2O_2, $KMnO_4$, etc. (Varekamp et al., 2000).

In order to extract the mercury species intact, several types of milder are used, such as citrate buffer and extraction with dithizone/chloroform (Hempel et al., 1995), $KBr/CuSO_4/H_2SO_4$ (Lambertsson et al., 2001), HCl/HNO_3 mixtures (Dietz et al., 2001), etc.

Solid sample preparation by acid or alkaline extraction with different heating sources (sonication, stream distillation, etc.) requires from 2 to 24 hours for complete recovery of mercury species (Horvat et al., 1993).

The microwave extraction of the mercury species is an extremely fast method (2-10 min) (Rodriguez Martin-Doimeadios et al., 2003a and b). Both open and closed systems are used for alkaline or acid extractions of mercury species from the sediments (Dietz et al., 2001; Tseng et al., 1997). This method should, however, be used with caution since it may also suffer, as for other techniques such as distillation-based methods (Hintelmann, 1999), from artifact formation of MeHg (Landaluze et al., 2004).

The use of speciated IDMS (isotope dilution mass spectrometry) has proven to be a powerful tool to check whether any interconversion is taking place. The use of Hg(II) and MeHg labeled with multiisotopes allows discovering the error of the specific steps and their contribution to the overall transformations of a known species (Hintelmann, 1999; Monperrus et al., 2004). The inconvenience is that usually a long time is needed for equilibration between the sample and the spikes.

Biological Tissues

Analysis of biological tissues is less affected by artifacts and interferences during extraction procedures and quantification as compared to sediments. The quantification requirements are easily met, since the levels of MeHg are usually high. To extract the mercury forms, similar methods as for sediments can be applied (Baeyens, 1992).

3.3. Methods for Mercury Species Determination

The speciation of an element can be defined as the determination of the individual physico-chemical forms of that element which together make up its total concentration in a sample (Liu and Lee, 1999).

In numerous methodologies developed to date for speciation analysis, a few analytical techniques are applicable for simultaneous separation and detection of multiple species of an element, or of multi-elements. Of these techniques, gas chromatography (GC) and liquid chromatography (LC) are most commonly used (Stojchev et al., 2006). Capillary electrophoresis (CE) is a comparatively new separation technique (Kuban et al., 2007).

It is well known that volatile organometallic compounds can be directly detected by GC, LC and CE. However, most metallic compounds have low volatility, and hence are not suitable for direct GC analysis. Additionally, the sensitivity achieved by direct detection sometimes does not meet the requirement in real sample analysis (Liu and Lee, 1999). Thus, chemical modifications of the target analytes are applied to facilitate their analysis.

Depending on the nature of analytes, sample matrix, and the analytical approach, chemical modification has found wide applications. Efforts have also been made to improve separation and enhance detection signals in speciation analysis by GC

and LC combined with common detection approaches (Liu and Lee, 1999; Stojchev et al., 2006).

Based on the nature of analytes and the products formed, different chemical modifications of analytes can be performed. The two most commonly used approaches relevant to specific interactions are derivatization and complexation. Derivatization is a more permanent modification than complexation since reactions which are taking place in derivatization are irreversible (Liu and Lee, 1999).

In relation to the separation process, chemical modification can be performed in three ways, namely, pre-column, on-column or post-column. “on-line” or “off-line” are also used to described the respective modification modes.

In GC, the initial sample volume is different, for on-line and off-line methods. In the former, as much as several hundred milliliters of sample may be used. With off-line hyphenated systems, the sample injected is limited to microliter volumes.

A problem encountered sometimes by using chemicals modification for speciation analysis is the possible loss of speciation information in the original sample.

Therefore, attention must be given in choosing modification methods and appropriate reagents, and in controlling operational conditions governing the modification process (Liu and Lee, 1999).

3.3.1. Comparison of modification techniques applied in CE, LC and GC

Chemical modification presents a valuable means for speciation analysis in CE, LC or GC, and bridges the three analytical techniques. By converting the analytes from their existing forms, chemical modification renders them suitable for separation and detection. Moreover, most of the modification methods can be used in combination with other sample treatment strategies such as extraction and enrichment procedures. Table 4.1 gives a brief comparison of speciation analysis using analytes modification by CE, LC and GC.

Chemical modification in speciation analysis by CE, LC or GC is distinguished, in purpose, reagent used, operation conditions, performance and application from one another, caused by their inherent features. In CE, separation is based on the difference in electrophoretic mobility under high voltage. In LC, the role of the modification is to affect partition coefficients of analytes between the mobile phase and stationary phase. For GC, chemical modification is usually performed to adjust the volatility of analytes to facilitate their analysis (Liu and Lee, 1999).

Table 3.1: Comparison of the speciation analysis using analyte modification by CE, LC and GC (after Liu and Lee, 1999)

Method	**Preferred operation media**	**Detection technique**	**Reagent for modification**	**Compatibility with other sample treatment procedures**	**LOD**	**Application**
CE	Aqueous	UV, FD	Derivatizing agents, Complexing agents, surfactants	LLE, SPE, FASI	Sub-µg/l-mg/l	Water
LC	Aqueous, non-aqueous	AAS, AED, FD, ICP-MS, UV	Derivatizing agents, complexing agents, surfactants	LLE, SPE	ng/l-sub-mg/l	Water, rock
GC	Non-aqueous	AAS, AED, ECD, FPD, ICP-MS, MS	Grignard reagents, $NaBEt_4$, $NaBH_4$	LLE,SPE, SPME, MAE, cryogenic trapping	Sub-ng/l-low-µg/l	Air, soil, rock, water, tissues

Numerous reagents have been used for chemical modification of metallic species in GC, LC and CE (Table 4.1). In GC, in accordance with hydride generation, ethylation, or alkylation, sodium borohydride ($NaBH_4$), sodium tetraethylborate ($NaBEt_4$), and various Grignard reagents are utilized, respectively. In LC and CE, a great variety of reagents has been used, and provides advantage procedures over GC. In general, complexation and derivatization procedures for speciation analysis by CE or LC are usually simple and easy-to-use.

In GC, they are composed of many steps, and thus more complicated. A typical procedure is to perform simultaneously in aqueous solution the microwave-assisted dissolution of sample, ethylation of analytes with $NaBEt_4$, and extraction of modified analytes with organic solvent (Liu and Lee, 1999).

In speciation analysis using chemical modification, an important aspect that differentiates CE from LC or GC is detection. The LOD reported by CE is in a similar range to GC (Table 4.1). However, GC is usually superior to CE by providing better detection sensitivity in terms of concentration. GC, and LC, is also more compatible to numerous element-specific detection techniques, e.g., AAS, AES, MS and ICP-MS.

Although CE, LC and GC have been used in the speciation analysis of inorganic and organic metal species, it is noted that most studies have been performed using GC as far as speciation of organometallic compounds is concerned.

In brief, of the three techniques that employ analyte modification for speciation analysis, GC has been the most successful and most frequently used approach. This is because of the technique's high separation efficiency, versatile detection methods, well-developed analyte-modification strategies, and a wide range of sample pretreatment procedures (Liu and Lee, 1999).

3.3.2. LC and GC in mercury speciation

For the analysis of mercury forms in natural waters by high performance liquid chromatography (HPLC) it is necessary to preconcentrate them before the injection in the chromatographic column (Shade and Hudson, 2005). Direct coupling of HPLC with the detector is not sensitive enough to analyze real water samples. For this reason, post-column vapor generation is used to improve the sensitivity and decrease the matrix effects (Shade and Hudson, 2005).

However, generation of cold vapor from organomercury species requires an extra step for conversion to Hg^{2+}; otherwise, the efficiency of cold vapor generation depends on the species present. The conversion is usually performed online by different approaches such as chemical oxidation with $K_2Cr_2O_7$ at ambient temperature which requires a long reaction time for efficient conversion (Wu, 1991). Thus, UV radiation, microwave heating, or an external heating source is used to facilitate the decomposition of organomercury species (Falter and Scholer, 1994).

A gas chromatograph (GC) is a chemical analysis instrument for separating chemicals in a complex sample. It uses a flow through narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling (stationary phase). The function of the stationary phase in the column is to separate different components, causing each one to exit the column at different time (retention time) (Wikipedia).

For the separation of mercury species at environmental levels, GC is most often used since it offers quantitative transfer to the detector. When analyzing volatile forms (Hg^0 and Me_2Hg) after fast desorption from the sampling traps (gold trap, carbotrap or Tenax) they are preconcentrated and separated directly without

derivatization. But, in most of the cases, it is necessary to derivatize the ionic mercury species in order to convert them to volatile forms, which are then separated by GC and detected by specific atomic detectors. During the hydride generation (HG) with sodium borohydride ($NaBH_4$), the Hg^{2+} is transformed to Hg^0, while MeHg forms MeHgH. The derivatization should be applied in inert atmosphere and should start at pH 1-2 because otherwise MeHg is reduced to Hg^0. The HG can therefore be directly applied for sea and estuarine waters (Tseng et al., 1998).

During the ethylation (Eth) with sodium tetraethylborate ($NaBEt_4$) the Hg^{2+} is transformed to $HgEt_2$, while MeHg forms MeHgEt (Equations 4.1 and 4.2).

$$Hg^{2+} + 2NaB(C_2H_5)_4 \rightarrow Hg(C_2H_5)_2 + 2Na^+ + 2"B(C_2H_5)_3" \qquad (4.1)$$

$$CH_3Hg^+ + NaB(C_2H_5)_4 \rightarrow CH_3HgC_2H_5 + Na^+ + "B(C_2H_5)_3" \qquad (4.2)$$

The ethylation is motivated by the higher stability of MeHgEt compared to MeHgH. In salty water one part of MeHg is reduced to Hg^0 during the ethylation. Thus, this procedure can be directly applied only to fresh waters (M. Ceulemans and F.C. Adams, 1996). As mentioned above, isotope dilution ICP-MS is a new powerful approach to solve the problems with the matrix and non-quantitative derivatization. A drawback of the Eth procedure is the impossibility to distinguish between Hg^{2+} and $EtHg^+$, both species that often coexist in the environment (Cai et al., 1997). An alternative is the use of the propylation as a derivatization technique with sodium tetrapropylborate as derivatizing agent which is more tolerant to interferences from chlorides (Demuth and Heumann, 2001). However, it was found that the propylation of extracts from soil samples suffers also from artifact formation of MeHg and especially of ethylmercury during the derivatization (Huang, 2005).

3.3.3. Detection of mercury species

As it was mentioned above, several element specific detectors are used for the speciation of mercury: Atomic Absorption Spectrometry (AAS), Atomic Fluorescence Spectrometry (AFS), Microwave-Induced Plasma Atomic Emission Spectrometry (MIP-AES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) etc.

ICP-MS is a very sensitive, element selective detection system, which hyphenated with a separation module such as HPLC, GC or CE becomes a very suitable tool for speciation analysis.

ICP-MS is routinely used in many diverse research fields such as earth, environmental, life and forensic sciences and in food, material, chemical, semiconductor and nuclear industries. The high ion density and the high temperatures in the plasma make it an ideal atomizer and element ionizer for all types of samples and matrices introduced by a variety of specialized devices. Outstanding properties such as high sensitivity (ppt-ppq), relative salt tolerance, compound-independent element response and highest quantization accuracy lead to the unchallenged performance of ICP-MS in efficiently detecting, identifying and reliably quantifying trace elements (www.speciation.net).

3.3.4. Coupling of GC with ICP-MS

ICP technology was built upon the same principles used in atomic emission spectrometry. Samples are decomposed to neutral elements in high temperature argon plasma and analyzed based on their mass to charge ratios. An ICP-MS can be thought of as four main processes, including sample introduction and aerosol generation, ionization by an argon plasma source, mass discrimination, and the

detection system (See figure 4.1). The schematic below illustrates this sequence of processes**.**

Unlike the atomic emission spectrometer, ICP-MS spectrometers can accept solid as well as liquid samples. Solid samples are introduced into the ICP by way of a laser ablation system which can usually be purchased as an accessory. Aqueous samples are introduced by way of a nebulizer which aspirates the sample with high velocity argon, forming a fine mist. The aerosol then passes into a spray chamber where larger droplets are removed via a drain. Typically, only 2% of the original mist passes through the spray chamber. This process is necessary to produce droplets small enough to be vaporized in the plasma torch (Worley and Kvech, 1998).

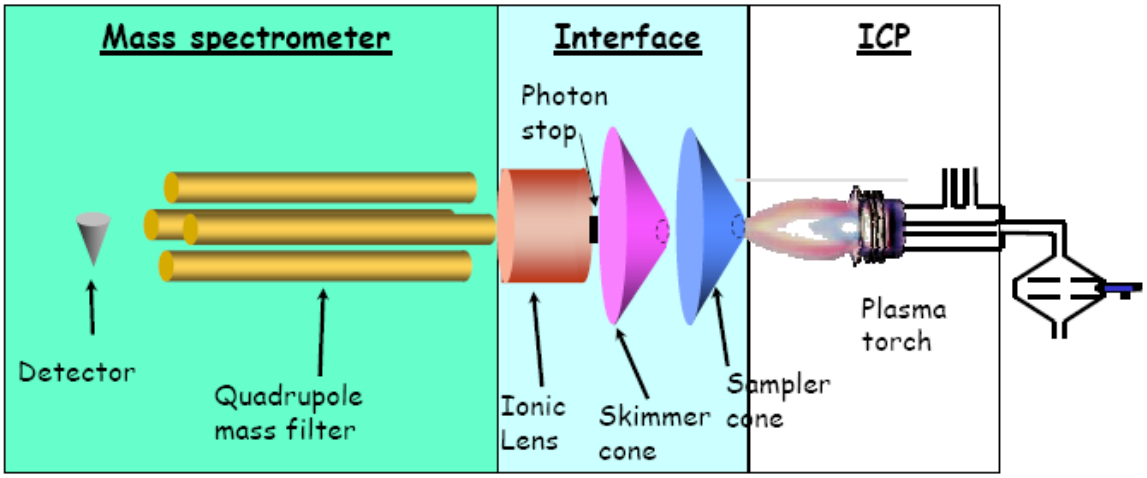


Figure 3.1: Schematic representation of an ICP-MS instrument

Because atomization/ionization occurs at atmospheric pressure, the interface between the ICP and MS components becomes crucial in creating a vacuum environment for the MS system. Ions flow through small orifices of approximately 1 and 0.4 millimeter in diameter, known as sampler and skimmer cones respectively, into a pumped vacuum system.

Several types of mass analyzers can be employed to separate isotopes based on their mass to charge ratio (Quadrupole, TOF, HR-MC,etc.).

Quadrupole analyzers are compact and easy to use but offer lower resolution when dealing with ions of the same mass to charge (m/z) ratio. Double focusing sector analyzers offer better resolution but are larger and have higher capital cost.

The quadrupole mass filter is made up of four metal rods aligned in a parallel diamond pattern (Figure 4.1).

After passing through the mass filter, the ions are either detected through direct current measurement on the ion collector (Faraday Cup) or the ions generate secondary electrons that are propagated in the multiplier. Together, both methods can cover an intensity range from a few ions/sec to 10^{12} ions/sec. Concentrations into the upper ppm ranges can be recorded with the Faraday Cup. Concentrations from ca. 10 ppm into sub-ppt levels can be measured with the secondary electron multiplier (SEM).

The objective of coupling GC with ICP-MS (Figure 4.2) is to analyze quantitatively the chemical forms of several isotopes and elements.

GC coupling allows transferring 100% of the sample to the ICP, while nebulization introduction only permits a few percent of the sample to reach the ICP. GC separation is performed by a temperature gradient and allows avoiding much isobaric or polyatomic interference.

The acquisition frequency of the detector is a major parameter when dealing with chromatographic transient signals. If the number of points (frequency) to define a peak is too low, a large uncertainty is produced on the peak integration and if the number of point is too high, the signal becomes noisy and thus lowers the detection limit. Therefore, a longer time taken in measuring each isotope (lower frequency) decreases the noise and increases the signal to noise ratio. As a consequence, the detection limit is decreased.

A compromise has than to be established between a good chromatographic peak resolution and good detection limits.

The acquisition frequency of ICP-MS detector can be improved by optimizing the dwell time.

Several coupling techniques are available for GC-ICP-MS:

- coupling between capillary GC and ICP-MS in dry plasma conditions;
- coupling between capillary GC and ICP-MS in wet plasma conditions;
- coupling between cryogenic GC and ICP-MS.

The dry plasma condition consists on a direct introduction of the carrier gas in the ICP. Thus, no desolvatation and vaporization of aerosols are needed. Besides, all the plasma energy is used to ionize the compounds eluted from the GC column.

This gives the possibility of working in cold plasma conditions (low power).

A drawback of this technique is the necessity of using an internal gaseous standard for optimization and of using oxygen to avoid carbon deposit on the cones.

In wet plasma condition a mixed introduction of the carrier gas and aerosols is produced by the nebulization chamber in the ICP.

Wet plasma presents several advantages than dry plasma conditions:

- easy to install since the nebulizer is not disconnected,
- easy to inject aqueous internal standards,
- possible use of non specific isotope dilution,
- no addition of oxygen to avoid carbon deposit (oxygen coming from water aerosols).

The main inconvenience of this technique is the possible increase of polyatomic interferences due to the aerosols.

Major challenges for interfacing GC to ICP-MS for speciation analysis are the avoidance of thermal degradation of the analyte species in the injector and column

and condensation at the interface during transport from the end of the column to the ICP torch. Today, GC-ICP-MS interfaces are commercially available.

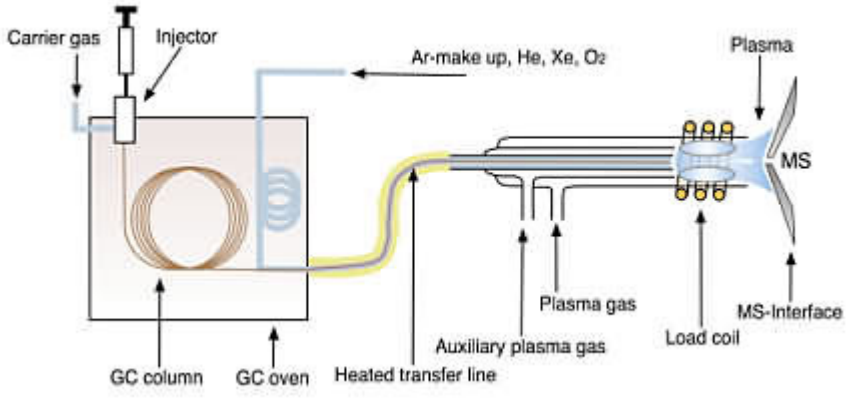


Figure 3.2: GC-ICP-MS hyphenated technique (www.speciation.net)

The advantages of such coupling techniques are essentially due to the quality of the ICP-MS as a GC detector: sensitivity, multielemental capacity and robustness.
As can be seen from the development of publications related to GC-ICP-MS, the technique suffers from its somewhat limited applicability (www.speciation.net). Within the field of speciation very few compounds fulfil the requirements for its application, namely volatility and thermal stability. As mentioned above, the analyst will have to resort to chemical reactions to transform nonvolatile compounds (usually ionic) into volatile thermally stable compounds (derivatization).
The main characteristic of GC-ICP-MS, in which it clearly differs from other atomic detection techniques such as MIP-AES, is its ability to provide isotopic information. This extra information can be used in two ways: to investigate the sources of elemental species, as in the studies performed on lead and sulfur, or to use isotope dilution methodology to study species transformation and to improve the quality of the analytical information obtained (www.speciation.net).

One of the great advantages of ICP-MS is extremely low detection limits for a wide variety of elements. Some elements can be measured down to part per quadrillion range (10^{-9} mg L^{-1}) while most can be detected at part per trillion levels. The table 4.2 below gives the limit of detection (LOD) of the most frequently used methods for the quantification of mercury.

Table 3.2: Most frequently used methods or quantification of Hg and their relative detection limit (Mason, 2006).

Method	Reported detection limit
Calorimetric methods	10 –100 ng g^{-1}
AAS graphite furnace (GF AAS)	1 ng g^{-1}
cold vapor (CV AAS)	0.01 – 1 ng g^{-1}
AFS cold vapor (CV AFS)	0.001 – 0.01 ng g^{-1}
NAA instrumental (INAA)	1 – 10 ng g^{-1}
Radiochemical (RNAA)	0.01 – 1 ng g^{-1}
GC Electron Capture Detector	0.01 – 0.05 ng g^{-1}
Atomic Emission Detector	~ 0.05 ng g^{-1}
Mass Spectrometer	0.1 ng g^{-1}
CV AAS/AFS	0.01 – 0.05 ng g^{-1}
HPLC UV	1 ng ml^{-1}
CV AAS	0.5 ng ml^{-1}
CV AFS	0.08 ng ml^{-1}
Electrochemical	0.1 - 1 ng ml^{-1}
ICP-MS	0.01 ng g^{-1}
GC-ICP-MS	$0.2.10^{-6}$ ng ml^{-1}
ICP-AES	2 ng ml^{-1}
X ray fluorescence	5 – 1000 ng g^{-1}
Electrochemical	0.1 – 1 ng g^{-1}
Gold-film analyzer	50 ng g^{-1}

3.4. Method validation

The phases involved when solving an analytical problem are as follows:

- problem definition and planning
- method selection
- method development
- method validation
- method application
- data evaluation
- data published

The purpose of method validation is to demonstrate that this method is fit for the purpose. This means that the method, as used by the laboratory generating the data, will provide data that meets the criteria set in the planning phase.
In the case of trace analysis, the following criteria are typically evaluated as part of the method development process:

- ***Specificity***: involves the process of line selection and confirmation that interferences for instrument (e.g. ICP-MS) measurement process are not significant..
- ***Accuracy or Bias***: can be best established through the analysis of a certified reference material (CRM).
- ***Repeatability*** (single laboratory precision): can be initially based upon an homogeneous sample and is measured by the laboratory developing the method. It is expressed as standard deviation (SD).
- ***Limit of detection (LOD)***: the detection limit of the method is defined as 3SD.

- ***Sensitivity or Delta***: is defined as $C=2(2)^{1/2}$ SD. This represents the minimum difference in two samples of concentration C that can be distinguished at the 95% confidence level.
- ***Limit of quantitation (LOQ)***: is defined as 10SD and will have an uncertainty of ~30% at the 95% confidence level.
- ***Linearity or Range***: is a property that is between the LOQ and the point where a plot of concentration versus response goes non-linear.

The regular use of certified reference materials and quality control (QC) materials with certified or information values for the total mercury concentration (Hg_{TOT}) or Hg_{TOT} and MeHg is indispensable in procedure development, validation, verification, and internal QC. Such materials could be found , together with original certificates , certification reports, and ordering information comprising matrices such as ethanol/water matrix, seawater, saline water, sediment, wastewater, etc (Stojchev et al., 2006).

CHAPTER 4

OBJECTIVES OF THE STUDY

As it was mentioned previously, comprehensive emissions data for various anthropogenic and natural sources of mercury within SA are not available.
To improve the quantitative environmental fate component of the risk assessment for mercury and its compounds, there is a need for more and better mercury emissions data and measured data near sources of concern, as well as a better quantitative understanding of mercury chemistry in the SA atmosphere, soils, water bodies and biota. Therefore, there is a great and urgent requirement to develop mercury analysis and speciation methods for the SA compartments since chemical and physical characteristics of mercury, as well as its toxicity, mobility and bioavailability depend on the chemical form that is present in a given environment. .

Validated methods, certified standards, field data (e.g. redox, pH, etc.) and skilled human resources are some of the requirements needed to obtain accurate, reliable, analytical data through appropriate sampling procedures (sampling handling and preparation) and analysis protocols (techniques) for the different mercury species.

The purpose of this study is the development of analytical methods and procedures for the speciation of inorganic and organic forms of mercury and their application to different environmental matrices collected in some SA compartments.
This will include:

- The development of sampling, extraction, separation and preconcentration methodologies for the speciation of mercury.
- The design, development and optimization of analytical methodologies to determine and quantify mercury species in

different environmental matrices such as water, soil, sediment, and biological samples.

- The application of developed methods for the speciation of mercury in some SA compartments affected by mercury pollution mostly from gold mining and chlor-alkali facilities.

CHAPTER 5

DEVELOPMENT OF INSTRUMENTAL METHODS FOR MERCURY SPECIES ANALYSIS

This chapter focuses on the instrumental design, development and optimization of methodologies for total mercury analysis and mercury speciation. The sample preparation is also discussed. The aim of this chapter is to provide details about the methodologies and instrumental parameters used before their application to real environmental samples.

5.1. Optimization of ICP-MS

ICP-MS instrument was optimized, before the hyphenation with a GC, by determining the total mercury concentration (Hg_{TOT}) using a certified reference material (CRM) of sediment.

Since the sample preparation is one of the main steps that lead to analytical errors, a particular care was taken during the preparation of the CRM prior to analysis, especially during sample extraction and the efficiency of the extraction technique used is also discussed.

5.1.1. Chemicals

All chemicals were pure analytical grade reagents and solutions were prepared in de-ionised water 18.3 MΩ cm (MILLIPORE). Concentrated nitric, hydrochloric, hydrofluoric and boric acids (Merck) were used for the sample extraction and a stock solution of mercury 100 ± 0.3 mg L^{-1} in 10% HNO_3 (Ultraspec) was also used for the preparation of standards.

5.1.2. Sample preparation

A rigorous cleaning procedure was followed in order to make sure that analysis will be done with a minimum risk of contamination. The following cleaning protocol was used for all experiments in this study:

- All vessels were first cleaned with a biocide detergent (1 or 2% in hot tap water) and stored for half an hour, rinsed thoroughly with tap water and then with MilliQ water.
- All vessels were then acid cleaned in a 10% (v/v) HNO_3 (technical acid) solution in MilliQ water and stored for 3 days. Bottles were then rinsed with MilliQ water.
- All vessels were finally acid cleaned in 10% (v/v) HCl (technical acid) solution in MilliQ water as described above for HNO_3. Bottles were then dried in an oven after being rinsed with copious amount of deionised water and stored in double plastic bags (zip lock).

The procedure used for the sample treatment was adapted from an existing method developed by the US Environmental Protection Agency (EPA 3052).

In order to get an optimized sample preparation procedure, four samples of CRM sediment (CRM015-050, RTC, USA), named samples A, B, C and D, were prepared or stored in different conditions.

For the sample extraction, 0.1 ± 0.005 g of sediment was digested in a closed microwave (Anton Paar, Multiwave 3000) at 800 W for 45 minutes using concentrated nitric (1 ml), hydrochloric (3 ml), hydrofluoric (1 ml) acids and hydrogen peroxide (1 ml). The temperature within the extracting vessels was between 167 and 184 ^{0}C. The microwave programme is presented in the table 5.1 below. Six millilitres of concentrated boric acid was added to each sample and all the samples were re-digested using the same microwave programme, except for sample A which was directly diluted with de-ionized water and stored in Teflon

bottles for mercury determination, this in order to study the impact of the emulsion formed after the first digestion. For the other samples a clear yellowish solution was obtained and all the samples were dilute with de-ionized water.

Table 5.1: Microwave programme for sample extraction

Sample weight: 0.1g

Phase	Power (W)	Ramp(min)	Hold(min)	Fan
1	800	15:00	30:00	1
2	0	0:00	0:00	2

p/rate: 0.3 bar/s IR:240^{o}C p:60 bar Drive: rot Stirrer: off

Reagent: HNO_3 (1ml); HCl (3ml); HF (1ml); H_2O_2 (1 ml)

Sample B was stored in ordinary volumetric flasks while samples C and D were stored in Teflon® bottles, and all samples were kept at 4^0C. In order to check the efficiency of storage conditions, samples B and C were analyzed a week after preparation while samples A and D were analyzed immediately after preparation.

5.1.3. ICP-MS analysis

An ICP-MS (SPECTROMASS 2000) instrument was used for the total determination of mercury in CRM sediment samples. To minimize sample contamination, cleaned torch and nebuliser were used. The spray chamber was also rinsed with de-ionized water. In order to reduce mercury adsorption in the walls of the sample introduction tubing, silicosteell tubing (Restek) was used instead of ordinary plastic tubing.

Instrument parameters such as ion optics voltages, mass scan, time scan, pump speed, argon flow were optimized for a better resolution and analyte-background intensity ratio and also to minimize the occurrence of oxides and non-singly

charged ions by two or more orders of magnitude compared to the analyte. The optimized instrument conditions are presented in table 5.2. The software used for data analysis was Smart analyzer provided by SPECTRO.

Table 5.2: ICP-MS parameters

Instrument Parameters	**SPECTROMASS 2000**
RF power	1350 W
Nebulizer gas flow	1000 ml min^{-1}
Coolant gas flow	3 scale units
Auxiliairy gas flow	1 scale units
Pump speed	2
Nebulizer step	1
Default dwell time	1s
Isotope monitored	^{199}Hg, ^{201}Hg, ^{202}Hg
Sampler	Nickel
Skimmer	Nickel
p Interface	2.013 mbar
p Quadrupole	5.46 x 10^{-6} mbar
Detection mode	SEM
Data analysis	Smart analyzer software

Mercury standards of 5, 10, 20 and 50 ng ml^{-1} in 1% HNO_3were prepared from a stock solution of 100 µg ml^{-1} for the instrument calibration and 3 replicates of each sample were ran. Results are presented as means of 7 measurements.

5.1.4 Results and discussion

An excellent linearity was obtained during the calibration for all the isotopes analyzed and the range was 0.06 to 60 ng ml^{-1} for ^{202}Hg which gave the best regression coefficient with R^2 = 0.9991 (see Table 5.3). The detection limit for ^{202}Hg isotope was then 0.06 ng ml^{-1} (ppb). The calibration curves are presented in the figure 5.1.

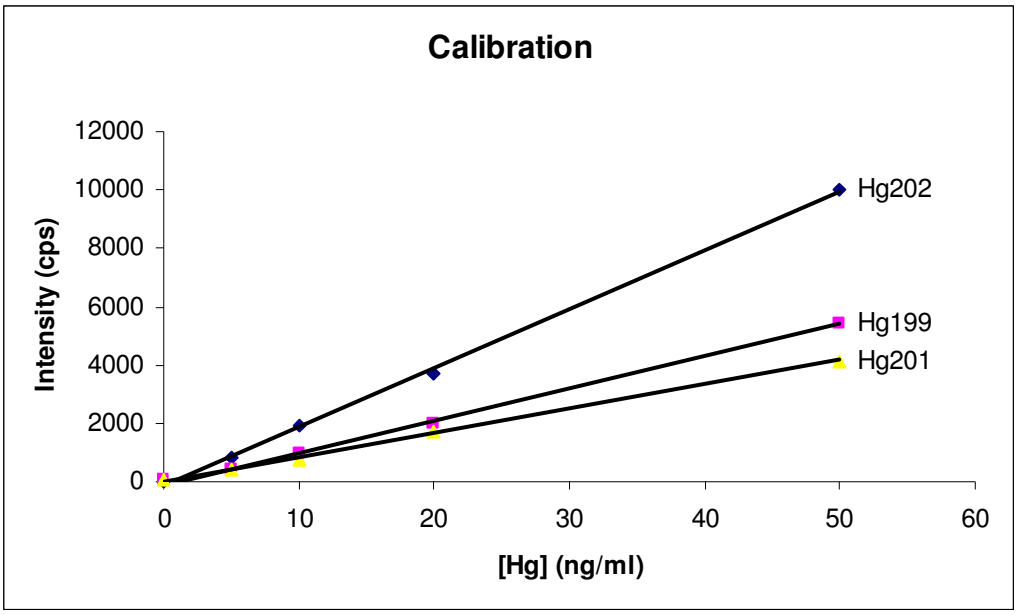


Figure 5.1: ICP-MS calibration for different mercury isotopes

Table 5.3: ICP-MS calibration parameters

	^{199}Hg	^{201}Hg	^{202}Hg
Range (ng g^{-1})	0.093 - 60.000	0.107 -60.000	0.067 – 60.000
DL (ng g^{-1})	0.093	0.107	0.067
BEC (ng g^{-1})	0.038	0.075	0.048
Correlation coefficient	0.9978	0.9982	0.991
Standard Error	0.156	0.122	0.099
Weighing	Manual	Manual	Manual

Table 5.4: Total Hg concentrations on CRM015-050 measured with ICP-MS and % recovery determined from the certified value

Sample	**[Hg] (mg L^{-1})**	**%RSD (n = 7)**	**SD (n=3)**	**Mean (95% CI)**	**Certified value (mg L^{-1})**	**Mean % recovery**
Sample A	0.129 0.144 0.098	8.2 6.6 7.7	0.019	0.124 ± 0.047	0.10 ± 0.02	124
Sample B	0.095 0.077 0094	3.2 10.1 3.9	0.010	0.089 ± 0.025	0.10 ± 0.02	89
Sample C	0.102 0.097 0.100	3.6 9.1 1.2	0.003	0.099 ± 0.007	0.10 ± 0.02	99
Sample D	0.110 0.110 0.105	1.1 3.9 8.3	0.001	0.108 ± 0.002	0.10 ± 0.02	108

The confidential interval given for the CRM was 0.99 to 0.11 mg L^{-1} (ppm). It can be seen from the Table 5.4 that excellent repeatability and reproducibility were obtained with samples C and D. Samples A and B presented a low reproducibility with outliers at 0.144 and 0.077 mg L^{-1} respectively. Discrepancies observed on sample A might be due to the matrix effect since these samples were not fully digested after the microwave extraction.

Sample B also has shown a low recovery (89%) compared to samples C and D even though they were all prepared in the same conditions. The loss of mercury in sample B can be explained by the adsorption in glass containers used for the storage of this sample. Storage conditions can also explain the within samples difference observed for sample B. The result obtained for samples C and D showed an excellent agreement with the certified value and confirmed not only the accuracy of the developed method but also the efficiency of Teflon® bottles for a long storage of mercury solutions.

5.1.5 Conclusion

The determination of total mercury concentration was done using an optimized ICP-MS instrument. This experiment has shown that ICP-MS is sensitive and specific enough for mercury determination and quantification. A sample preparation procedure was also developed demonstrating the efficiency of the closed microwave extraction technique with percentage recovery close to 100%. The necessity of using appropriate containers for a long-term storage was also demonstrated.

5.2. Coupling of GC with ICP-MS

The present study was aimed to develop an analytical method for the determination of organomercury species in different environmental matrices. The approach to this problem was to couple a gas chromatograph (GC) for the separation of the different forms of mercury to an inductively coupled plasma mass spectrometry (ICP-MS) instrument for the metal-specific detection and quantification. The first goals on the way to the realization of such hyphenated system were as follows:

- to obtain clear peak resolutions of the different mercury forms;
- to arrive at the capability of detecting multi-forms of mercury;
- to achieve a good detection power for the analyzed species and
- to be in the position to carry out quantitative analysis on the data.

The development of GC-ICP-MS is described for the determination and quantification of inorganic and organic mercury species, especially methylmercury (MeHg). A certified reference material (CRM 463 tuna fish, BCR) of tuna fish was used for this purpose.

5.2.1 Design, development and optimization of hyphenated GC-ICP-MS

5.2.1.1 Instrumental design

A schematic view of the instrumental set-up is presented in figure 5.2. A gas chromatograph was equipped with a capillary column and coupled to an inductively coupled plasma mass spectrometer (SPECTROMASS 2000) via a transfer line made of stainless steel. The transfer line was wrapped with thermal ceramic fibres (Cerablanket) to avoid the loss of heat (Figure 5.3). The transfer configuration allowed working in wet plasma conditions.

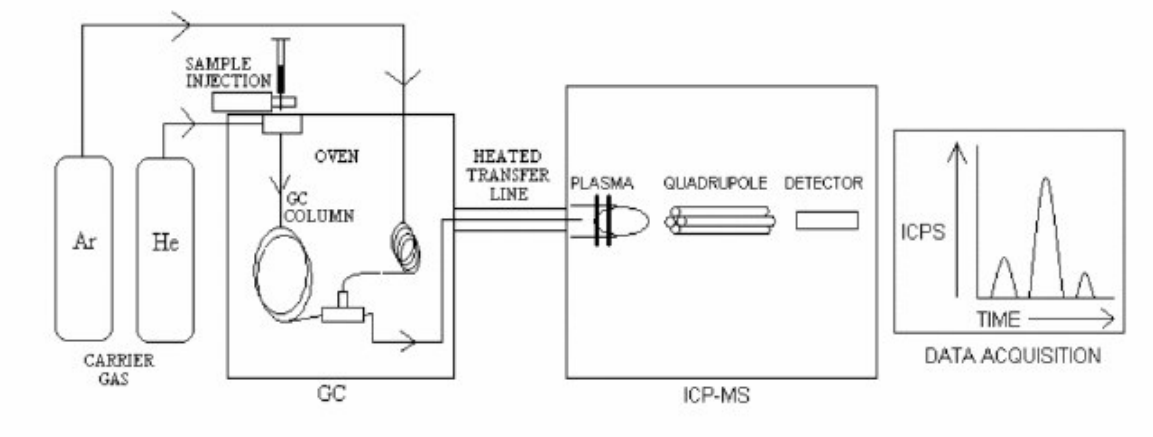


Figure 5.2: schematic representation of the used hyphenated technique (Nsengimana, 2007)

The transfer line was inserted into the torch injector, and the connection to the torch was realized by means of glass T-piece. A cooled spray chamber and a conventional Babington nebuliser were connected to this T-piece (See figure 5.4). This configuration allowed optimization of instrument performance since it enables the use of internal standards for mass bias correction during the chromatographic run by a continuous aspiration from the ICP sample introduction system. Other advantages of wet plasma conditions were presented in chapter 4.



Figure 5.3: GC-ICP-MS coupled with the transfer line on top of the GC (in white).

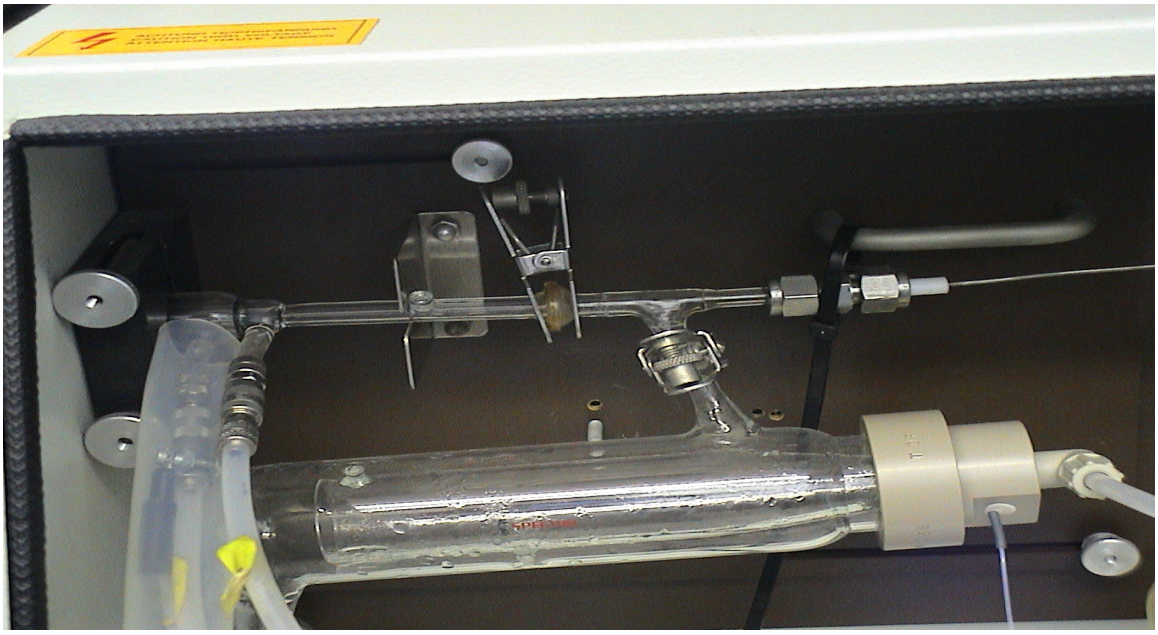


Figure 5.4: “T-piece” glass used for the connection of the transfer line (top right) and the nebuliser to the plasma torch (top left).

5.2.1.2 Method development and optimization

GC-ICPMS Conditions

The organomercury compounds investigated included the following: ethylated methylmercury and inorganic mercury species (MeHg, IHg). As mentioned, the objective of GC-ICP-MS optimization was to conduct simultaneous detection of different mercury forms in a reasonable time with optimized peak profile for accurate species measurements. The principal chromatographic parameters that were taken into account during the optimization of the separation conditions for the above organomercury compounds were the effect of the baseline perturbation on the chromatographic signal and the peak shape and intensity.

GC separation parameters were optimized with a standard solution containing MeHg, IHg, to obtain symmetrical peaks. GC conditions were chosen in a way that the elution of the species is sufficiently away from the zone disturbed by the solvent elution (isooctane). The temperature program, controlled by a programmable temperature controller (REX-P48/96, RKC Instrument), and gas flow were optimized to get good chromatographic resolution for all the species. All the species were separated to baseline within a chromatographic run of only 2 minutes.

Peak shape was first improved by heating the transfer line. To avoid decomposition of the coating of the transfer capillary and to ensure that the matrix compounds do not condense on the column wall when real samples are analyzed, a transfer-line temperature of 200°C was chosen for analysis.

The makeup gas flow through the transfer line was optimized to obtain both best symmetric peak shape and high sensitivity. The makeup gas flow changed slightly, because both the GC and ICP-MS instruments were frequently coupled and uncoupled, to enable their use for other applications. The maximum values

obtained for the makeup gas flow was 300 ml min^{-1}. The optimized operating conditions of the GC and ICPMS are presented in Table 5.5.

The raw data of the transient isotope signals for the different species were further processed using Excel software to obtain the peaks intensities of the corresponding isotopes.

Table 5.5: ICP-MS and GC operating conditions

ICP-MS

Instrument Parameters	SPECTROMASS 2000
RF power	1350 W
Nebulizer gas flow	1000 ml min^{-1}
Coolant gas flow	3 scale units
Auxiliairy gas flow	1 scale units
Pump speed	2
Nebulizer step	1
Dwell time	500 ms
Isotope monitored	^{199}Hg, ^{202}Hg
Sampler	Nickel
Skimmer	Nickel
p Interface	2.013 mbar
p Quadrupole	5.46 x 10^{-6} mbar
Detection mode	SEM
Data analysis	Smart analyzer software

GC

Gas-chromatograph	Home made
Column	DB 1701; 30 m; 0.32 mm i.d.; 0.25 µm coating
Temperature programme	60-200 ^{0}C, 30 ^{0}C min^{-1}
Oven temperature	200 ^{0}C
Transfer line temperature	200 ^{0}C
Carrier gas/ml min^{-1}	Helium/ 5
Makeup gas/ ml min^{-1}	Argon/300
On-column injection volume/µl	1

The temperature of the plasma is affected by the RF power (Heisterkamp and Adams, 2001). Applying a low forward power can reduce the background significantly, because formation of argon-based polyatomic ions is reduced.

But, reducing the forward power also affects the stability of the plasma, resulting in destabilization during elution of the solvent. Hence, normal plasma conditions with a forward power of 1350 W were chosen to ensure the stability of the plasma after elution of the solvent

The integration time (dwell time) also has to be optimized, since it affects both peak shape and signal-to-noise ratios (Heisterkamp and Adams, 2001). Increasing the integration time improves the signal-to-noise ratio, but reduces the number of measurement points leading to the possibility of the deterioration of peak shapes. In this study a dwell time of 500 ms was used for all the experiments.

A chromatogram reflects the integrated area of the mass spectrometric peak of that isotope during the integration time. The background signal can contribute considerably to the mass spectrometric peak. Hence, background subtractions were performed during data analysis to obtain the net signal and, in addition, to correct for slight in-between measurement deviations in the background level.

All experiments were performed with a narrow (0.32 mm i.d.) wall coated capillary column (DB 1701) of 30 m in length and with a film thickness of 0.25 µm. A carrier gas flow of 5 ml min^{-1} was set to obtain fast chromatograms with capillary GC (CGC) by isothermal separation of the mercury species at 60 ^{0}C.

5.2.2 Material and Reagents

The reagents used were of the highest purity available unless stated otherwise.

De-ionised water was provided by a Milli-Q system (MILLIPORE). HNO_3 (Fluka), H_2O_2 (Merck), tetramethylammonium hydroxide (TMAH, Aldrich) was used for the microwave extractions. Methanol (Merck), sodium tetraethylborate ($NaBEt_4$ 97%, Aldrich), acetic acid glacial (ACE, analytical grade), acetate buffer (Aldrich) and isooctane (2,2,4-Trimethylpentane, CHROMASOLV® Plus, 99.5%, Aldrich) were used for the derivatization and stock solutions of inorganic mercury and methylmercury were prepared from mercury chloride ($HgCl_2$, Merck) in 1%

HNO_3 (v/v) and methylmercury chloride (CH_3HgCl, 13% Cl_2, Aldrich) in 5% methanol respectively. Working standard solutions were prepared daily by appropriate dilution of the stock standard solutions in1% HNO_3 (v/v) and all solutions were stored at 4^0C in the dark using Teflon® bottles or borosilicate bottles with Teflon® lined cap. Finally, 10 µl Syringes (HAMILTON Microliter™ syringes) were used for the sample injection into the GC.

5.2.3 Sample preparation

For Hg_{TOT} analysis a microwave digestion was done using the program presented in the table 5.6 below modified from the study done by Lee and Suh (2005).

Table 5.6: Optimized digestion parameter for the determination of Hg_{TOT} in CRM 463 tuna fish.

Microwave program

Sample weight: 0.25 g

Phase	Power (W)	Ramp (min)	Hold (min)	Fan
1	250	3:00	2:00	1
2	450	3:00	2:00	2
3	600	3:00	2:00	3

Reagent : HNO_3 (4 ml) ; H_2O_2 (1 ml)

The fish extraction procedure for mercury speciation using microwave heating has been described elsewhere (Monperrus et al., 2003). This method was developed by the Laboratoire de Chimie Analytique Bio-inorganique et Environnement (LCABIE, CNRS, France). Several modifications were done to the existing procedure. Briefly, 0.5 g of sample, a certified reference material of tuna fish

(CRM 463 N^0 407) from the Community Bureau of Reference (BCR), was accurately weighed in the extraction vessel. Then 2.5 ml of TMAH 25% (v/v) were added. Care was taken to minimize sample losses after adding TMAH by closing properly the vessel. The mixture was gently stirred, placed in the microwave (Anton Paar, Multiwave 3000), and extracted for 3 min at 40 W. After the extraction period, the sample was allowed to cool at room temperature. The extract was centrifuged for 5 minutes at 2500 rpm and the supernatant was then transferred to a clean bottle with a Teflon® cap and submitted to ethylation.

The organomercury species had to be derivatized in order to obtain volatile species. Concentrated ammonium hydroxide and 5 ml of acetic acid/sodium acetate buffer (0.1-0.2 M) were added to 3 ml of the extract to set the pH at 3 to 4. Two milliliters of isooctane and 5 ml of 0.5 to 1% (w/w) sodium tetraethylborate ($NaBEt_4$) were added. The bottle was immediately capped and hand shaken for 5 minutes. The organic phase was finally transferred to an injection vial and stored at -18 °C until measurement.

5.2.4 Analysis

For Hg_{TOT}, standard solutions of 5, 10, 15, 20 and 25 ng ml^{-1} at 1% HNO_3 (v/v) were prepared from a mercury certified standard (ULTRASPEC) of 10 mg L^{-1} in 10% HNO_3 (v/v) solution.

Three replicates were analyzed for each sample and results are presented as the means of the triplicates of the ^{202}Hg isostope.

External calibration was carried out for the speciation. Standards, of three calibration levels (IHg/MeHg of 0.1/0.2; 0.5/0.5 and 1/0.1 mg L^{-1} (ppm)), were prepared from stock solutions of CH_3HgCl and $HgCl_2$. A blank was also analyzed. All experiments were carried out on triplicate. The blank and the standards were treated in the same manner as described above (See section 5.2.3).

5.2.5 Results and Discussion

5.2.5.1 Analytical instrument

The capillary column chromatograms were obtained by use of the settings given in Table 5.5. An example of chromatogram obtained under optimal GC-ICP-MS conditions for a standard solution containing 1 mg L^{-1} MeHg and 0.1 mg L^{-1} (ppm) IHg is presented in Figure 5.5. It can be seen from this chromatogram that a clear separation of the different species has been achieved, good peaks were also obtained and measurements of mass separation (isotope specific detection) and of species separation (compound specific detection) can be achieved (See also Figure 5.6).

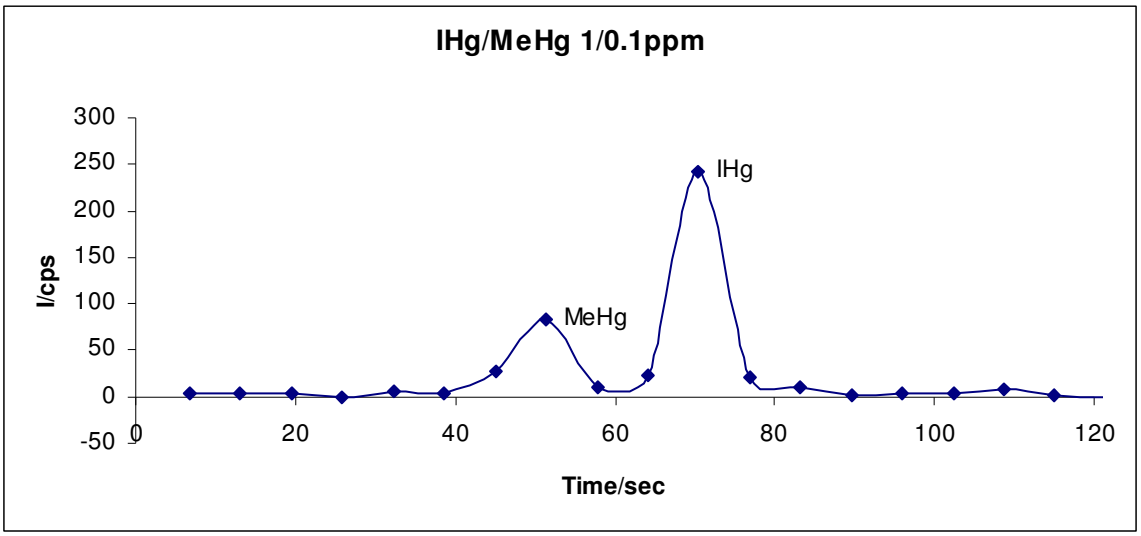


Figure 5.5: Example of Chromatogram of inorganic and organic mercury standard (1 and 0.1 mg L^{-1} respectively)

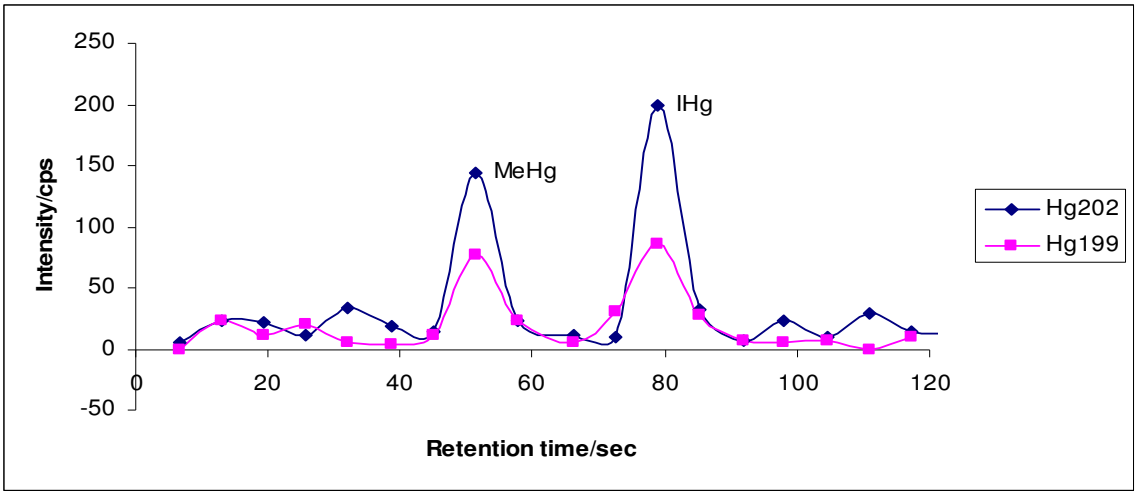


Figure 5.6: Example of Chromatogram of Hg isotopes 202 and 199 without baseline correction (IHg/MeHg:1/0.1 mg L^{-1}).

5.2.5.2 Linearity

Figure 5.9 presents the result of the external standard calibration obtained for the ^{202}Hg isotope (See also figures 5.7 and 5.8a, b and c). A linearity is obtained for the steps which included dilution, injection, column, transfer-line, plasma, interface and MS detection. It shows the excellent linearity of the GC-ICP-MS coupling system over a range of more than 3 orders of magnitude (0; 0.1; 0.2; 0.5; 1 mg L^{-1} of mercury standards were injected).

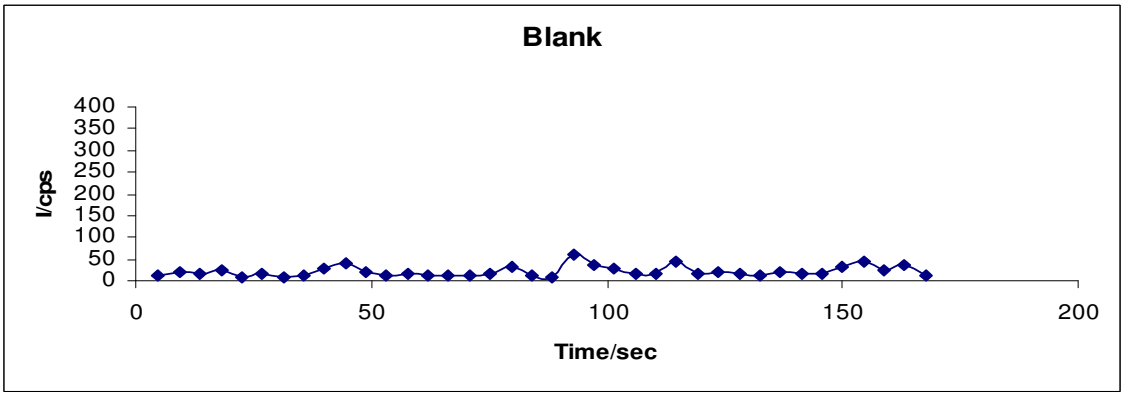


Figure 5.7: Chromatogram of a blank

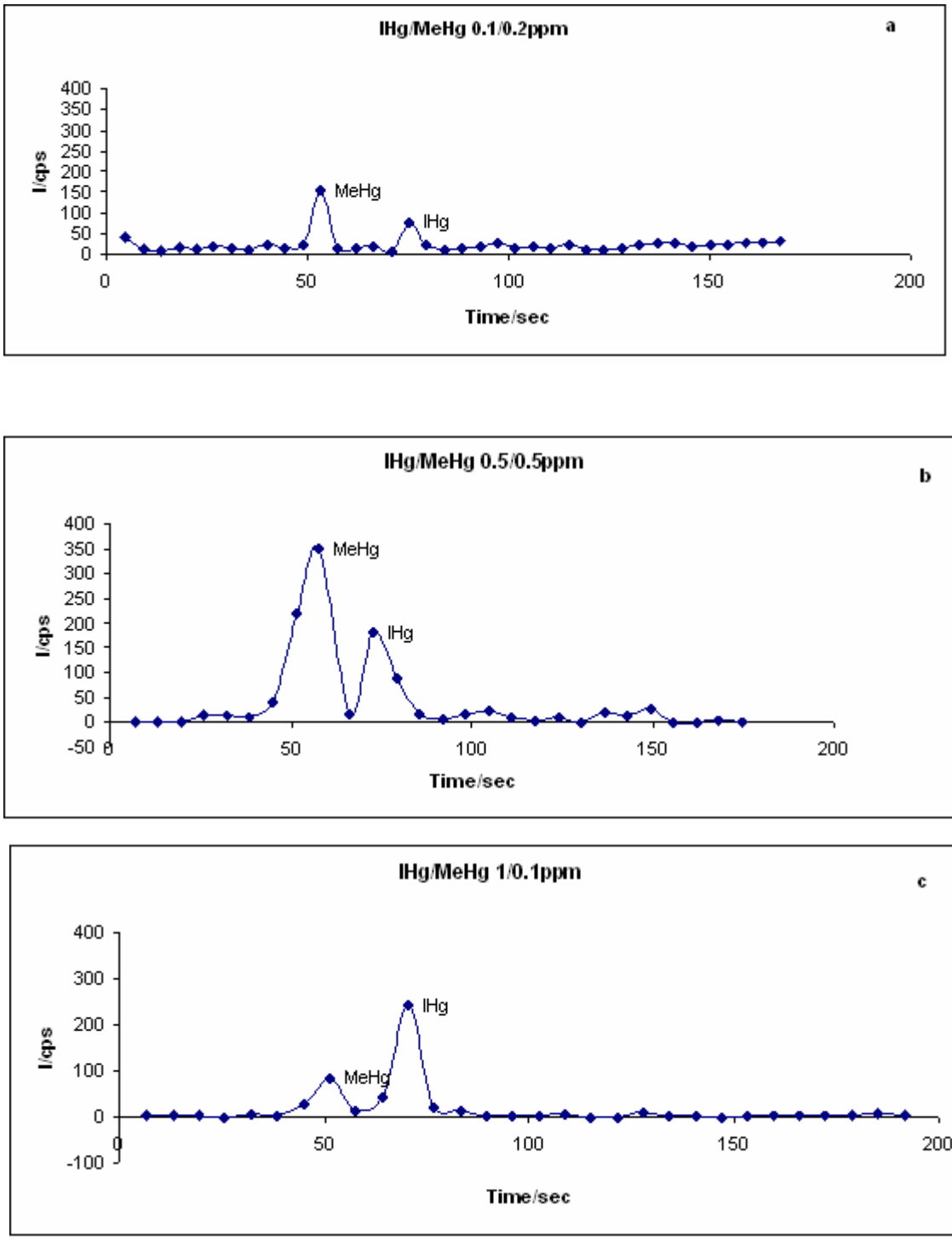


Figure 5.8a, b and c: Chromatograms of calibration standards

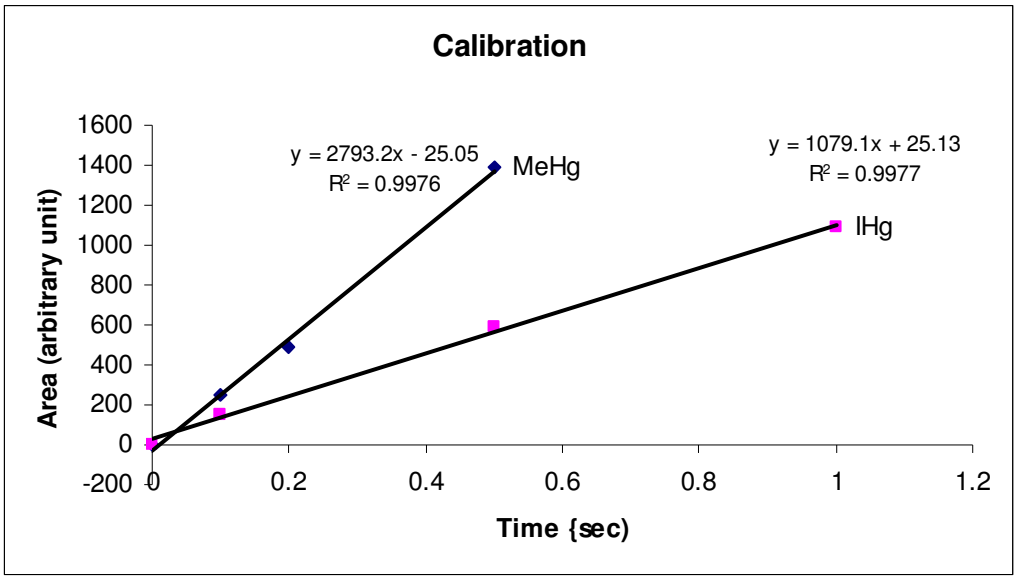


Figure 5.9: Calibration for inorganic and organic mercury species

5.2.5.3 Repeatability and detection limit

Peak heights and acquisition times are critical for the determination of isotope concentrations. Table 5.7 shows the RSD for seven replicates (n=7) of IHg and MeHg for the isotope 202. The RSD for the retention time is around 0.1% and 5.3% and the peak height RSD varies between 11.6% and 13.3% for MeHg and IHg respectively. It can be seen from the result that the excellent repeatability was obtained for the retention time and, although the RSD for peak intensity is beyond 10% it is still in the range of acceptable RSD at µg L^{-1} (ppb) level.

The peaks obtained during the calibration correspond to concentrations in the nanogram range. The instrumental detection limits were estimated by the equation DL = 3σ/p, where DL is the detection limit, σ the standard deviation of 6 blanks measurement and p the slope of the calibration curve. These limits depend only to a small extent on the individual species. The LOD for was 1.36 µg L^{-1} for both IHg and MeHg.

Table 5.7: RSD (%) of 0.5 mg L^{-1} IHg and 0.2 mg L^{-1} MeHg.

Retention time/sec		**Peak intensity/cps**	
IHg	MeHg	IHg	MeHg
79.15	51.49	187.72	172.36
79.15	51.49	139.07	148.69
79.15	51.49	176.89	190.92
76.26	51.31	198.91	144.62
78.97	51.31	161.56	172.76
76.26	51.31	199.76	189.91
84.31*	71.38*	129.97*	122.04*
RSD (%)=5.26	RSD (%)=0.10	RSD (%)- 13.32	RSD (%)= 11.61

*: These values were considered as outliers and, thus were not taken in account for the calculation of the RSD.

5.2.5.4 Analysis of the certified reference material

Three replicates of CRM 463 were analyzed and the result is presented in table 5.8. The extraction of mercury compounds in biological samples by microwave systems has been previously examined (see chapter 4). For this purpose, TMAH was found to be an excellent extractant. Tseng et al. (1997) reported a complete optimization of the different extraction parameters (TMAH concentration, heating time, microwave power). A 25% TMAH solution was found efficient to ensure complete solubilization after heating for 2-4 min at a power of 40-60 W. In this work, taking into account the previous optimizations and the fact that TMAH allows complete alkaline hydrolysis of biomaterials (i.e., proteins, lipids, and sugars) (Tseng et al., 1997) this extractant was chosen.

Two different determinations were done after ethylation with $NaBEt_4$ at pH 5 (acetate buffer 0.2M) and pH 4 (acetate buffer 0.1M) (See figure 5.10). The

former has leaded to a lower recovery compared to the latest. The same observation was made by Rodriguez Martin-Doimeadios R.C. et al. (2002) who demonstrated that a degradation of MeHg is occurring at pH 5. Thus, a non quantitative ethylation at pH 5 seems to be responsible for this low recovery.

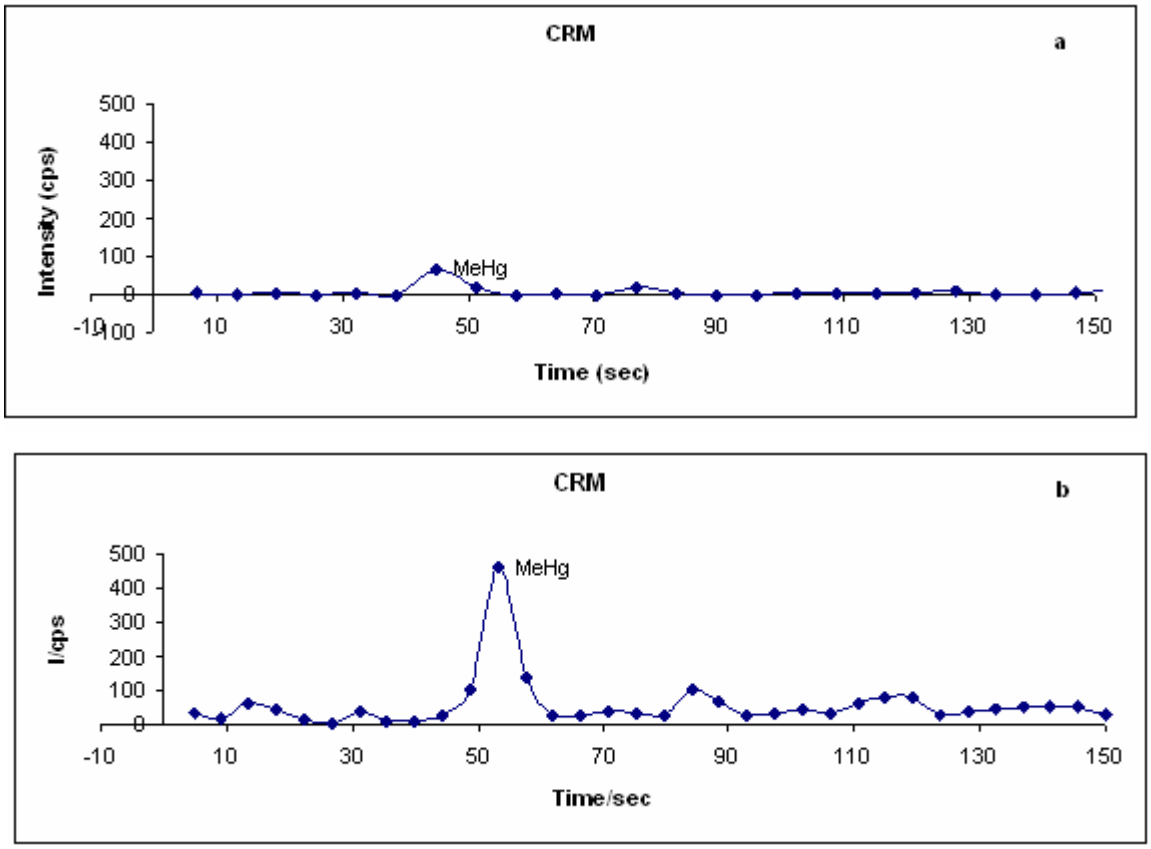


Figure 5.10a and b: Chromatograms of CRM 463 at different extraction conditions (pH 5 and 4 respectively) (MeHg: 0.3 mg kg^{-1}).

It has to be noticed that some interferences in biological matrixes may also decrease the ethylation yield (Rodriguez Martin-Doimeadios et al., 2002).

Table 5.8: MeHg concentration determined in CRM 463 Tuna fish by GC-ICP-MS

Sample	[MeHg] ($mg\ kg^{-1}$)	RSD (%) (n= 3)	Mean ± σ ($mg\ kg^{-1}$)	Certified value ($mg\ kg^{-1}$)	% recovery
Sample 1	2.87	6.1			94.4
Sample 2	2.97	5.9	2.91 ± 0.18	3.04 ± 0.16	97.7
Sample 3	2.90	2.9			95.4

The results obtained for the CRM 463 exhibited good agreement with the certified values for MeHg, quantified by external calibration. Recoveries were between 94.4 and 97.7 % and agreed within the limits of the certified values given by the Community Bureau of Reference. Extraction using TMAH appeared to be efficient for mercury species in biological tissues.

The concentration of total mercury concentration obtained using ICP-MS was 2.92 ± 0.12 mg kg^{-1} (x=3; 95% CI) with a recovery of 102.5% compared to the certified valued of 2.85 ± 0.16 µg g^{-1} (95% CI).

5.2.6 Conclusion

In summary, it can be stated that our initial efforts to develop a GC-ICP-MS method has resulted in a suitable coupling device for a fast, accurate, and precise simultaneous determination of mercury species in biological tissues. The detection limit is in an acceptable range and can be improved. The quality of the sample preparation method must also be underlined since reliable results were obtained from this method. The study has demonstrated the possibility of radically accelerating a critical step of sample preparation for speciation analysis, namely sample decomposition or extraction, by carrying them out in a low-power focused

microwave field. The three minutes single-step sample preparation used was equivalent in terms of recovery and accuracy to hours-long multisteps procedures commonly reported in the literature for speciation analysis. This study is the first, in our knowledge, to present the hyphenation of GC-ICP-MS for mercury species determination done within a South African laboratory.

5.2.7 Recommendations

A significant improvement in the precision of MeHg determination using external calibration can be done using the isotope dilution technique which will help in monitoring the artifact methylation that may occur during sample preparation.

The use of an internal standard is advisable for a better monitoring of the signal and for mass bias correction during analysis. Several parameters need also to be improved such as the peak intensity which was lower than in other works and thus had an influence in the detection limit. This can be done by either improving the extraction (derivatization) step or by modifying instrument parameter such as the dwell time. Further study must take in account the improvement of the detection limit for the quantification of very low mercury concentrations, such as in natural water samples, and the development of techniques for other matrices such as air samples.

Finally, the acquisition of commercially available software will help for a better data analysis.

CHAPTER 6

APPLICATION OF DEVELOPED METHODS

6.1 Introduction

Until recently, risk assessment of mercury pollution has mainly been based on total element concentrations. Unfortunately, the determination of such a virtual sum parameter provides limited information only, since chemical and physical characteristics, biological activity or toxicity, mobility and bioavailability do not depend on the presence and concentrations of elements but rather on chemical species. Therefore, knowing the distribution of different types of mercury can enhance and enable conclusions. For this reason, all current research recommends mercury speciation analysis instead of total mercury analysis.

Today, advances in analytical methodology make it possible to perform speciation analysis, and to utilize the enhanced information provided by speciation analysis for adequate risk assessment, optimization of remediation strategies and for solving other mercury related problems.

It was the major objective of this chapter to apply the methods developed in chapter in real environmental samples in order to assess their efficiency and the reliability of results obtained by using these methods. Different studies were done on speciation of mercury in water, soil, sediment samples and in biological tissues such as hair and fish, all collected at different environmental compartments affected by mercury pollution.

This chapter also will highlight the value of the enhanced information provided by speciation analysis and will demonstrate how this information can be used to tackle mercury related problems.

6.2 Mercury determination in a dismantled chlor-alkali plant

Chlor-alkali plants are one of the most important anthropogenic sources of mercury contamination in the environment.

The term chlor-alkali refers to the two chemicals which are simultaneously produced as a result of the electrolysis of a saltwater. Thus, the most common chlor-alkali chemicals are chlorine and sodium hydroxide (caustic soda) but can include potassium hydroxide and muriatic acid. There are 3 types of electrolytic processes used in the production of chlorine and caustic soda: the diaphragm cell process, the mercury cell process, and the membrane cell process.

The membrane cell is the most modern and has economic and environmental advantages. The other two processes generate hazardous wastes containing mercury or asbestos (World Bank Group, 1998).

The mercury cell process takes place in an electrolytic cell, where liquid mercury acts as a cathode. It attracts sodium (or potassium) cations with which it forms an amalgam. Chlorine gas collects at the anode (graphite). When the amalgam is added to water, the sodium (or potassium) reacts with the water to form sodium hydroxide and hydrogen, leaving the mercury, which can then be reused. Because mercury is highly volatile, mercury contamination occurs throughout the process, commonly leading to both the product (caustic soda) and the wastewater stream containing small amounts of mercury.

Mercury contamination and the potential risk of it conversion to methylmercury in soil was evaluated in a dismantled chlor-alkali facility by measuring the mercury species (IHg and MeHg) concentration in soil and water samples collected within the site. This site was selected since it presented some features that made it useful for the study purpose which consisted on the application of a developed and optimized method for mercury speciation in water and soil.

Unfortunately, details about the sampling site location and history are confidential. A sketch of the sampling site is given in figure 6.1 and a description of the collected samples is presented in Table 6.1.

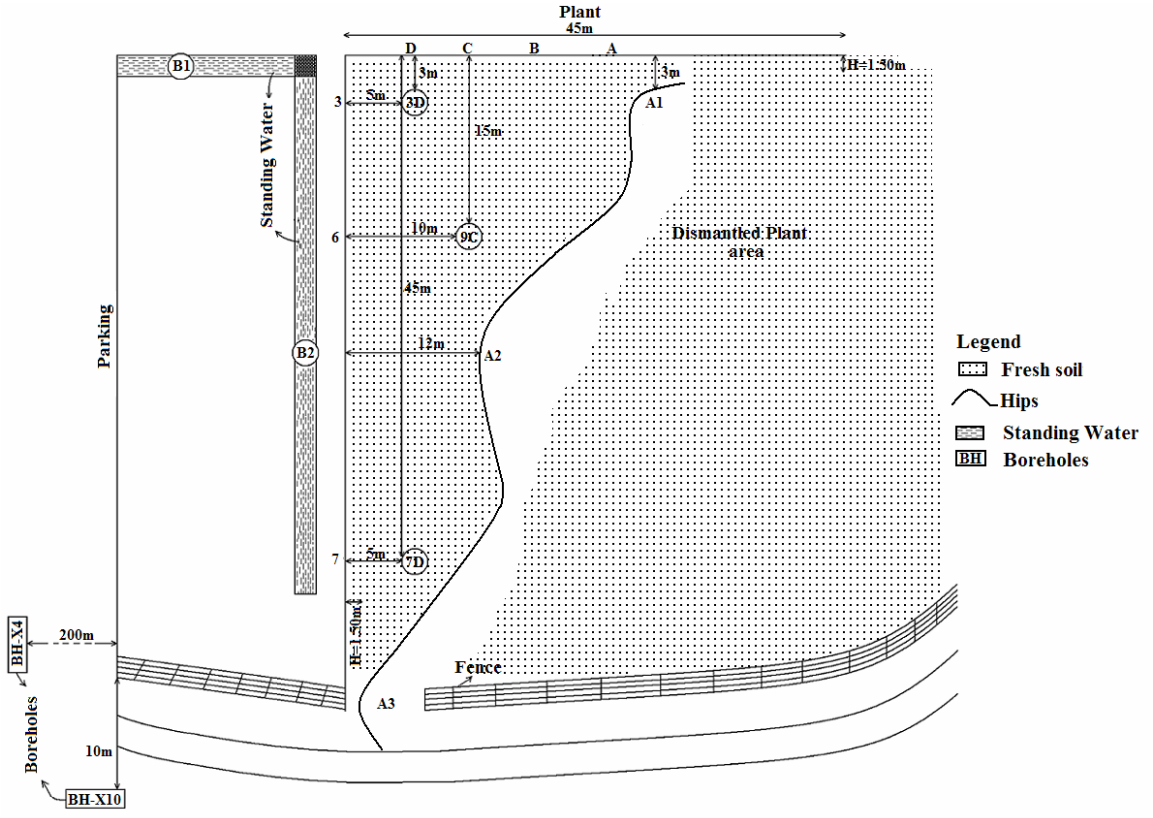


Figure 6.1: Sketch of the sampling site with indications where soil and water samples were collected.

Table 6.1: Description of collected samples

Sample name	Description
B1	Standing water(~100 m at the right side of the dismantled plant)
B2	Collected in standing water behind blocks 1 to 7
BH-X4	Borehole X4
BH-X10	Borehole X10
3D0	Collected at the intersection of blocks D and 3. Depth:0 cm
3D0-30	Intersection 3D. Depth:0 to 30 cm
3D30-60	Intersection 3D. Depth:30 to 60 cm
3D60-90	Intersection 3D. Depth:60 to 90 cm
7D0	Intersection blocks D and 7. Depth:0 cm
7D0-30	Intersection 7D. Depth:0 to 30 cm
7D30-70	Intersection 7D. Depth:30 to 70 cm
7D70-100	Intersection 7D. Depth: 70 to 100 cm
9C0	Intersection blocks C and 6. Depth:0 cm
9C0-30	Intersection 6C. Depth:0 to 30 cm
9C30-60	Intersection 6C. Depth:30 to 60 cm
9C60-80	Intersection 6C. Depth:60 to 80 cm
A1	Hips close to block A
A2	Hips close to 6C
A3	Hips close the site entrance

6.2.1 Material and Reagents

The reagents used for this study were of analytical grade level obtained from Sigma-Aldrich.

Mortars and pestles, filter papers, plastic bags, borosilicate bottles with Teflon lined cap, meters for field measurements, cooling boxes, nitrile gloves, masks, etc. were also used during the experiment.

6.2.2 Sampling and sample preparation

The sampling was done in November (wet season) on a wide bare field which used to contain a chlor-alkali facility and consisted on fresh soil at the surface. The presence of the fresh soil was due to the work which was done during the sampling period in order to balance the soil level throughout the site. Caterpillars were used for this purpose and, according to what was seen on the sampling day, soil was removed from the position 3D to 7D i.e. to the site entrance (see figure 6.1). The remaining soil, after balancing the level, was collected as hips named A1, A2 and A3 (see table 6.1) and corresponding samples were collected at the top of the hips.

It has to be noticed that, due the removal of the soil by the caterpillars, the actual surface of the soil was at 1.50 m depth from the foundation. Therefore, the analysis of hips was found necessary in order to obtain information about the mercury concentration at the original surface.

6.2.2.1 Soil samples

Soils Samples were collected at the dismantled chlor-alkali plant down to 80, 90, and 100 cm depth, in order to assess mercury concentration variability on the contaminated site. All physico-chemical or field parameters (temperature, pH,

conductivity and redox potential) measured *in situ* using appropriate instruments (WTW multi-parameter instrument pH/Cond 340i and ORP).The electrodes were checked using a standard buffer solution and the meters were calibrated according to the manufacturer's instructions prior to sampling. All reported redox potentials were corrected relative to the standard hydrogen electrode (SHE).

Soil samples were collected in clean plastic containers and were also rapidly frozen i.e. stored at 4^0C in the dark for few hours using cool boxes.

6.2.2.2 Water samples

Water samples were collected from two boreholes (BH-X4 and BH-X10) and two standing waters, probably from rain, named B1 and B2 and located near the dismantled plant (see figure 6.1). Field parameters were also measured *in situ*.

The sampling comprised the collection of 3 samples at each site. Cleaned borosilicate bottles with Teflon® lined cap were used for the sampling. The equipment used for sample collection was rinsed with the water to be sampled just before the water samples were collected and the rinse water was discarded away from the sampling point. This was done so as to condition, or equilibrate, the sampling equipment to the sample environment and to help ensure that all cleaning-solution residues had been removed before sampling (H Tutu, Thesis 2005). The samples were acidified on the field at 1% (v/v) with ultra pure HCl. Unacidified and unfiltrated samples were kept for anions test (Cl^-, F^-, NO_3^-, SO_4^{2-}) using ion chromatography. Blanks were also collected using de-ionized water to estimate the potential contamination due to the container and the storage. Sampling bottles were stored in double plastic bags, in the dark and at 4^0C.

Due to the presence of suspended particles in the samples from standing waters, it was found necessary to proceed to a filtration for B1 and B2. A blank was also filtrated in the same conditions than for the samples above.

6.2.2.3 Sample preparation

Once in the laboratory, 5g of soil sample were grinded using clean mortar and pestle. Then the grinded soils were freeze-dried using a lyophilizer, and finally, stored in a freezer at approximately -15^{0}C.

For the total mercury analysis, 0.1 g of the freeze-dried samples were digested in a microwave digester (Anton Paar Multiwave 3000) using a mixture of concentrated acids. The microwave program is presented in table 6.2.

A clear yellowish solution was obtained after digestion and 6 ml of concentrated boric acid was added to the digested samples. All the samples were re-digested at the same conditions and then diluted with de-ionized water and stored in Teflon® bottles at 4^{0}C.

Table 6.2: Microwave program for soil digestion

Reagent: HNO_3 (1ml); HCl (3ml); HF (1ml)				
Weight: 0.1g				
Phase	Power (W)	Ramp(min)	Hold(min)	Fan
1	800	15:00	30:00	1
2	600	10:00	20:00	2

p/rate:0.3 bar/s IR:240^{o}C p:60 bar Drive: rot Stirrer: off

For the mercury speciation, 1 g of freeze-dried samples were digested in a microwave (Anton Paar Multiwave 3000) at 60 W for 4 minutes using 10 ml of concentrated HNO_3 and the digested samples were filtrated, diluted with de-ionized water and finally stored in Teflon® bottles.

For total mercury (Hg_{TOT}) determination using ICP-MS (SPECTRO 2000), soil samples were directly analyzed after digestion without any further treatment while a derivatization step was necessary for the speciation using $NaBEt_4$ before introduction to the GC and the determination was done with ICP-MS. The following procedure was used for the derivatization of soil and water samples:

- Take 3 ml of digested sample and transfer to acetate buffer (0.1M)
- Adjust pH at 3.9 to 5 (Use acetic acid if necessary)
- Add 1ml NaBEt4 1% + 1ml isooctane
- Collect organic phase and transfer 1µl into GC vial

The 3:1 ratio (3ml sample and 1ml isooctane) in the preconcentration step was used for a better resolution since it was found that a 1:1 ratio was giving peaks with intensities to close to the background and than difficult to be analyzed.
Water samples were also ethylated in the same conditions as for soil samples. Figures 6.2 and 6.3 summarize the different sample preparation procedures used in this chapter.

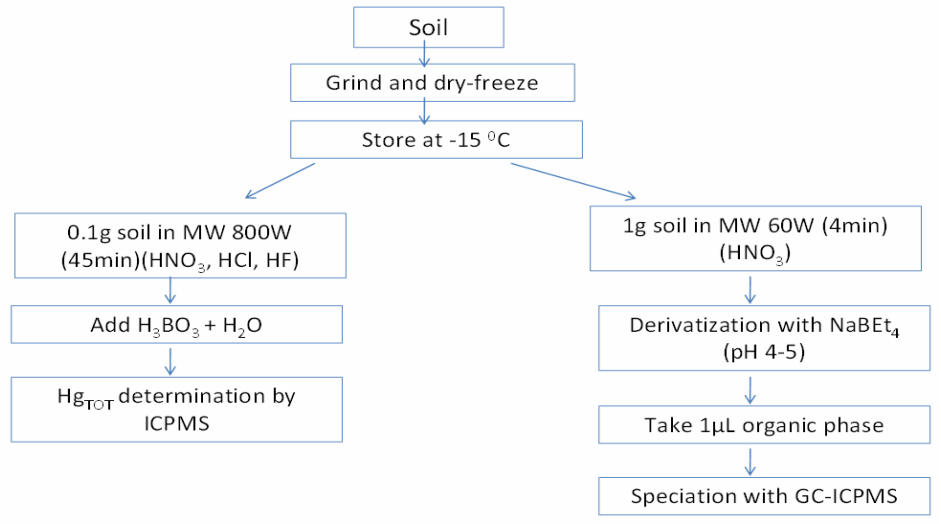


Figure 6.2: Sample preparation chart for mercury determination in soil

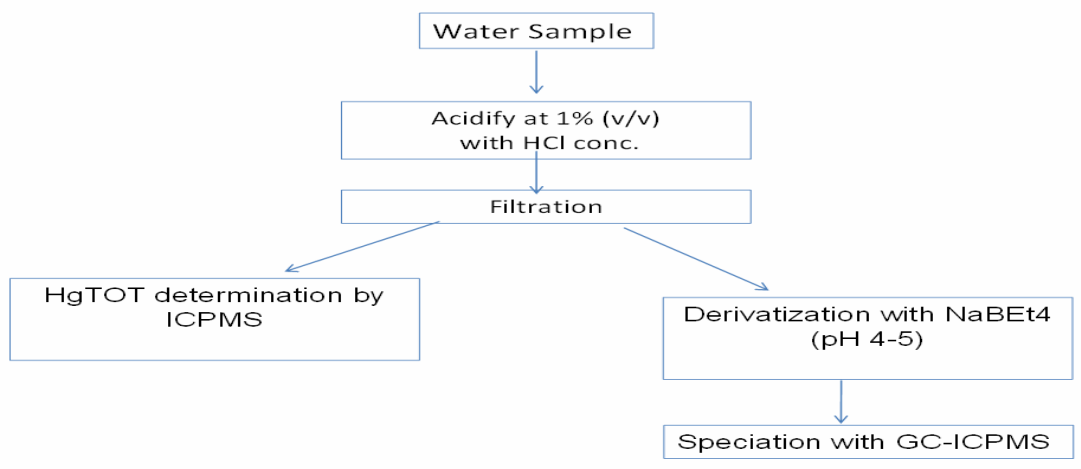


Figure 6.3: Sample preparation chart for mercury determination in water

6.2.3 Analysis

Soil and water samples were analyzed using ICP-MS and GC-ICP-MS for total mercury and for the speciation of mercury, respectively.

6.2.3.1 Total mercury

Mercury standard solutions of 0.5, 1.0, 1.5 and 2.0 mg L^{-1} at 1% HNO_3 (v/v) were prepared from a mercury certified standard (ULTRASPEC) of 10 mg L^{-1} in 10% HNO_3 solution. The obtained calibration curves for mercury are presented in figures 6.4 and 6.5.

Three replicates were analyzed for each sample and seven measurements were done on each replicate for statistical purposes. Results are presented as the mean values of the triplicates.

Blanks were analyzed in the same conditions as with real samples and a standard was run before, between and after samples analysis. Results are presented in table 6.4 and reported results correspond to the ^{202}Hg isotope which has given the best R^2 value for the standard calibration.

6.2.3.2 Mercury speciation

The speciation of mercury was done using a coupling of GC with an ICP-MS instrument. An external calibration was carried out. Standards, of three calibration levels, were prepared from stock solutions of CH_3HgCl and $HgCl_2$. A blank was also analyzed. All experiments were carried out on triplicate. The blank and the standards were treated in the same manner as described above (See section 6.1.2.3).

6.2.4 Results and Discussion

6.2.4.1 Standard calibration

The standard calibration for Hg_{TOT} was done at ppm level since high mercury concentrations were expected from samples to be analyzed. The calibration has shown a good linearity ($R^2 = 0.9991$ for ^{202}Hg isotope) on a range of 0.5; 1.0; 1.5; 2.0 mg L^{-1} (table 6.3 and figures 6.4 and 6.5).
The detection limit was about 0.003 mg L^{-1} (ppm). All the measurements for each triplicate have shown a good repeatability with a RSD always below 10% (table 6.3).

The instrument parameters used for the speciation of mercury were the same as for the CRM fish analysis. Once again the GC chromatograms for mercury standards have shown good sharp peaks (figure 6.6).
Figure 6.7 presents the result of the external standard calibration obtained for the ^{202}Hg isotope using GC-ICP-MS. This shows an excellent linearity for both inorganic and methyl-mercury standards in the range of 0.001 to 1 mg L^{-1}.
The instrumental detection limit (LOD) for both IHg and MeHg was about 0.0014 mg L^{-1}.

Table 6.3: ICP-MS Standard calibration parameters

	^{199}Hg	^{202}Hg
Range (mg L^{-1})	0.00471-1.80000	0.00318-1.80000
DL (mg L^{-1})	0.00471	0.00318
BEC (mg L^{-1})	0.00485	0.00090
Correlation coefficient	0.9989	0.9991
Standard Error	0.00276	0.00249
Weighing	Manual	Manual

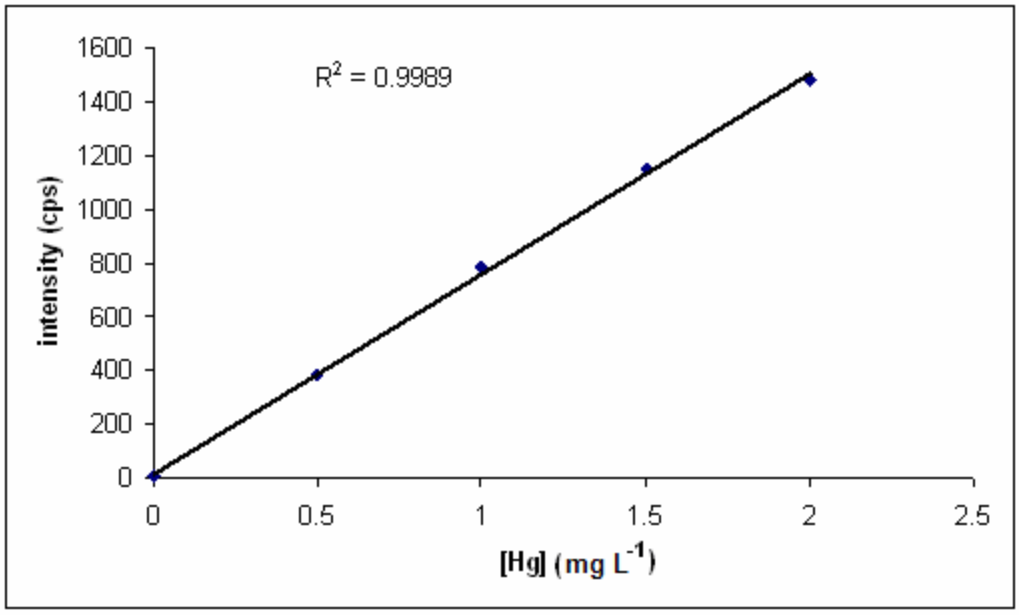


Figure 6.4: ICP-MS calibration for ^{199}Hg isotope

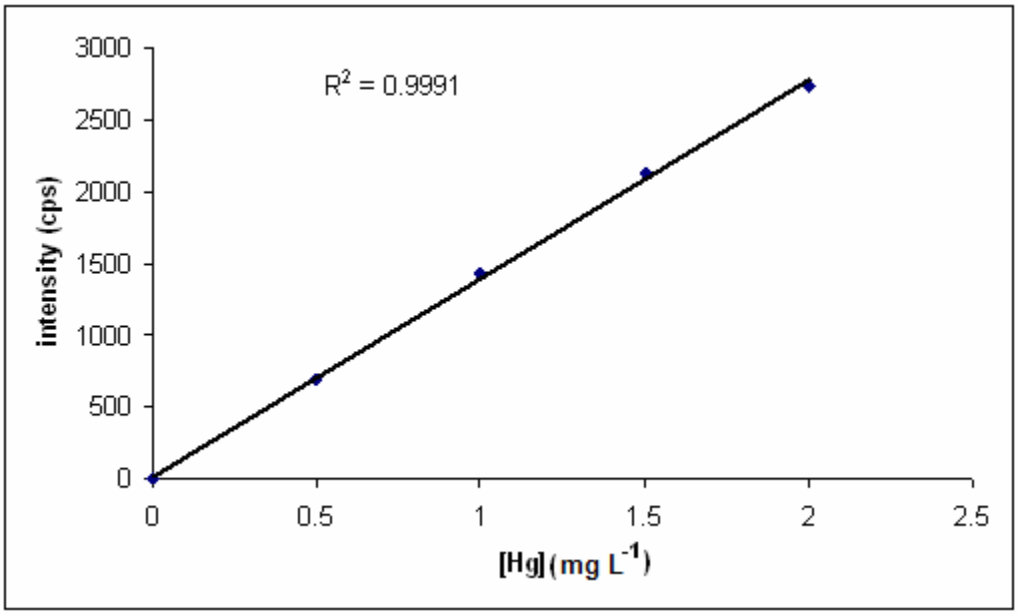


Figure 6.5: ICP-MS calibration for ^{202}Hg isotope.

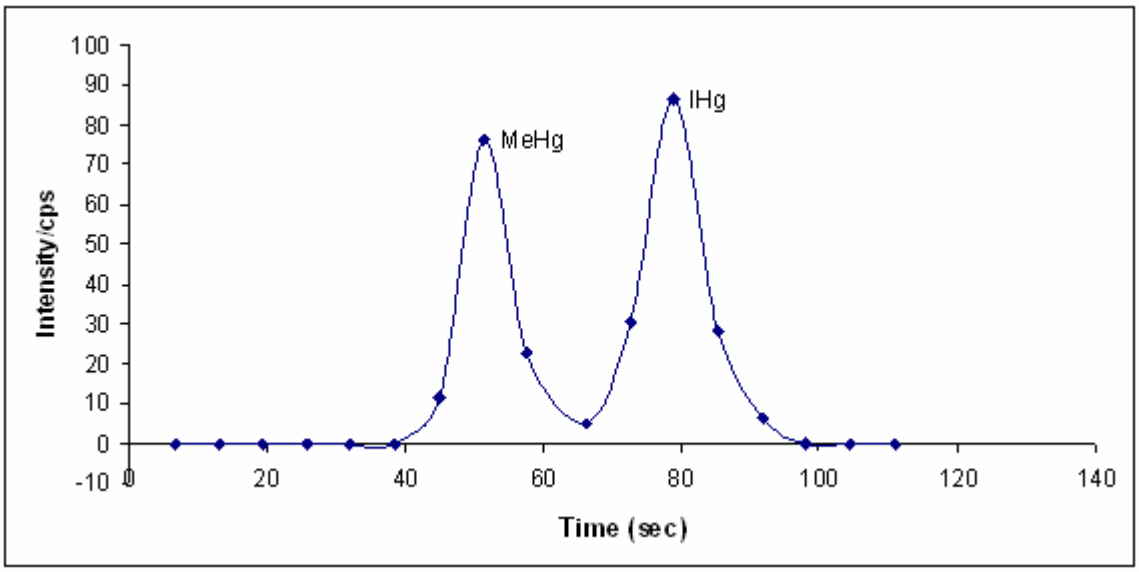


Figure 6.6: Chromatograms of Hg standards.

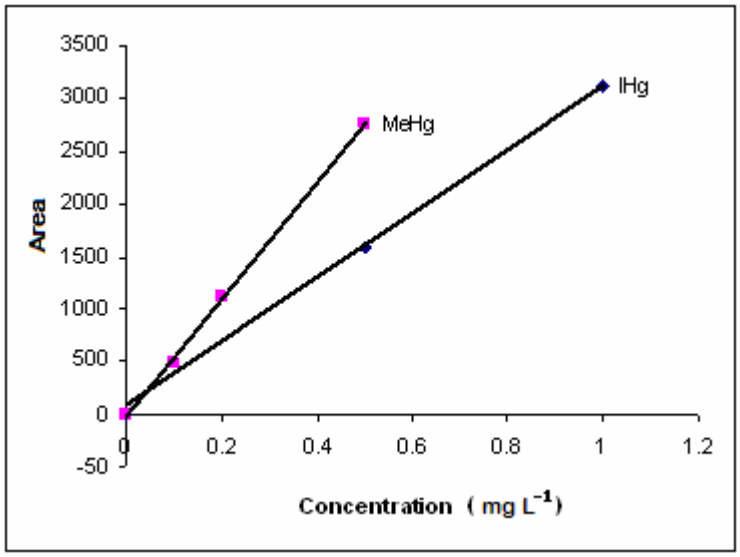


Figure 6.7: GC-ICP-MS calibration for ^{202}Hg isotope

In order to assess the reliability of the results obtained by this technique, a 0.5 mg L^{-1} spiked sample of MeHg was analyzed since a certified reference material for mercury speciation in soil was not available when the analysis was done. The analysis has given a MeHg concentration of 0.47 ± 0.07 mg L^{-1}(n=3; 95%CI) with a mean percentage recovery of 94%. This result showed a good agreement with the spiked concentration and confirmed the reliability of the developed method.

Good chromatographic resolutions were also obtained during analysis of soil and water samples (Figures 6.8 and 6.9), and no major matrix interference was observed.

Table 6.4 presents a summary of results obtained for mercury determination in water and soil samples collected from the contaminated site including field parameters measurements. It has to be noticed the good precision (repeatability) obtained for all the measurements since the percentage RSD was always below 10%.

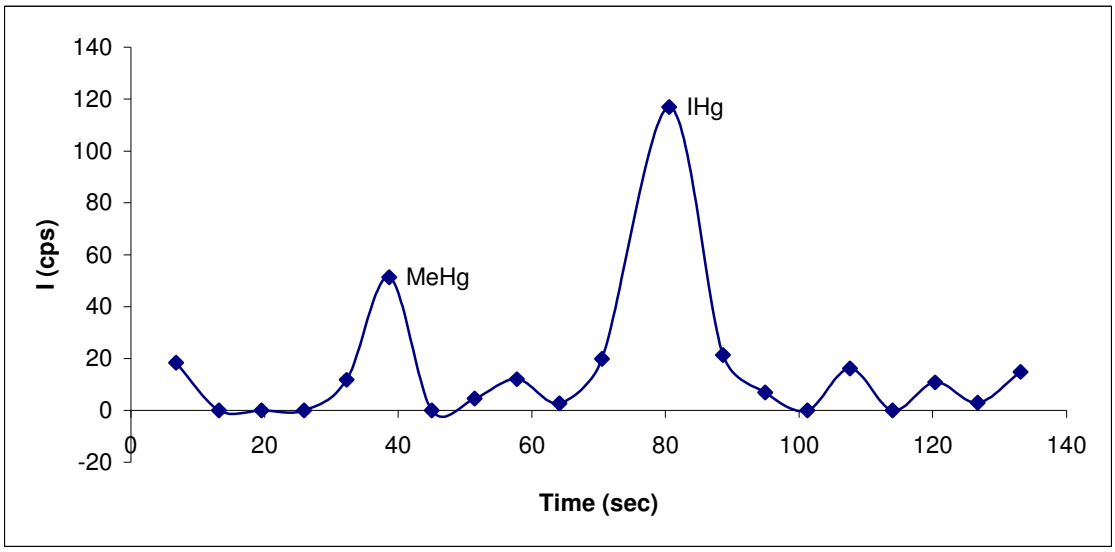


Figure 6.8: Example of soil chromatogram obtained with GC-ICP-MS.

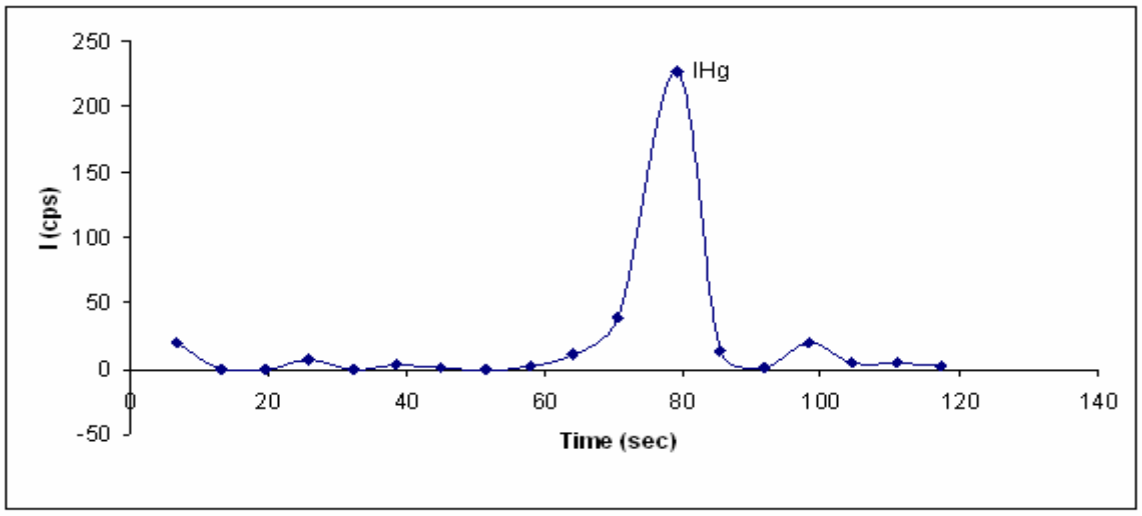


Figure 6.9: Example of water chromatogram obtained with GC-ICP-MS.

6.2.4.2 Soil samples

The total mercury concentration in soil samples varied between 0.64 to 89.68 mg kg^{-1} (Table 6.4). The inorganic mercury content in soil is in the range of 0.63 to 89.84 mg kg^{-1}. Rule and Iwashchenko (1998) have reported mercury concentration in the range of 0.24–6.6 mg kg^{-1} in contaminated soils from chlor-alkali plants

while Maserti and Ferrara (1991) have reported high mercury concentration ranging from 80.5 to 104 mg kg^{-1}. The results obtained showed a good agreement with the observation made by Revis et al. (1989) who stated that approximately 1-3% of the total mercury in surface soil is in the form of methylmercury. The other 97-99% of total soil mercury can be considered largely as Hg(II) complexes, although a small fraction of mercury in typical soil will be Hg^0.

Table 6.4: Samples measurement results obtained with ICP-MS (Hg_{TOT}) and GC- ICP-MS (IHg and MeHg)

Sample	T (^{0}C)	pH	ORP vs SHE (mV)	Conductivity (µS cm^{-1})	Hg_{TOT} (mg kg^{-1})	%RSD (n = 3)	IHg (mg kg^{-1})	%RSD (n = 3)	MeHg (mg kg^{-1})	%RSD (n = 3)
Water samples										
B1	22.7	8.95	386.4	611	0.005	5.7	0.006	7.2	nd*	-
B2	22.8	9.20	367.5	535	0.082	2.8	0.082	2.4	nd	-
BH-X4	23.1	6.04	412.2	27.2	0.343	2.3	0.359	4.3	nd	-
BH-X10	24.3	5.28	403.9	14.19	0.158	0.6	0.172	2.8	nd	-
1st soil profile										
3D0	23.3	9.53	371.6	21	4.606	6.5	4.572	8.1	0.042	9.5
3D0-30	22.4	9.68	350.4	23	0.640	7.0	0.627	9.5	0.017	5.8
3D30-60	22.7	9.78	329.4	32	nd	-	nd	-	nd	-
3D60-90	23.3	9.95	297.9	53	nd	-	nd	-	nd	-
2nd soil profile										
7D0	23.0	9.86	339.9	85	2.511	6.3	2.217	1.5	0.026	5.0
7D0-30	23.3	9.64	337.6	90	0.969	4.3	1.067	3.5	nd	-
7D30-70	23.0	9.73	335.2	103	nd	-	nd	-	nd	-
7D70100	22.8	9.85	337.8	95	nd	-	nd	-	nd	-
3rd soil profile										
9C0	23.8	9.58	358.6	100	89.683	1.0	89.842	8.1	0.045	7.5
9C0-30	23.9	9.99	337.4	82	85.914	1.5	86.359	6.8	nd	-
9C30-60	23.9	10.31	299.0	187	1.951	4.5	1.895	7.2	nd	-
9C60-80	24.0	10.65	271.9	319	10.578	4.4	10.958	3.8	0.074	8.5
Hips										
A1	23.8	9.41	345.5	45	9.757	5.0	na**	-	na	-
A2	23.8	9.14	379.4	32	15.256	3.5	na	-	na	-
A3	23.6	9.94	310.1	88	7.620	3.2	na	-	na	-

(*): nd = not detected ; (**): na = not analyzed

The methylmercury percentage obtained in this study was 0.91%, 1.04% and 0.05% for samples 3D0, 7D0 and 9C0 respectively. This percentage can exceed 3% in soil with high organic content under slightly acidic conditions (Cappon,

1987). This was not the case for the samples analyzed in this study since the pH of the soil was alkaline with an average of 9.8.

It needs to be mentioned that the sum of IHg and MeHg concentrations was sometimes exceeding the concentration of Hg_{TOT}. This is probably due to analytical errors and fortunately the excess was mostly below 5% (only 7D0-30 has given a difference of about 10%). This level of error, instead of negatively affecting the results, confirmed the precision of the used method.

It was shown in chapter 2 that some of the major inorganic mercury species behave differently at different pH. For example at low pH in oxidising conditions $HgCl_2$ is the predominant species, whilst at higher pH, $Hg(OH)_2$ is the main species (Figures 6.10 and 6.11).

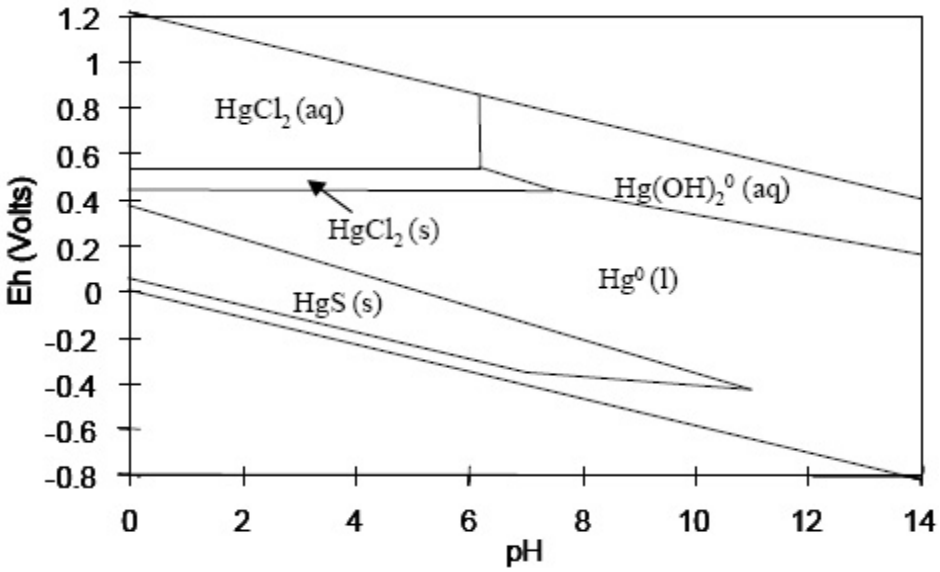


Figure 6.10: Eh-pH diagram for some of the most important chloride and sulphur mercury species (Beak International Inc., 2002).

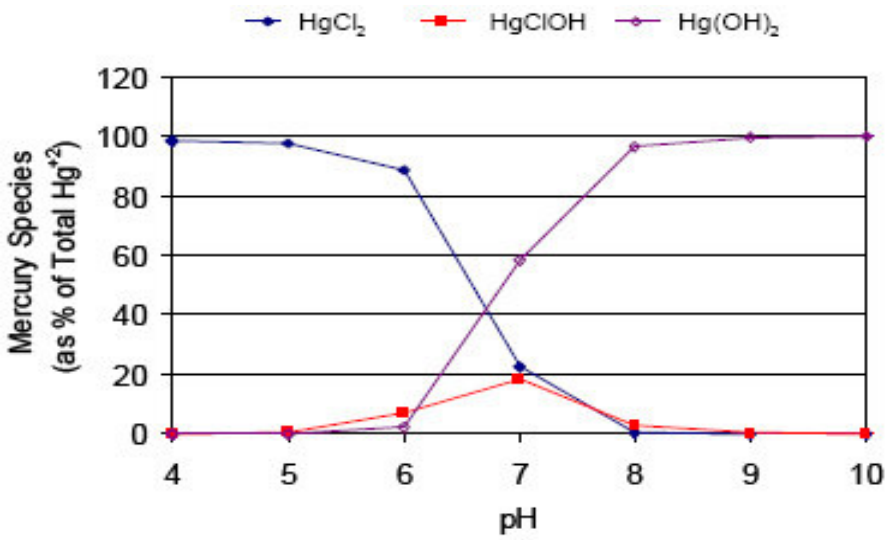


Figure 6.11: Predominant Hg(II) species with pH (R. Lewis and J. Plant, 2005).

As mentioned above, all soils had an alkaline pH due to the former activity of chlor-alkali plants (Table 6.4 and figure 6.12). Hempel et al. (1995) also reported alkaline pH values ranging from 8.6 to 9.2 in 46 soil samples collected around former caustic soda plants in East Germany. The pH of the soil samples was alkaline and had a tendency to increase with the depth except for 7D which showed higher pH at 30 cm depth than at the surface (Figure 6.12). The abnormal trend in the profile 7D will be discussed later.

Redox potential in the soil was decreasing with the depth except again for 7D which showed an almost constant Eh with a difference of only 2.1 mV from the surface to 100 cm depth (Figure 6.13).

On an Eh-pH diagram (Figure 6.10) all the samples plot to the stability field of elemental mercury (Hg^0). The contamination of soils in the vicinity of a chlor-alkali plant is due to emissions containing Hg^0, which is almost insoluble and can react with metallic oxides and organic matter (Schuster, 1991; Stein et al., 1996).

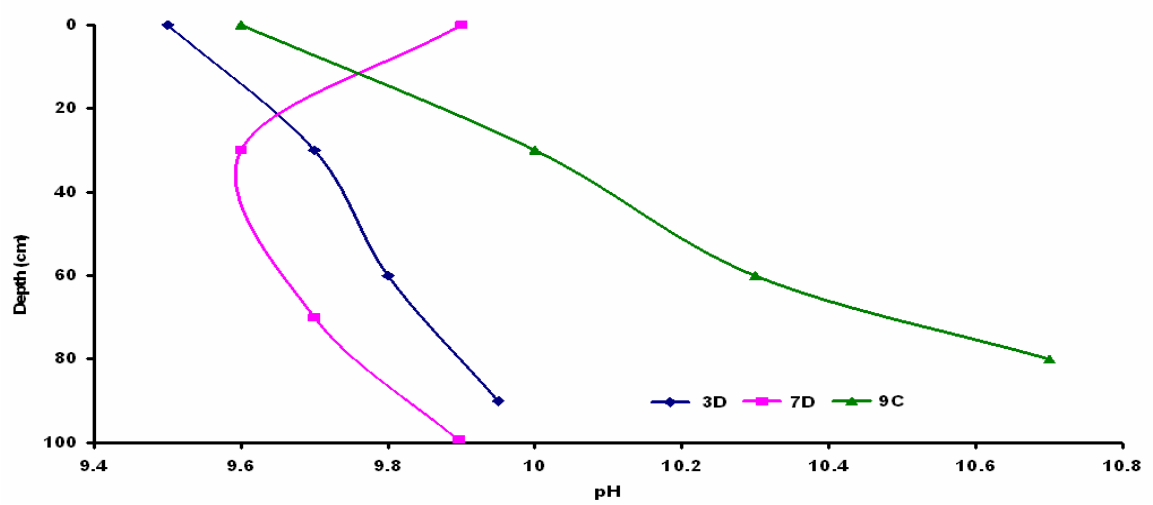


Figure 6.12: Variation of pH with depth for the different profiles.

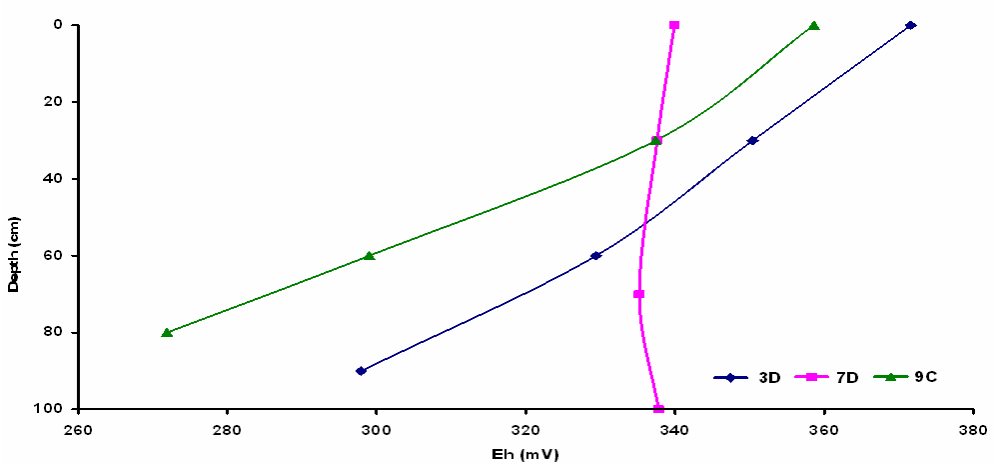


Figure 6.13: Variation of redox potential in soil with depth.

Neculita et al. (2005) have reported high concentrations of volatile mercury in soil samples affected by a chlor-alkali plant, representing sometimes 98 % of total mercury (pH 9.11 ± 0.03). They suggested the presence of very high concentrations of Hg^0 or potentially volatile $HgCl_2$ in soils. Such levels of volatile mercury in soils are unusual, but not odd, because mercury is used in the elemental form as a cathode in caustic soda productions. Biester and Nehrke (1997) reported that on the average, 34% of all Hg occurred as Hg^0 in a highly contaminated soil

(1717 mg kg^{-1}) by emissions from a chlor-alkali plant. It has to be noticed that spots of elemental mercury were visible at the sampling site.

The conductivity was generally increasing with the depth except for the profile 7D (see figure 6.14). The average values were 32, 93 and 172 µS cm^{-1} for profiles 3D, 7D and 9C respectively with a maximum of 319 µS cm^{-1} at 80 cm depth for 9C.

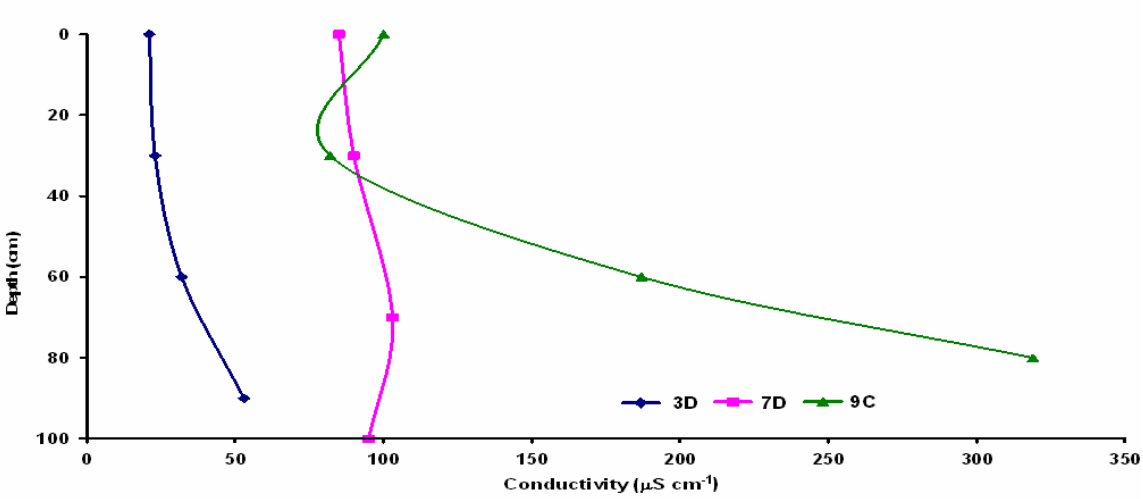


Figure 6.14: Variation of the conductivity with the depth

For a better interpretation of the results, the main ions contents in the soil and water samples were analyzed using ICP-OES for cations determination and ion chromatography for anions. The same trends as for the conductivity were observed for Al, Ca, Fe and Na ions contents for the profiles 3D and 9C (Table 6.5 and figure 6.15). 9C showed highest concentrations at the bottom level (80 cm depth) for almost all the analyzed metals. It has to be mentioned that all the metals analyzed were showing the same decreasing concentration with depth for 7D.

The chloride concentrations ranged to 38.8 to 417.2 mg kg^{-1}. This was especially high because naturally occurring chloride concentration in soils is about 3.5 mg kg^{-1} (Schuster, 1991). Neculita et al. (2005) have also measured a chloride

concentration of 35 mg kg^{-1} in highly contaminated soil. The high chloride concentration is probably due to the former activities of the chlor-alkali plant.

In general, mercury concentrations are very low in soil solution and groundwater, and mercury transport in lower soil horizons and groundwater is limited. Concentrations of total mercury in upland forest soils across the upper Midwest of the US range from about 100–250 µg kg^{-1} dry weight for the surface humus layers, and only 15–30 µg kg^{-1} for the underlying mineral horizons (Nater and Grigal 1992). More recent studies have determined similar values (Hintelmann *et al*. 2002, Grigal, 2003). Hintelmann *et al*. (2002) found strongly decreasing concentration gradients from the surface organic layer (>200 µg kg^{-1} d.w.) down to only 15 cm below the surface (<25 µg kg^{-1}) in multiple cores from upland soils in northwestern Ontario. Dominant soil solution flux was horizontal, not vertical and over three-fourths of the downslope transport of mercury occurred in the upper 50 cm (Grigal, 2003).

Even extreme laboratory situations have failed to find significant vertical transport of mercury through soil. Hogg et al. (1978) applied $HgCl_2$ to undisturbed soil cores and leached them with sewage effluent of relatively high pH (8.5) and high concentrations of cations and anions compared to waters in undisturbed systems. They failed to find movement of Hg below 20 cm, but other cations including $Ca^{2+,}$ Mg^{2+}, K^{+}, and Zn^{2+} were progressively leached in a similar experiment. There are reports of vertical mercury movement in the field, but also under extreme conditions.

Table 6.5: Mercury and total ions concentrations(*) in water and soil samples

Sample	Hg_{TOT}	IHg	MeHg	Al	Ca	Co	Cu	Fe	K	Mg	Mn	Na	Ni	Ti	Zn	Cl^-	F^-	SO_4^{2-}	NO_3^-
Water samples																			
B1	0.005	0.006	nd	2.05	41.6	n.d.	0.05	0.26	7.3	10.8	0.06	84.1	0.03	n.d.	0.06	38.9	0.3	1.6	0.3
B2	0.082	0.082	nd	1.8	24.5	n.d.	0.04	2.0	6.3	6.5	0.1	74.9	0.03	0.06	0.06	32.1	0.2	1.1	-
BH-X4	0.343	0.359	nd	0.59	129.4	0.01	n.d.	0.29	38.1	94.0	2.2	6335	0.04	n.d.	0.05	146.5	0.9	11.6	2.3
BH-X10	0.158	0.172	nd	0.47	132.3	n.d.	n.d.	0.27	35.8	87.4	2.6	6507.0	0.04	n.d.	0.05	167.8	0.8	10.8	2.0
1st soil profile																			
3D0	4.606	4.572	0.042	8486.0	6653.8	15.4	17.4	18225.6	3278.7	2893.0	192.9	7328.8	n.d.	8582.4	25.1	59.7	38.0	113.9	208.1
3D03	0.640	0.627	0.017	9162.5	8768.5	13.8	12.8	20492.6	4335.0	2955.7	197.0	9753.7	n.d.	8374.4	33.8	38.8	9.3	200.5	79.1
3D36	nd	nd	nd	8097.6	8292.7	4.9	13.7	13170.7	3219.5	3512.2	97.6	7317.1	n.d.	5268.3	17.6	271.5	6.5	70.4	145.2
3D69	nd	nd	nd	9831.5	5805.2	35.6	16.9	26310.9	2996.3	2715.4	374.5	7303.4	n.d.	15262.2	25.3	115.8	11.4	44.7	154.5
2nd soil profile																			
7D0	2.511	2.217	0.026	13944.6	11174.8	9.6	14.3	21012.4	5539.6	4393.5	286.5	13085.0	n.d.	9742.1	9.6	39.0	9.8	84.8	63.9
7D03	0.969	1.067	nd	13726.7	15823.8	37.2	12.4	25260.9	4956.8	7435.3	381.3	14012.6	n.d.	12773.4	33.4	64.7	24.4	76.1	126.4
7D37	nd	nd	nd	10129.1	10029.8	15.9	15.9	16087.4	4270.1	4468.7	198.6	7249.3	n.d.	6057.6	22.8	82.3	46.9	141.6	216.3
7D710	nd	nd	nd	10029.5	5506.4	10.8	12.8	15732.5	2753.2	2261.5	196.7	4424.8	n.d.	5506.4	16.7	39.6	52.1	56.0	78.1
3rd soil profile																			
9C0	89.683	89.842	0.045	12936.8	5665.7	21.7	47.2	22663.0	2927.3	2644.0	197.4	8687.1	n.d.	9726.2	22.7	176.8	32.0	70.4	69.9
9C03	85.914	86.359	nd	11876.2	8782.4	35.9	15.0	25648.6	3592.8	3293.4	399.2	6986.0	n.d.	13972.0	25.9	40.9	11.5	77.3	46.9
9C36	1.951	1.895	nd	13702.7	9037.9	32.1	19.4	27599.7	3692.9	3401.4	388.7	6705.6	n.d.	13022.4	30.1	247.4	14.0	247.9	174.6
9C68	10.578	10.958	0.074	14146.3	10634.1	2.9	16.6	28682.9	3804.9	4000.0	292.7	7024.4	n.d.	10048.8	27.3	417.2	3.5	334.3	161.2

(*)All values are in mg kg^{-1} (ppm) level

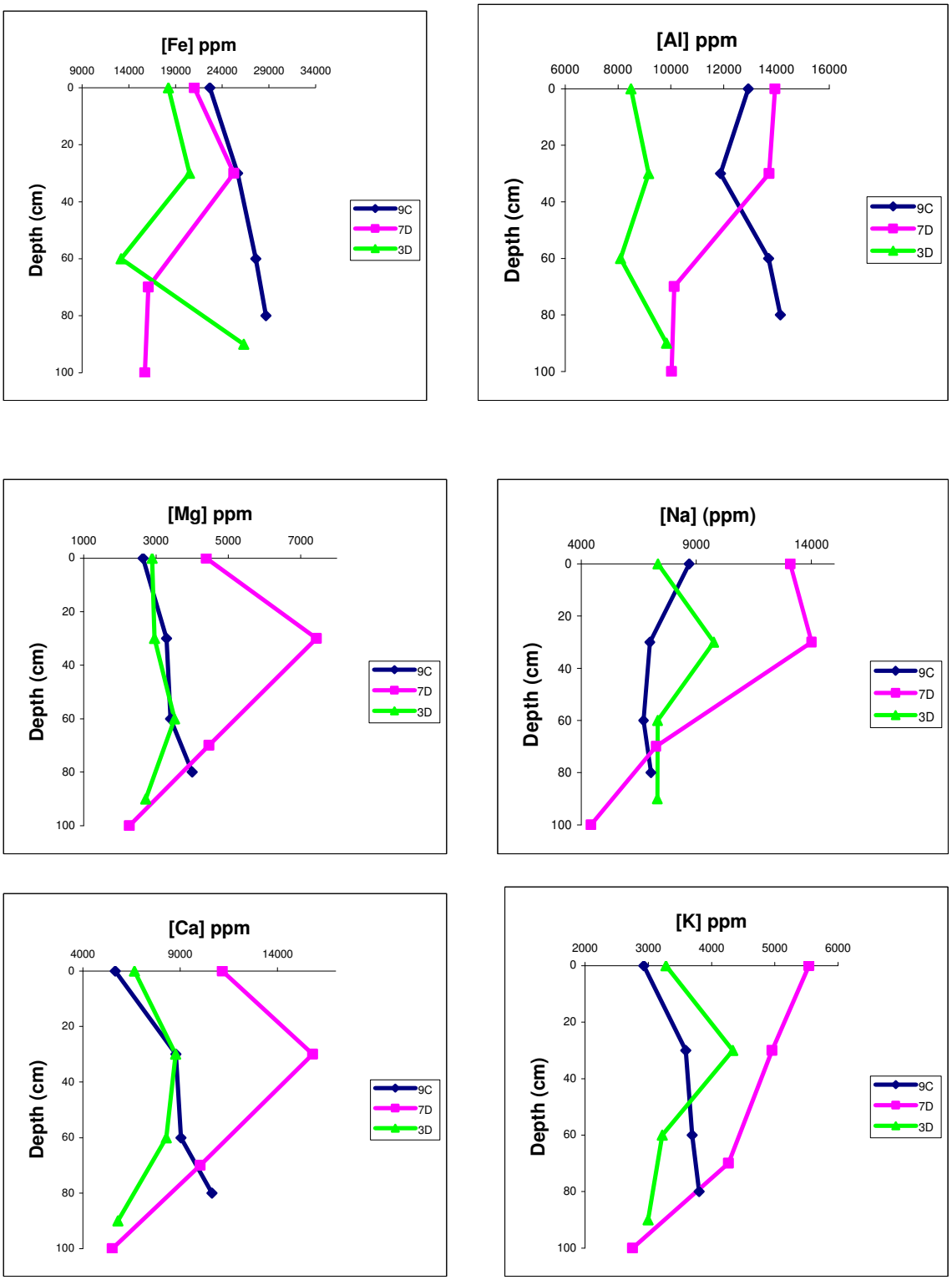


Figure 6.15: Examples of metal concentrations with depth.

In brief, soil solution that is low in DOM is also likely to be low in mercury so that vertical transport of mercury is minimal. However, a caveat must be attached to that statement. There is the possibility that mercury, with DOM, could be transported quite deeply in highly structured soils through preferential flow paths (Jardine et al. 1989*a*). This mercury could reach groundwater, at least during storms (Jardine et al. 1990). There is also evidence, however, that in most cases this DOM and presumably associated mercury will be adsorbed by the soil matrix as the solutions move through the soil (Jardine et al. 1989*b*). Similarly, although vertical mercury flux was minimal in moist peat soils, drying led to shrinkage of the soils, and resulting cracks provided pathways for significant mercury flux (Lodenius et al. 1987).

The concentration of IHg for the soil analyzed in this work varied in the range of 2.2-89.8 mg kg^{-1} at the surface and of about 0.64-1.90 mg kg^{-1} at 60 cm depth (Figure 6.16). This agrees with the general observation made by scientists, such as those mentioned above, that mercury concentration decreases significantly with depth. It needs also to be mentioned the likely vertical, and not horizontal, transport of mercury in the site since the concentration of IHg increases for about forty times at the surface from 3D to 9C (see figure) which represent a distance of about 10 m (See map site).

The analysis of hips (A1, A2 and A3) has shown the highest mercury concentration with A2 (15.26 mg kg^{-1}) which is close to position 9C. Thus, rain wash from A2 might explain the high mercury concentration observed at the surface of 9C. It has to be recalled that A1, A2 and A3 were collected on top of the hips. Therefore, higher mercury concentration could be expected in the bulk of hips.

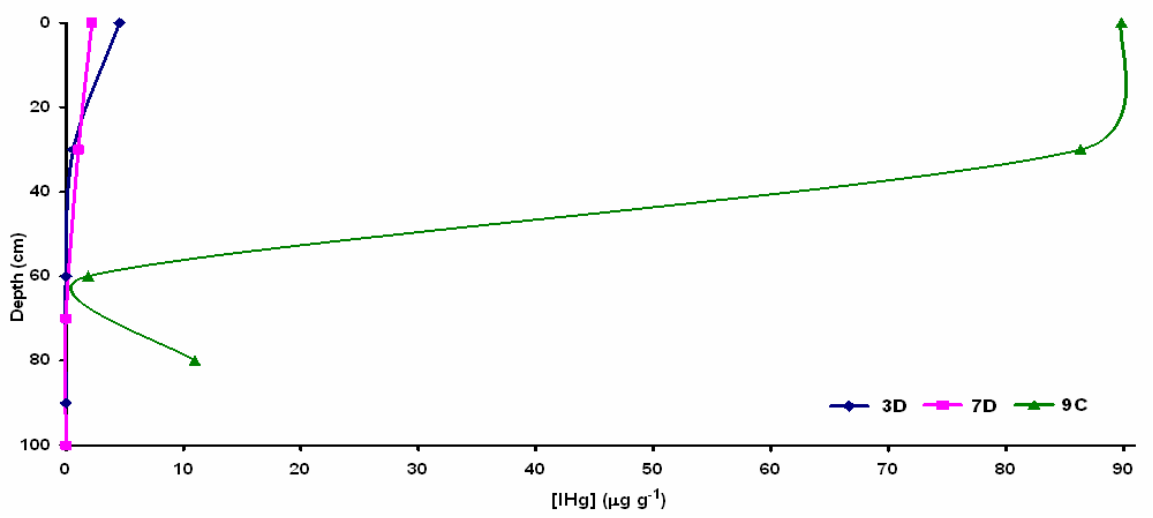


Figure 6.16: Concentration of inorganic mercury with depth.

The mercury concentration of 10.58 mg kg^{-1} at 80 cm depth of the profile 9C can be explained by the nature of the soil as shown by Lodenius et al. (1987) cited above. Soil movement due to work done on the site might also caused a vertical transport of mercury. Further studies, such as the determination of the geological structure of the soil, need to be done for a better understanding of mercury behavior in this site.

There are limited data on MeHg concentrations in soil solution (Grigal, 2002). Similar to Hg_{TOT}, observations of higher MeHg concentrations in soil solution near the surface (e.g., Branfireun et al. 1996) imply that the major source of MeHg is via surficial or near-surface processes. In northern Sweden, concentration of both Hg_{TOT} and MeHg in soil solution declined with depth, with MeHg about 0.24 µg kg^{-1} (ppb) at the surface to about 0.01 µg kg^{-1} at 50 cm (Bishop et al. 1998), and ratios of MeHg to Hg_{TOT} ranging from 0.05 to 0.2%, with a tendency for a decrease with depth (Bishop et al. 1998).

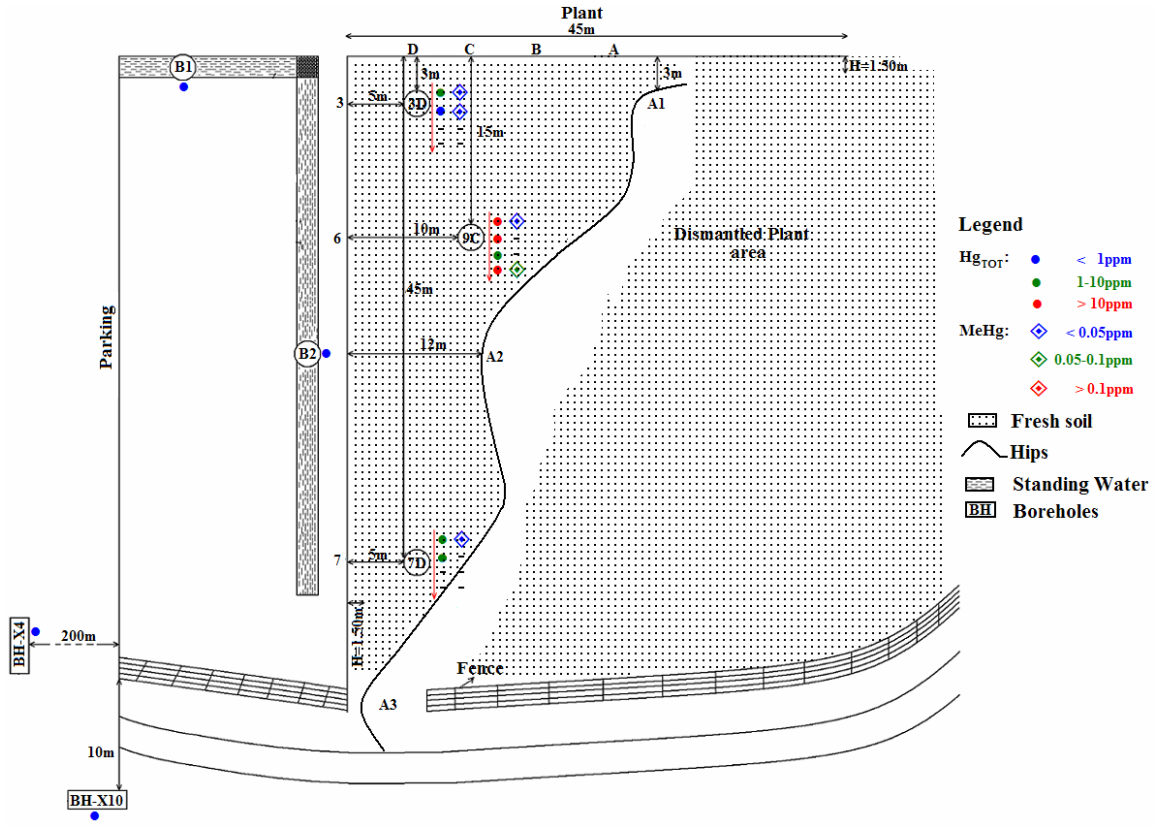


Figure 6.17: Mercury concentrations in the sampling site.

Grigal (2002) has reported that the ratio of MeHg to Hg_{TOT} in soil water can be expected to range from about 0.15 to 15%.

The result obtained from this study gives a ratio of MeHg to Hg_{TOT} in the range of 0.06 to 3.2% and it was also found a decrease in MeHg concentration with depth (Figure 6.18). The methylmercury concentration ranged between 0.017 to 0.074 mg kg^{-1}. Hempel et al. (1995) reported methylmercury concentration of 0.400 mg kg^{-1} (<0.04% of total Hg) whereas Bloom et al. (2003) determined methylmercury concentration of 0.010 mg kg^{-1} (<0.0001% of total Hg) in polluted soils near chlor-alkali plants, stressing the site-dependent aspect of the methylmercury.

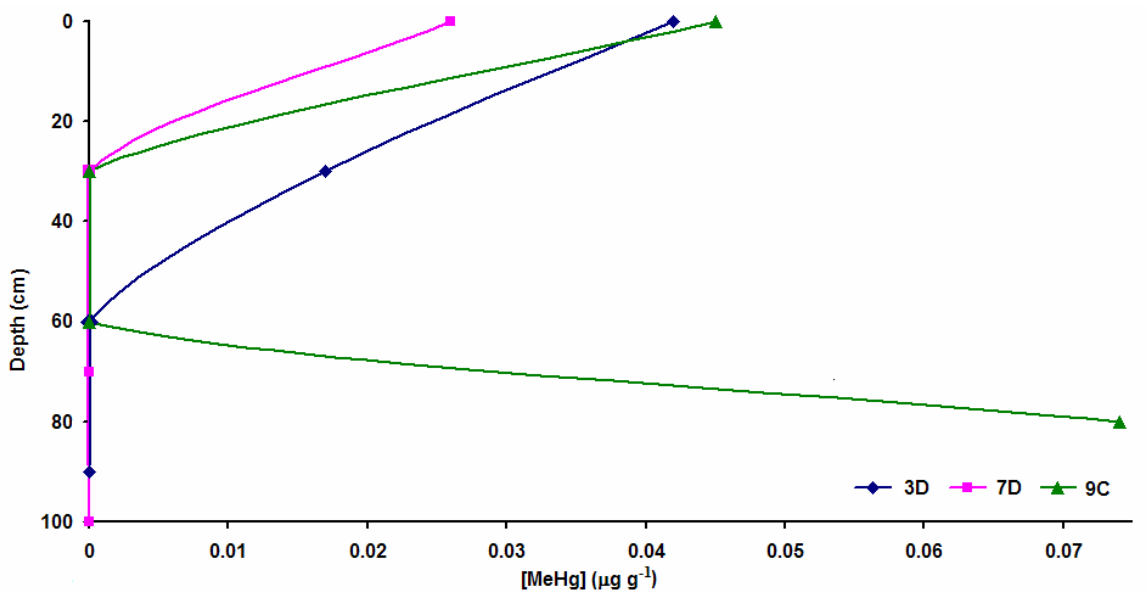


Figure 6.18: MeHg concentration at different profiles.

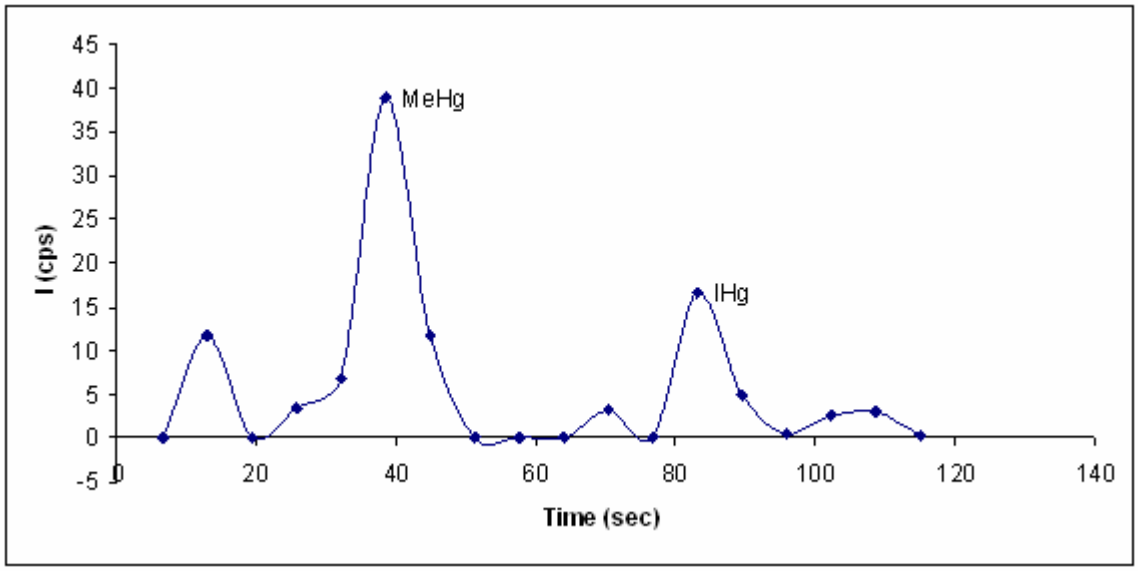


Figure 6.19: Chromatogram of sample 9C60-80.

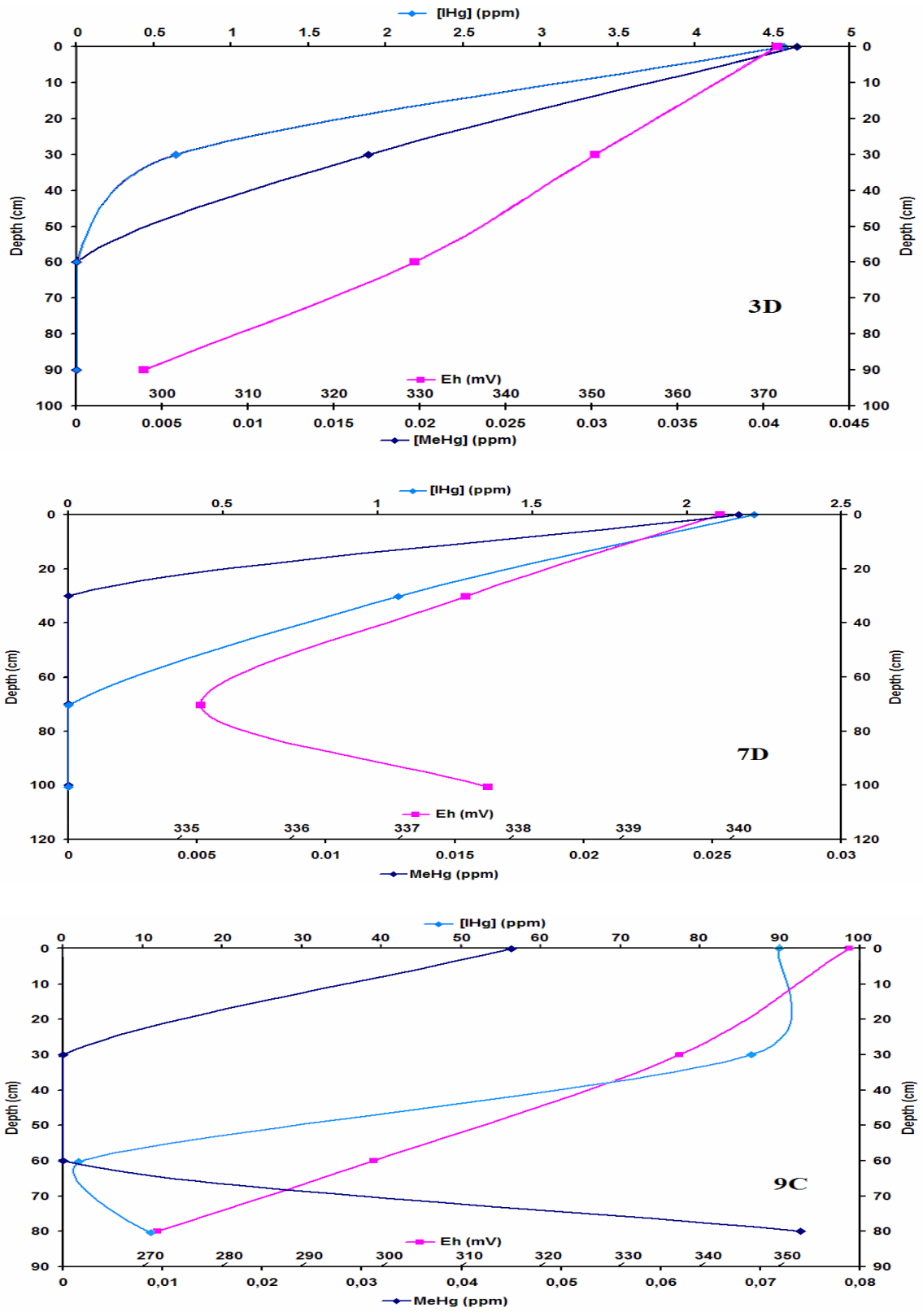


Figure 6.20: Variation of IHg, MeHg concentrations and redox potential with depth.

Rodriguez Martin-Doimeadios et al. (2004) have demonstrated that Hg^{2+} methylation showed a strong dependence on redox conditions and biological activity of the different variables tested, the combination of anaerobic and biotic conditions was the most conducive to methylmercury production. This might explain the presence of MeHg for the profile 9C at 80 cm of depth since the redox potential was the lowest at this level (Figures 6.19 and 6.20).That is a favorable condition for anaerobic bacteria to convert IHg to MeHg.

An abnormal trend was observed on the profile 7D in terms of field measurement values or total metal concentrations. Samples close to surface had the lowest pH and conductivity and also the highest redox potential for 3D and 9C while for 7D the highest pH was measured at the surface and no significant changes was observed with the redox potential (Table 6.4). This might be due, as mentioned above, to the fact that bulldozers were used on the site during the sampling period in order to balance the soil level after dismantling the plant. The mix up of the soil is likely to be responsible of disturbances observed for the profile 7D.

6.2.4.3 Water samples

Concentrations of mercury were about 0.16 and 0.34 mg L^{-1} for boreholes (BH-X4 and BH-X10 respectively) and about 0.005 and 0.082 mg L^{-1} for standing water samples B1 and B2, respectively. MeHg was not detected in water samples. Thus, all the mercury was assumed to be in the form of inorganic mercury species. Chromatograms obtained with water were of best quality (Sharp peak, better intensity and low background) in comparison with those obtained from soil samples (Figure 6.9). This is probably due to the nature of the matrix, water being a less complex matrix than soil.

The concentration levels of mercury species in natural water samples are usually low, in the order of ng L^{-1} (picogram) (Stojchev et al., 2002; Amouroux et al.,

1998; Monperrus et al., 2005). Once more the result obtained confirmed the pollution of this site by the dismantled plant. It could not be explained why the concentration of mercury was higher in BH-X4 than in BH-X10 which is closer to the dismantled plant. Once again, a geologic study of the underground water pathway should provide more information for this purpose.

The pH was slightly acidic in both boreholes (about 5 to 6) and the redox potential was about 0.4V. This presumes the presence of the $HgCl_2$ species contrary to the standing waters were the pH was 8.95 to 9.20 and redox conditions were slightly more oxidative than in the soil but less than in boreholes.

In order to predict the predominant mercury species in these water systems a geochemical speciation modeling was done based on the concept of partial equilibrium. The model has been constructed based on four water samples, namely B1, B2 and BH-X4 and BH-X10. Average concentrations and parameters of these samples were considered. The model predicts that mercury speciates as Quicksilver or elemental mercury. Obviously, this will change if the concentration of Cl^- were to increase for instance (Figures 6.21a and 21b).

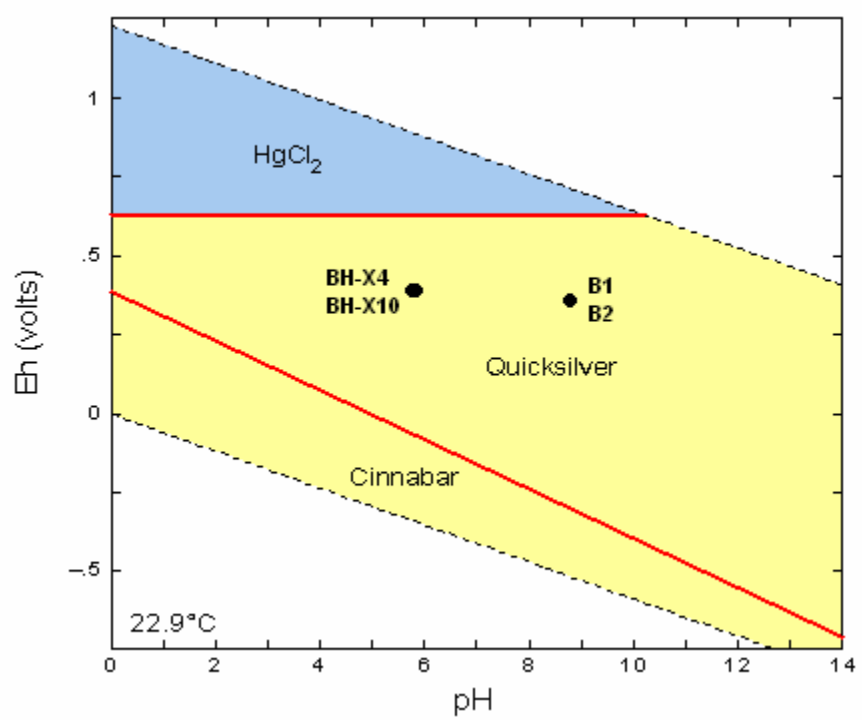


Figure 6.21a: Water speciation by Geochemist's Workbench (-Log a Cl^- = 0.143)

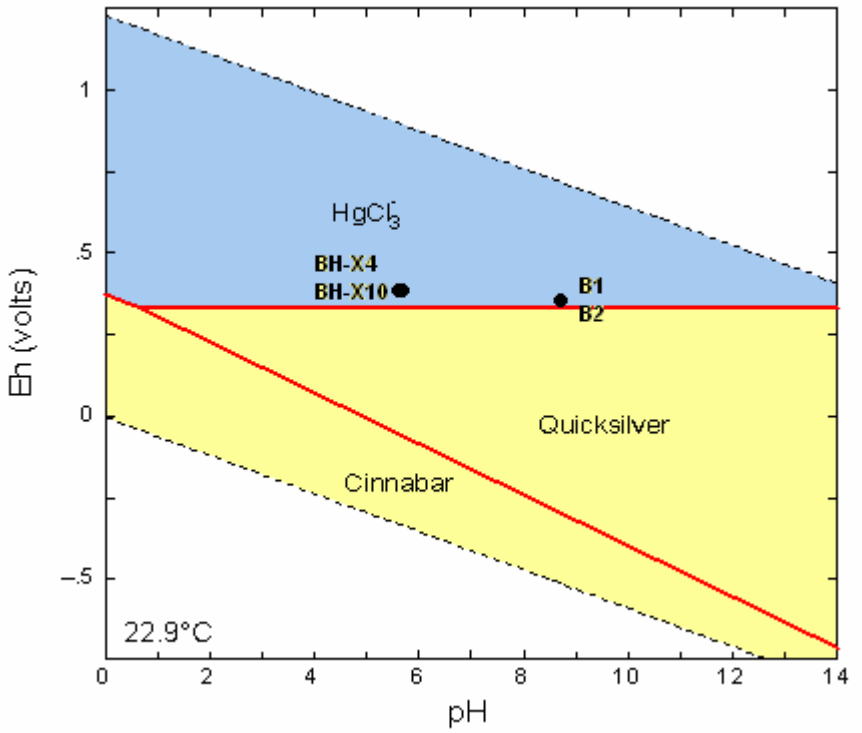


Figure 6.21b: Water speciation if Cl^- concentration is increased to - Log a = 0.8

6.2.5 Conclusion

To assess mercury distribution in highly contaminated field-collected soils and water affected by the activity of a chlor-alkali plant, total, inorganic and methylmercury concentrations were determined using ICP-MS and the hyphenation of GC and ICP-MS.

The soil characterization revealed moderately to extremely contaminated soils (up to 90 mg kg^{-1}) having an alkaline pH and high chloride concentrations.

Methylmercury concentrations were low in all soils (up to 0.074 mg kg^{-1}).

The profile 9C has shown the highest concentrations of total and methylmercury whereas these concentrations were much lower in the other two profiles.

Some abnormalities were observed in the profile 7D. This can be related to work done within the site in order to balance the soil level.

Mercury concentrations in water samples were also high (up to 0.340 mg L^{-1}). Methylmercury was not detected in all water samples. A model based on the ions contents in water samples predicted that mercury speciates as elemental mercury in the studied water systems.

These results were in good agreement with previously published studies.

The very high total mercury in studied soils and waters, as well as the results of the mercury speciation, suggest a potential ecotoxicity via the soil solution and water. Further studies need to be done on this site to confirm the likely uptake of various mercury species by organisms and plants.

6.3 Ecotoxicology assessment in three villages affected by mercury pollution

This part of the study focused on the application of the developed analytical methods for the determination of mercury (Hg_{TOT} and speciation) in biological tissues.

It is generally recognized that the general population is primarily exposed to MeHg through consumption of fish, and that the analysis of human hair is the most appropriate method for monitoring the intake of MeHg in persons at risk (Akagi and Naganuma, 2000) since MeHg is incorporated into the forming hair, and correlates with the MeHg concentration in blood (Kehrig et al., 1997). Thus, the concentration profile along the hair strand represents a recapitulation of previous blood concentrations, i.e. previous MeHg exposure (WHO, 1990).

The research was conducted together with the Medical Research Council South Africa (MRC SA), Environment and Health Research Unit (E & HRU). The aim was to evaluate ecotoxicology by mercury in villages alongside the Inanda Dam in Kwazulu-Natal, South Africa (http://www.mrc.ac.za). The study population included 97 hair samples collected from people living in three different villages alongside the Inanda Dam (Figure 6.22) namely Mshazi, Nqetho and Madimeni. Ten fish species taken from the Inanda Dam were also analyzed.

Numerous reports in the media, between 1986 and 1990, have referred to discharges of mercury waste from Thor Chemicals in KwaZulu-Natal, into two river systems. A study conducted in 1990 found that Thor Chemicals was the source of this pollution and that excessive levels of mercury were detected in the vicinity of the plant.

In 2002, Barrat and Combrink (2002) published a paper concerning a study which included the area directly below Thor Chemicals to the top end of Inanda Dam and showed that mercury levels in sediment below the plant were of a similar magnitude to those detected 8 years previously. However, sediment and algae

levels further downstream were considerably lower. A 98% increase in mercury sediment levels was noted at a sample site 10 kilometres downstream from the chemical plant. This may suggest that a degree of biomagnification may have taken place, although other factors could also be responsible for this increase. Despite mercury levels in fish collected from Inanda Dam, situated approximately 35 km downstream from Thor Chemicals, being below statutory levels, there is a concern that the fish eating population and high risk groups may be exposed to excessive levels of mercury in their diet. It was then recommended that a risk assessment had to be conducted on the fish eating population of the region.

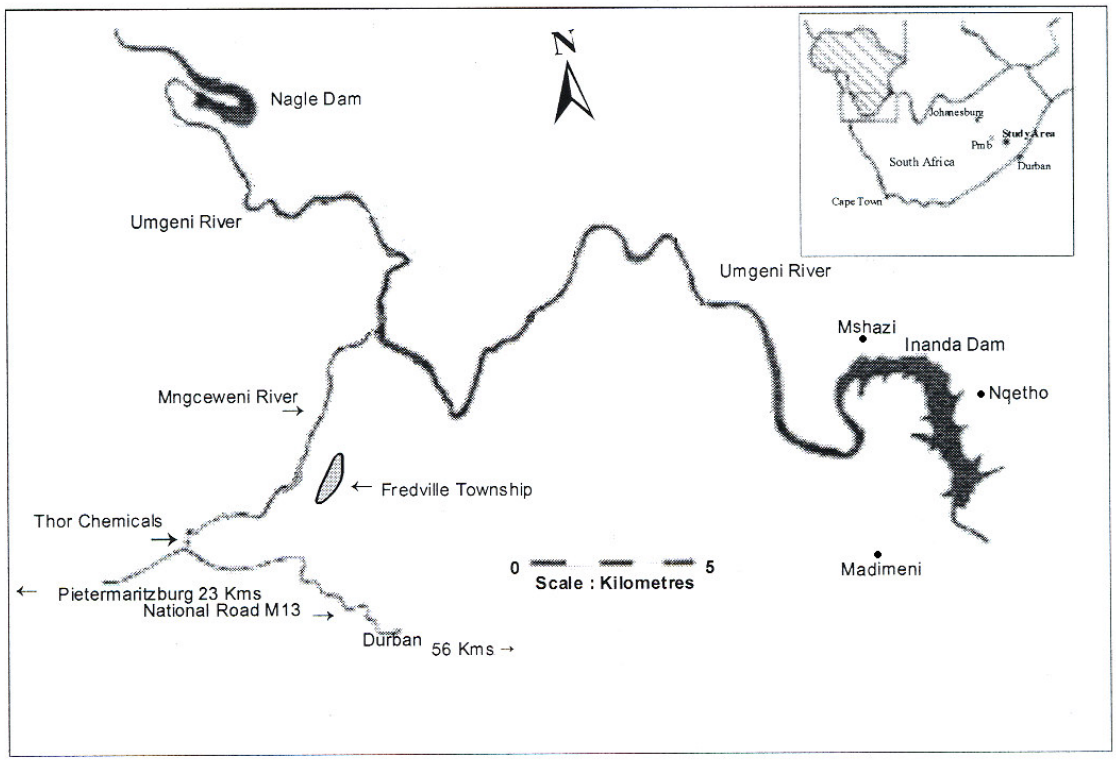


Figure 6.22: Map of the sampling site.

6.3.1 Sample preparation

The chemicals used were of analytical grade obtained from Aldrich and Merck. Concentrated HNO_3 and H_2O_2 30% (v/v) were used during the microwave digestion of fish and hair samples. The same reagents, as for the speciation in soil samples, were used for the derivatization before analysis by GC-ICP-MS.

6.3.1.1 Hair samples

Before being digested, hair samples were treated according to the following washing procedure after Akagi et al. (1995):

- Wash with detergent and water
- Wash with acetone
- Dry and cut finely with scissors

For Hg_{TOT} determination, 0.1 g of dried hair sample was digested in a microwave digester (Table 6.6). A clear yellowish solution was obtained and diluted with de-ionised water prior to analysis with ICP-MS.

Table 6.6: Optimized digestion parameters for the determination of Hg_{TOT} in hair.

Microwave program

Phase	Power (W)	Ramp (min)	Hold (min)	Fan
1	250	3:00	2:00	1
2	400	3:00	2:00	2
3	600	3:00	2:00	3

Reagent : HNO_3 (2 ml) ; H_2O_2 30% (1 ml)

For mercury speciation, an overnight cold digestion was done as described by Morton et al. (2002) using the same amount of sample and reagent as for the total analysis. This was followed by a derivatization with $NaBEt_4$ before determination with GC-ICP-MS.

Blanks were also prepared and analyzed for Hg_{TOT} and mercury speciation following the same procedures. A chart of sample preparation methods used is presented below.

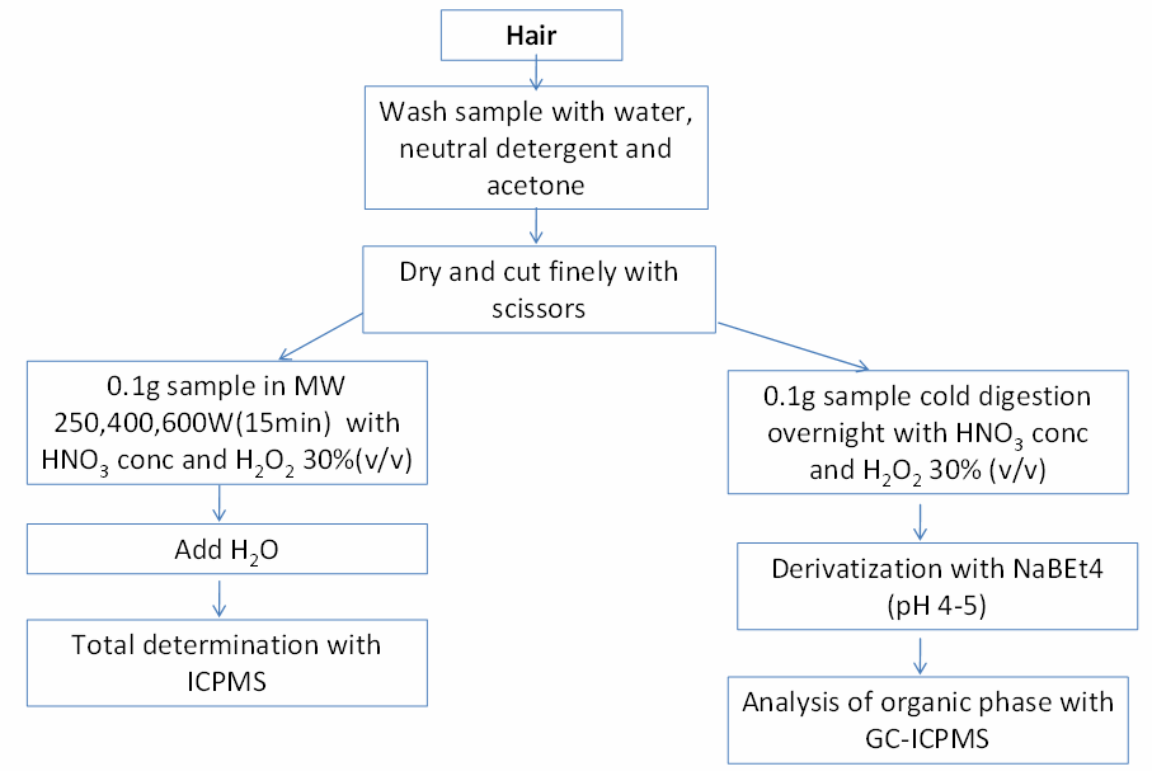


Figure 6.23: Sample preparation for the determination of mercury in hair.

6.3.1.2 Fish samples

Catfish and carp ranging between 80 and 5450 g in mass, and between 29 and 68 cm in length, were caught from Inanda Dam. Once transported to the laboratory

the samples were kept in a freezer at -20 ^{0}C prior to preparation for chemical analysis.

To avoid cross-contamination all equipment used in sample handling were thoroughly cleaned before each sample was processed. Stainless steel blades, aluminum foils and borosilicate bottles were used and before the next sample was processed, instruments were washed with detergent solution, rinsed with tap water and finally rinsed with de-ionized water. The work surface was also washed with de-ionized water and allowed to dry completely.

A fillet, which includes the flesh tissue and skin from head to tail beginning at the mid-dorsal line from the left side of each fish including the belly flap, was removed from each sample. Filleting was conducted on a cutting board covered with heavy duty aluminum foil, which was changed between samples. Excess aluminum foil was used to carefully fold and wrap the fillet samples. The foil wrapped sample was then placed in a secured plastic bag and kept in a freezer for several minutes. The frozen tissue was ground using cleaned mortar and pestle, freeze-dried with a lyophilizer and then stored in a fridge.

The same microwave digestion programmes and ethylation were used as for the analysis of the CRM. GC-ICP-MS parameters were also similar.

6.3.2 Results and discussion

Hair and fish samples were analyzed using ICP-MS for total mercury analysis and the hyphenation of GC with ICP-MS for inorganic and methylmercury determination. Instrumental parameters were the same as for the determination of mercury in soil samples.

6.3.2.1 Speciation calibration

An external standard calibration for the GC-ICP-MS analysis was done. Once again a good linearity (R^2= 0.997 and 0.998 for IHg and MeHg respectively) was obtained for a range of 10 to 1000 µg L^{-1}. The detection limit was 1.30 µg L^{-1} and the limit of quantification 4.30 µg L^{-1}. The reported results concern the isotope ^{202}Hg.

6.3.2.2 Hair samples

As mentioned above, a total of 97 hair samples were collected from inhabitants of three villages alongside the Inanda Dam: 20 from Mshazi, 48 from Nqetho, and 29 from Madimeni. Most of the samples provided were having a mass below 50 mg. This was an important inconvenience since it was shown in the previous works that a minimum of 100 mg of a sample is required for a good determination of mercury in hair (Morton et al., 2002).

The determination of total mercury was also done on hair samples collected from people living in non exposed area (in the Gauteng province) and samples were also provided by the MRC SA. Analytical results are presented in tables 6.7 to 6.10.

Table 6.7: Determination of Hg_{TOT} in hair samples collected from a non- exposed area

Sample ID	Hg_{TOT} (mg kg^{-1})	%RSD (x=3)
HLT	0.075	5.8
HA	0.165	7.2
HN	0.028	4.7
HD	0.032	3.6
HMG	0.039	9.4
HZN	0.037	2.2
HVN	0.033	8.4

* ppm= mg kg^{-1}

Table 6.8: Hg_{TOT} in samples collected in Mshazi

Sample	Hg_{TOT} (mg kg^{-1})	% RSD (n=3)
H1	6.29	8.5
H2	3.03	1.2
H3	2.10	1.4
H4	nd*	-
H7	11.11	0.6
H8	0.69	10.7
H12	1.05	9.5
H13	5.98	1.0
H15	1.54	3.3
H18	29.54	3.5
H19	nd	-
H21	20.18	1.5
H22	10.07	3.0
H23	1.88	6.8
H24	1.83	8.6
H25[a]	1.02	11.5
H25b	0.11	12.9

*nd= not detected or below detection limit

Table 6.9: Hg_{TOT} in samples collected in Nqetho

Sample	Hg_{TOT} (mg kg^{-1})	%RSD (n=3)
H44	0.53	1.5
H45	0.56	12.7
H49	45.02	1.9
H51	16.93	1.8
H56	1.34	4.8
H57	nd	-
H60	3.25	10.2
H61	2.10	4.4
H62	nd	-
H63	0.87	11.4
H64	1.9	8.7
H65	0.81	6.8
H66	0.26	6.6
H67	1.03	11.2
H71	0.41	11.0
H73	nd	-
H77	6.89	0.8
H78	0.40	9.4
H80	0.52	10.4
H81	0.03	6.6
H82	0.06	8.2
H83	19.18	2.3
H85	17.44	5.8
H86	0.70	11.6
H87	0.30	12.4
H90	1.02	10.8
H91	0.03	7.3
H92	4.73	7.4
H93	nd	-
H96	0.97	6.9
H101	0.13	19.4
H102	0.18	10.1
H107	nd	-
H110	1.58	8.4
H111	0.19	16.1
H112	17.58	1.3
H113	0.28	4.6
H114	0.04	9.0
H116	1.18	8.5
H117	2.81	2.6
H118	0.98	11.4
H120	0.68	9.4
H121	1.57	6.2
H192	nd	-
H199	0.29	15.9

Table 6.10: Hg_{TOT} in samples collected in Madimeni

Sample	Hg_{TOT} (mg kg^{-1})	%RSD (n=3)
H75	4.38	7.6
H122	0.08	11.6
H123	nd	-
H126	1.07	5.8
H128	6.06	3.0
H129	1.32	7.43
H133	4.33	1.8
H135	nd	-
H138	0.23	11.8
H149	0.51	12.8
H150	0.69	13.0
H152	9.57	7.3
H153	0.44	8.0
H156	0.41	13.0
H157	27.55	1.6
H158	2.23	5.2
H160	0.51	14.2
H162	1.35	8.2
H165	nd	-
H167	nd	-
H168	10.4	4.7
H169	0.36	10.5
H171	2.04	3.4
H177	51.74	4.9
H178	3.43	1.6
H181	54.76	8.8

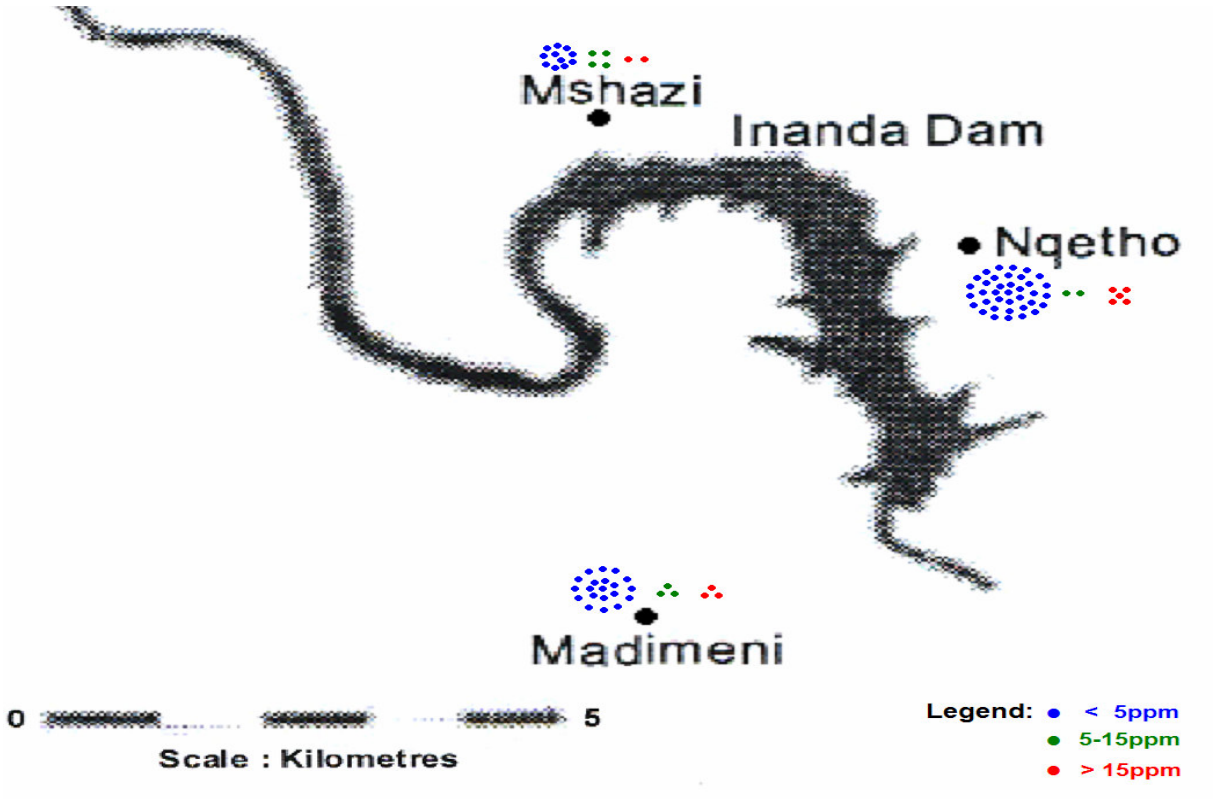


Figure 6.24: Total mercury concentration in hair for each village.

It has to be noticed that it was difficult to collect hair from the local population due to their belief in witchcraft. Most of the collected samples had very low masses, with sometimes less than 5 mg, making the mercury determination difficult to carry out in all the samples. Therefore, the speciation of mercury was performed for only 9 hair samples of the highest masses with a mean of 70 mg per sample. An example of the chromatograms obtained is shown in figure 6.25 and the results are presented in the table 6.11 below.

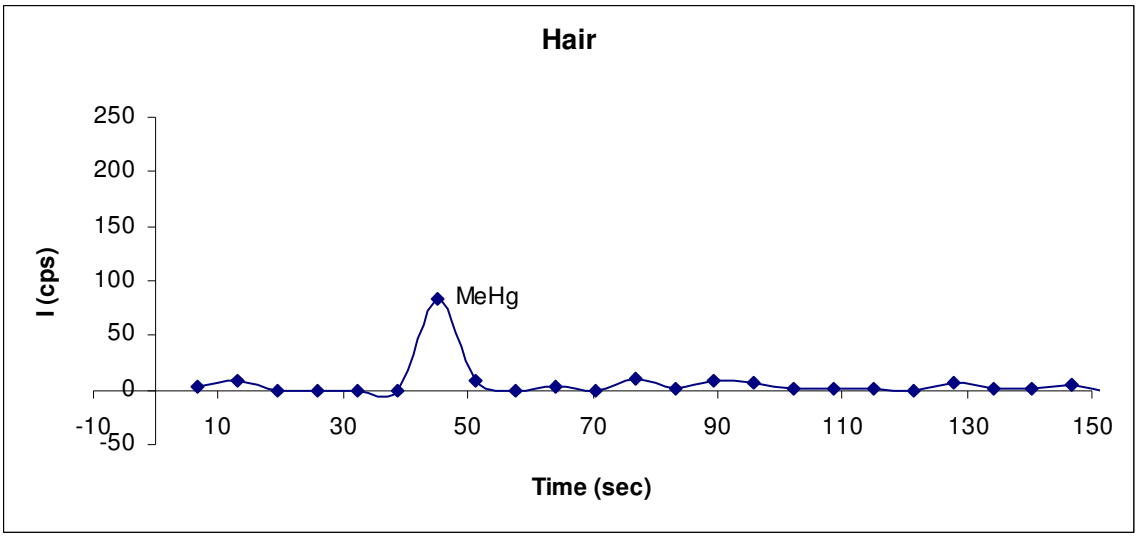


Figure 6.25: Example of chromatogram of hair sample (H74).

Table 6.11: Inorganic and mono-methylmercury concentrations in hair samples (%RSD in brackets).

Hg in hair samples		
Sample	MeHg (mg kg^{-1})	IHg (mg kg^{-1})
H13	6.01 (6.8)	bdl*
H20	0.77 (3.1)	bdl
H23	1.81 (9.5)	bdl
H42	nd	bdl
H74	0.25 (5.8)	bdl
H79	1.93 (2.2)	0.225 (7.7)
H125	2.13 (8.6)	bdl
H141	0.87 (5.0)	0.164 (4.1)
H177	34.67 (5.3)	16.36 (7.5)

*bdl = below detection limit

It has to be noticed the good RSD obtained for each triplicate for both total mercury and speciation analysis except for very low concentration were sometimes it exceeded 10%.

In order to check the fiability of the obtained results, a comparison was done between the total mercury analysis and the speciation results on three samples (Table 6.12) assuming that the major mercury species in the total mercury concentration in hair were in the form of IHg and MeHg.
The highest difference obtained was 1.37% which confirms the reliability of the used methods.

Table 6.12: Comparison between total analysis and speciation result on 3 hair samples.

Sample	**Speciation (IHg+MeHg) (mg kg^{-1})**	**Total Hg (mg kg^{-1})**	**Difference (%)**
H13	6.01	5.98	0.50
H23	1.81	1.88	0.96
H177	51.03	51.74	1.37

Hair samples contained total mercury at median levels of 4.21 and 6.43 mg kg^{-1} for inhabitants of Nqetho and Mshazi , respectively, while much higher levels of Hg_{TOT} were observed for inhabitants of Madimeni with a median value of 8.37 mg kg^{-1} (Table 6.13).

Table 6.13: Summary of total Hg concentration in hair from inhabitants of the 3 villages.

Village	**N**	**Hg_{TOT} (mg kg^{-1})**		
		Median	**Range**	**Mean**
Mshazi	17	6.43	0.11-20.18	5.67
Nqetho	44	4.21	0.03-17.58	3.51
Madimeni	26	8.37	0.08-54.76	7.08

Methylmercury analysis in hair showed that the average proportion of MeHg to total mercury was more than 90%, indicating that MeHg was the predominant form of mercury in hair samples. Akagi et al. (1995) and Malm et al. (1990) also observed similar levels of total mercury in some hair samples in Brazil. The abnormally high concentrations of mercury observed in their studies were considered to be the result of exposure to MeHg through the consumption of contaminated fish.

Mercury concentrations of more than 50 mg kg^{-1} were measured in two hair samples, namely H177 (51.74 mg kg^{-1}) and H181 (54.76 mg kg^{-1}), which represent 2.1% of the studied population. Both samples were collected in Madimeni which is located far from the dam compared to Nqetho and Mshazi (Tables 6.8 and 6.9). The results obtained for Madimeni will be discussed further. Barbosa et al. (1998) also have reported mercury concentrations above 50 mg kg^{-1} in hair collected from indigenous women living along the Madeira River and Fresco River in the Amazon basin (Brazil).

Hair is subject to shampoos, colorants, hair dryers, traditional treatments using natural substances which are potential mercury containing substances, and host of other treatments. Substances are regularly removed from the hair by these treatments and similarly, other substances, including heavy metals, can actually be added by some of these processes. With some substances being added and others being removed, it is clear that the relative concentration of any particular substance, especially a metal, can be sensitively affected (Baratz, 2005). Hair mercury level is often not correlated with blood mercury concentration or symptoms of mercury toxicity, and reports of hair contamination by exogenous mercury are not uncommon (Nuttall, 2006). The concentration of mercury measured in the two samples mentioned above can be explained by the application of traditional substances to the hair.

6.3.2.3 Fish samples

The results of measurements of total mercury and MeHg in fish from the Inanda Dam are presented in table 6.14 and figure 6.26 shows an example of chromatogram obtained with GC-ICP-MS.

Table 6.14: Fish mercury levels, Inanda dam, KwaZulu-Natal, South Africa (% LOD in brackets)

Sample Species	**Weight (g)**	**Length (cm)**	**Total-Hg (mg kg^{-1})**	**Me-Hg (mg kg^{-1})**
Catfish	1800	59	1.15 (4.3)	1.14 (7.8)
Catfish	2300	42	0.70 (2.8)	n.a.
Catfish	1700	53	0.26 (1.5)	n.a.
Carp	2050	48	1.78 (8.1)	1.77 (8.2)
Carp	80	29	0.34 (9.7)	n.a.
Carp	5450	68	0.79 (8.1)	0.80 (8.5)
Carp	3800	57	0.49 (10.9)	n.a.
Carp	2250	43	0.61 (7.2)	n.a.
Carp	1200	35	0.31 (11.5)	n.a.
Carp	3250	48	0.27 (3.8)	n.a.

n.a.: not analyzed.

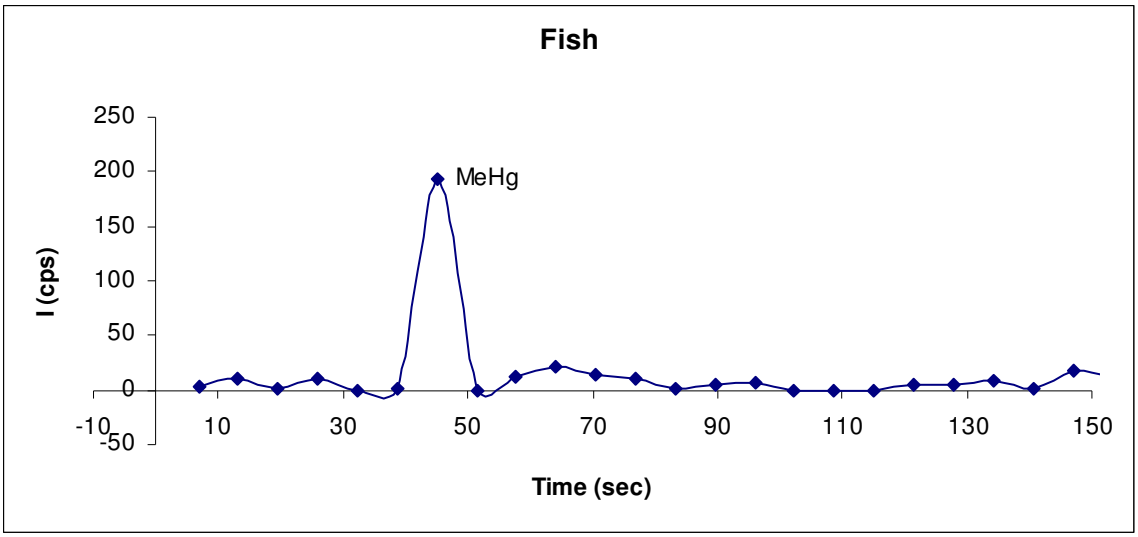


Figure 6.26: Example of chromatogram of fish sample (carp fish).

Total mercury in fish ranged from 0.26 to 1.78 mg kg^{-1}, and 5 of the 10 fish samples analyzed had relatively high mercury concentrations exceeding 0.5 mg kg^{-1}, which is the allowable limit for mercury concentration in fish according to the South African Food Stuffs, Cosmetics and Disinfectants Regulations (FCD Reg., 1994). The limit for the US Food and Drugs Agency Requirements is 1 mg kg^{-1} (US EPA, 1997). The average values obtained for the analyzed fish species were 0.70 mg kg^{-1} for catfish and 0.66 mg kg^{-1} for carp fish. The predominant form of mercury was also found to be MeHg in all samples analyzed. The relatively high values suggest high and widespread contamination of these species in the Inanda Dam. Moreover, the mercury concentrations obtained during the previous study done by Barrat and Combrink (2002) ranged from 0.05-0.25 mg kg^{-1}. Results obtained during the present study are 5 to 7 times higher than the previous ones confirming the increase of the methylation in the site.

No particular relationship was found between the mercury concentration and the fish size. But, when comparing the concentration of mercury in similar size for carp and catfish in the study area, an interesting trend emerges between the mean mercury concentration of 0.93 mg kg^{-1} for the catfish (59 and 42 cm length) and

the mean value of 0.55 mg kg^{-1} for carp (57 and 43 cm length). Barrat and Combrink (2002) have reported a mean concentration of mercury for catfish twice that of the carp. This implies that the catfish are storing greater amounts of mercury. The higher concentrations of mercury in the catfish may be explained by the fact that they are bottom feeders and may ingest sediment and mercury while feeding and that they are also predators and could be ingesting mercury from other organisms as well, unlike the carp that are predominantly omnivores only (Skelton, 1993). Mercury from the sediment is influenced by a low pH (5.8 to 5.4) which may allow the mercury to be taken up by higher trophic levels in the aquatic system (Panda et al.,1990); Hollsbeek et al., 1996).

The MeHg levels in fish should be reflected in the levels of mercury in hair of people which consume fish in the region. To confirm and elucidate the pathway of the MeHg from fish to human, epidemiological studies are needed on eating habits, and fish consumption, together with further surveys on the mercury pollution levels in various fish species.

According to the information obtained from the MRC SA, a questionnaire was administered to all subjects for hair sampling as a trial. The questionnaire contained eating habits including frequency of fish consumption, occupation and other details. The questionnaire revealed, in Madimeni, a good relationship between the high concentrations of mercury observed and the vegetarians. Vegetables in this area are watered with boreholes water and, probably due the porosity of the soil, the boreholes were contaminated by the water from the Inanda Dam. Figure 6.27 presents the probable pathway of the pollution of the boreholes from the dam. Further investigations, such as the analysis of plants collected from the same sampling site, are necessary for a better understanding of the result.

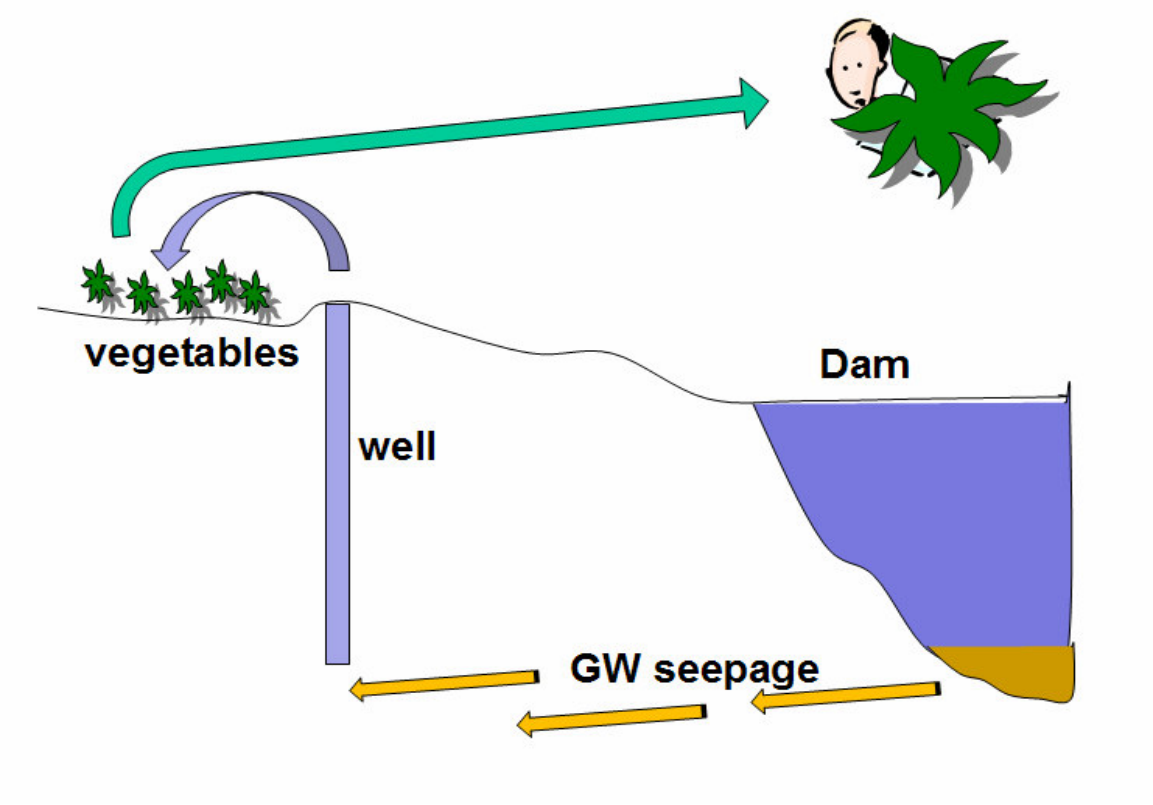


Figure 6.27: Eventual pathway of mercury contamination of vegetarians living in Madimeni.

These data together with the fact that human hair samples contain relatively high levels of mercury mostly in the methylated form suggest strongly that mercury released into the Mngceweni River by the former chemical processing plant (Thor Chemicals) in the nineties is transformed into MeHg in the aquatic environment, probably in the bottom sediments, and ultimately accumulates in fish bodies by biomagnifications in these ecosystem. Studies done by Barrat and Combrik (2002) on the rivers in the vicinity of Thor Chemicals confirmed the occurrence of this biotransformation.

6.3.3 Conclusion

Total mercury analysis and mercury speciation, by ICP-MS and GC-ICP-MS respectively, were carried out in human hair and fish samples collected from inhabitants living along side the Inanda Dam and in the dam, respectively.

The analysis has shown that 78.2% of the studied hair samples had a mercury concentration below 5 mg kg^{-1}, 10.3% had mercury concentration ranging between 5 to 15 mg kg^{-1} and 11.5% showed mercury level above 15 mg kg^{-1} reaching sometimes more than 50 mg kg^{-1}. The mercury contents on hair from the inhabitants of Madimeni were higher than those of Mshazi and Nqetho which are the closest to the Inanda Dam. This was related to the consumption of vegetables watered from boreholes water eventually contaminated by the dam. The speciation analysis indicated that methylmercury was the predominant form of mercury in hair samples. This suggests exposure to methylmercury rather than inorganic mercury and this is mostly due to fish consumption, although the epidemiological information collected does not support this link. This is somewhat surprising and certainly there should be more study of this.

The lowest mean value obtained (3.51 mg kg^{-1} for inhabitants of Nqetho) was higher than for people living in area non-exposed to mercury pollution (0.058 mg kg^{-1}).

The MeHg content in fish from 5 out of 10 samples (50% of the analyzed fish samples) exceeded the limits (< 0.5 mg kg^{-1} in SA) for mercury in edible fish tissues. Mean mercury concentration in catfish was 1.7 times greater than in carp of similar size. This was in good agreement with results obtained by Barrat and Combrink (2002) who reported a mean concentration of mercury for catfish twice that of the carp.

This study indicated that the Inanda Dam is affected by mercury pollution and could be a source of bio-accumulation and mobilisation of mercury into the

ecosystem. Therefore, the so-called reservoir effect (mentioned in chapter 2) could still be impacting fish concentration.

The contaminated zone around Thor Chemicals should be clearly identified since the region is also industrialized with other potential mercury sources besides the old Thor facility and, the Inanda Dam being very far from the site of the Thor Chemicals plant, it appears unlikely that the contamination signal could be transported that far in sufficient quantities to impact the fish in the dam. Sewage inputs could also be a source of methylmercury.

Unfortunately, the different pathways could not be discussed deeper since samples were provided by the MRC SA and we did not get access to vegetables, ground water or soil samples for further analyses.

A risk assessment study needs to be conducted on the fish-eating population, in particular where catfish may be consumed on a regular basis. Monitoring of fish mercury levels in the Inanda Dam should be encouraged in the future as a routine precaution.

Finally, the analysis of plants from the study area is recommended for a better understanding of the results and also in order to assess the bioavailability of mercury in the study site.

6.4 Mercury from gold tailings

The Central Rand Goldfield of the Witwatersrand basin has an important place in the history of gold mining in SA, as it is here that gold was first discovered in 1886 and where gold mining first commenced in the world's largest goldfield (Mphephu, 2004).

Gold was initially extracted using a mercury amalgam method up until the McArthur-Forrest process of gold extraction, using cyanide, was developed and was successfully applied to Witwatersrand ores (Naicker et al., 2003).

Ore mined underground was brought to the surface, were it was milled to a fine sand and, was exposed to a film of mercury spread on copper plates. These were periodically removed and the mercury-gold amalgam scraped off and distilled to recover the gold. The residual tailings were transported to dumps near the extraction plant, producing the so-called “sand dumps” (Naicker et al., 2003). The mercury amalgam process is highly selective for gold, and other ore minerals were unaffected, and hence reported to the tailing dumps.

Although having been of inestimable value of the economy of SA, mining activities on the Witwatersrand goldfield have left a legacy which has, in certain instances, impacted negatively on the surrounding environment and nearly communities. These effects are largely the result of the establishment of tailing dams. Due to inadequate design, poor management and neglect, these tailing dams have been subjected to varying degrees of water and wind erosion. Mining operations in the Johannesburg area continued until the early 1960s. Many of tailing dumps have remained undisturbed in the Johannesburg area for almost a century, during which time they have been exposed to rainwater and wind.

As the mining industry grew, secondary industries were established on the Witwatersrand, creating a vast mining-industrial complex. Most of the development was located in one sub-basin of the Vaal River system, namely the Klip River basin, and it is this sub-basin that receives the bulk of the run-off from the Witwatersrand mining-industrial complex (McCarthy and Venter, 2006).

Run-off consists of surface water from rain, treated sewage, treated and untreated discharges from industrial sources. Studies have reported the existence of acid mine drainage on the Witwatersrand (Wittmann and Forstner, 1976; Naicker et al., 2003), and the presence of enhanced concentrations of heavy metals in surface water and sediments in the Klip River basin (Naicker et al., 2003). The source was assumed to be from surface run-off from the main tailing dumps in the catchment as well as pumped water from mining operations (Marsden, 1986). Later studies showed that the dumps, particularly those consisting predominantly of sand sized

tailings, were polluting the underlying ground water which in turn was entering the surface water environment via ground-water seeps (Mphephu, 2004).

Sediments are important carriers for trace metals in the environment and reflect the current quality of the system. Analysis of the development of concentrations of metals along sediment cores therefore makes it possible to determine the history of metal contamination for a certain region. The metals can derive from direct discharges or from diffuse pollutant sources like atmospheric deposition (De Lacerda et al., 1991).

The present study focuses on the determination of total mercury concentration and its speciation in surface water and sediment profiles in order to investigate the effect of prolonged exposure to polluted discharges emanating from the Witwatersrand industrial-mining complex.

6.4.1 Sampling area

The sampling site is located in the upper Klip River catchment due south of the Johannesburg suburb of Kibler Park (Figure 6.28). The southern part is the area of heavy industrial activities with predominantly gold mines and resulting tailings dams. The Klipriver catchment finish further south in the Vaal Dam which provides drinking water for the greater Johannesburg metropolitan area with few million population.

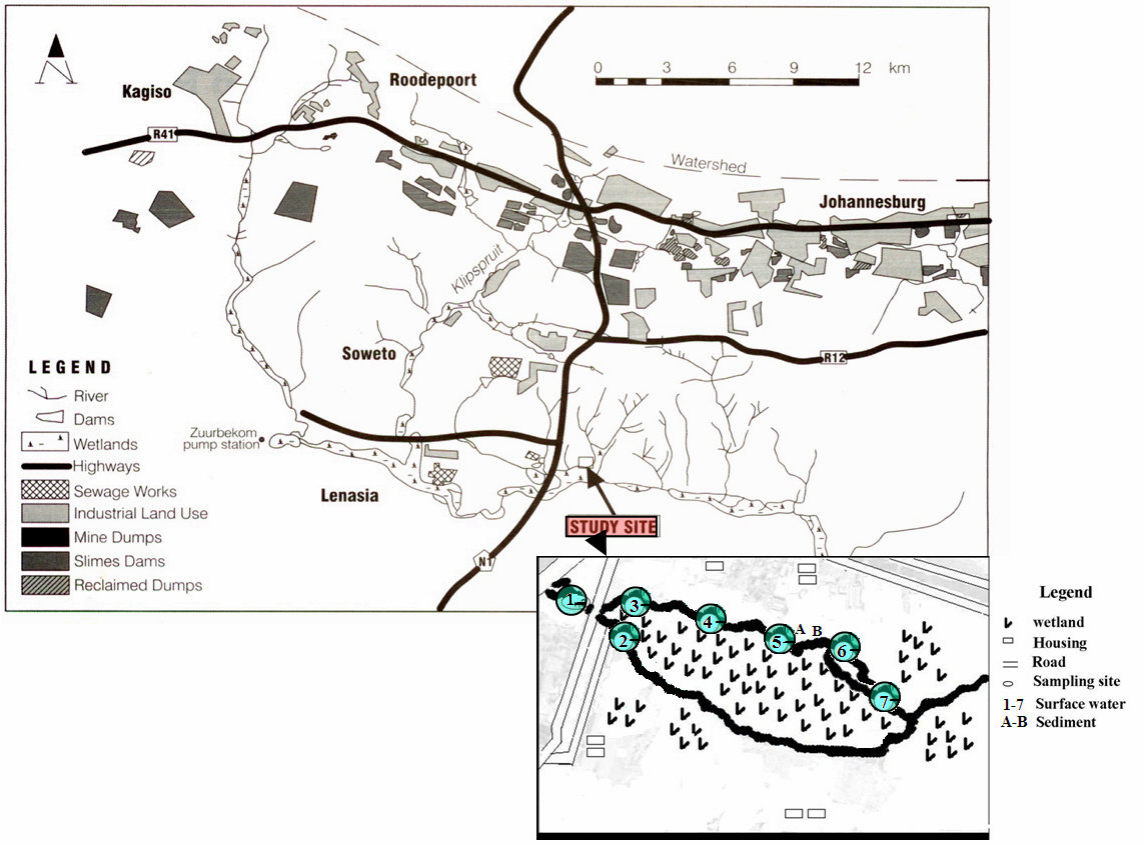


Figure 6.28: Map of the sampling site.

Sampling was done during dry season in order to assess the effect of seasonal change on the mercury behavior since a previous study was done on the same site during the wet season (Nsengimana, 2007).

The site was selected on the basis of accessibility and its downstream location relative to major sewage treatment plants.

6.4.2 Sampling and sample preparation

The sampling procedures for water and sediment were the same as those described previously for water and soil (See case I). Seven water samples were collected and the sediments (an auger profiles) were collected at 20, 40, 60, 80 and 100 cm depth. Table 6.15 gives details on the location of collected samples.

The pH, redox potential, conductivity and temperature were measured on the site using field meters. Once in the laboratory, the sediments were dry freeze and stored in the dark at 4°C prior to analysis.

Table 6.15: Sampling GPS data

Sample	GPS (South)	GPS (East)
Water samples		
W1	26°19'32.60"	27°59'15.85"
W2	26°19'33.50"	27°59'16.70"
W3	26°19'32.48"	27°59'16.93"
W4	26°19'31.18"	27°59'20.37"
W5	26°19'32.63"	27°59'26.86"
W6	26°19.355'	27°59.472'
W7	26°19.569'	27°59.503'
Sediment samples		
A1 (0-20 cm)	26°19.541'	27°59.439'
A2 (20-40 cm)	26°19.541'	27°59.439'
A3 (40-60 cm)	26°19.541'	27°59.439'
A4 (60-80 cm)	26°19.541'	27°59.439'
B1 (0-20 cm)	26°19.542'	27°59.441'
B2 (20-40 cm)	26°19.542'	27°59.441'
B3 (40-60 cm)	26°19.542'	27°59.441'
B4 (60-80 cm)	26°19.542'	27°59.441'
B5 (80-100 cm)	26°19.542'	27°59.441'

Water and sediment samples were also treated in the same way as water and soil samples in section 6.1. The total mercury concentration was determine by ICP-MS while inorganic and methylmercury were analyzed, after sample derivatization with $NaBEt_4$, using the coupling of GC with ICP-MS.

6.4.3 Results and discussion

Field measurements and mercury concentrations determined in water and sediment samples are presented in table 6.16.

It has been noticed during the analysis of field parameters that the sediment profile A was showing abnormal trends (Figure 6.29). These abnormalities are likely to be

caused by disturbances on the sediment profile. Disturbances might be due to anthropogenic activities such as those observed on the day of the sampling when caterpillars (probably from the near brick factory) were dumping stones on the sampling site. A mixture of different layers during sample collection can also contribute to the perturbation of the profiles composition and characteristics.
For this reason, results discussed below for sediment samples will focus only on the sediment profile B which showed more reliable trends.

The relatively high pH observed on both water and sediment samples is due to the presence of large tracts of Malmani dolomite, $CaMg(CO_3)_2$, in the studied site. The dolomite, being alkaline in nature, leads to an increase of pH. This also contributes to the improvement of the water quality since the high pH will enhance the precipitation of pollutants such as iron (Tutu, 2005).
The pH of the sediment profile B ranged from 6.72 to 7.26 and the redox potential from 436 to -28 mV. pH and conductivity had a tendency to increase with depth whereas the redox potential was decreasing to reach negative values at 60-80 cm depth. The uppermost sediment was characterized by the lowest pH and conductivity while the highest conductivity value was observed at the bottom sediment, suggesting an increase in ions concentrations with depth.

Table 6.16: Field parameters and mercury concentrations in water samples and sediment profiles.

Sample	pH	T (ºC)	ORP (Ag/AgCl)	ORP vs SHE (mV)	Conduct. (µS/cm)	HgTOT /ppb	%RSD (n=7)	MeHg (µg kg^{-1})	%RSD (n=3)	IHg (µg kg^{-1})	%RSD (n=3)
W1	7.5	14.2	200	414	634	0.69	5.0	nd(*)	-	nd	-
W2	7.97	14.2	174	388	693	1.20	1.3	nd	-	nd	-
W3	7.92	13.9	166	380	626	0.77	1.3	nd	-	nd	-
W4	7.83	16.2	169	383	670	0.39	2.0	nd	-	nd	-
W5	7.61	14.1	254	468	619	0.69	1.7	nd	-	nd	-
W6	7.87	14.5	228	442	643	1.93	0.7	nd	-	nd	-
W7	7.91	13.8	194	408	625	0.95	2.3	nd	-	nd	-
A1	7.16	13.9	244	458	283	168.84	1.2	1.53	8.2	149.74	5.6
A2	7.13	14.2	255	469	204	179.71	1.7	2.13	7.8	189.04	4.4
A3	7.04	14.3	-63.3	150.7	360	147.74	4.2	2.79	6.1	138.21	5.1
A4	7.26	14	-14	200	244	103.51	0.4	nd	-	104.87	6.8
B1	6.72	13.9	222	436	223	541.71	5.2	2.19	7.6	498.35	1.9
B2	7.24	12.6	93.8	307.8	221	139.02	0.6	4.45	5.1	132.40	5.3
B3	7.21	12.5	-58	156	270	178.50	0.3	3.69	5.3	155.07	3.3
B4	7.28	12.2	-224	-10	397	331.80	2.1	nd	-	314.68	2.6
B5	7.26	13.6	-242	-28	531	295.92	1.9	4.09	6.2	290.57	2.5

(*) nd = not detected; (**) na = not analyzed

Table 6.17: Ions concentrations(*) and % carbon on the sediment profile B

	Al	Au	Co	Cu	Fe	K	Na	Ni	Zn	F^-	Cl^-	PO_4^{3-}	SO_4^{2-}	% C
B1	28979.84	51.14	200	79.45	27364.32	254.47	1425.73	289.5	386.3	0.123	0.832	2.272	2.212	0.844
B2	9563.09	48.96	82.86	36.72	13935.03	915.25	1189.27	62.14	175.14	0.066	0.446	1.503	1.403	0.652
B3	8611.11	44.26	65.91	35.78	7720	720	938.8	36.72	124.29	0.044	0.42	1.37	1.138	0.545
B4	8751.78	34.51	60.21	40.38	10983.78	987.51	770.92	43.32	127.02	n.d	n.d	n.d	0.248	0.635
B5	25111.86	50	68	66	11384.19	951.98	835	221.778	286.71	0.069	0.56	1.327	1.605	1.013

(*): All concentrations are in mg kg^{-1}

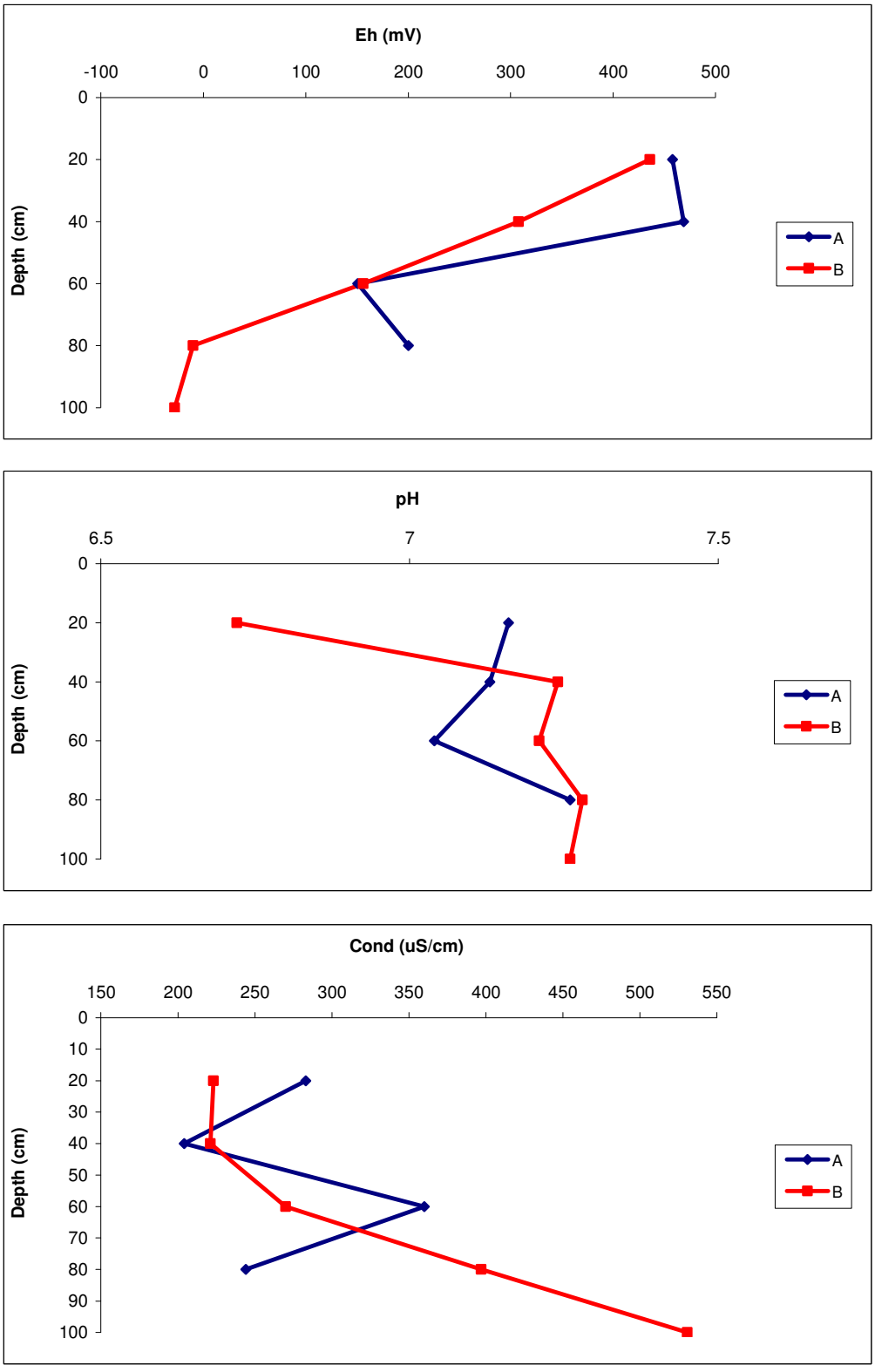


Figure 6.29: Redox potential, pH and conductivity of sediment profiles.

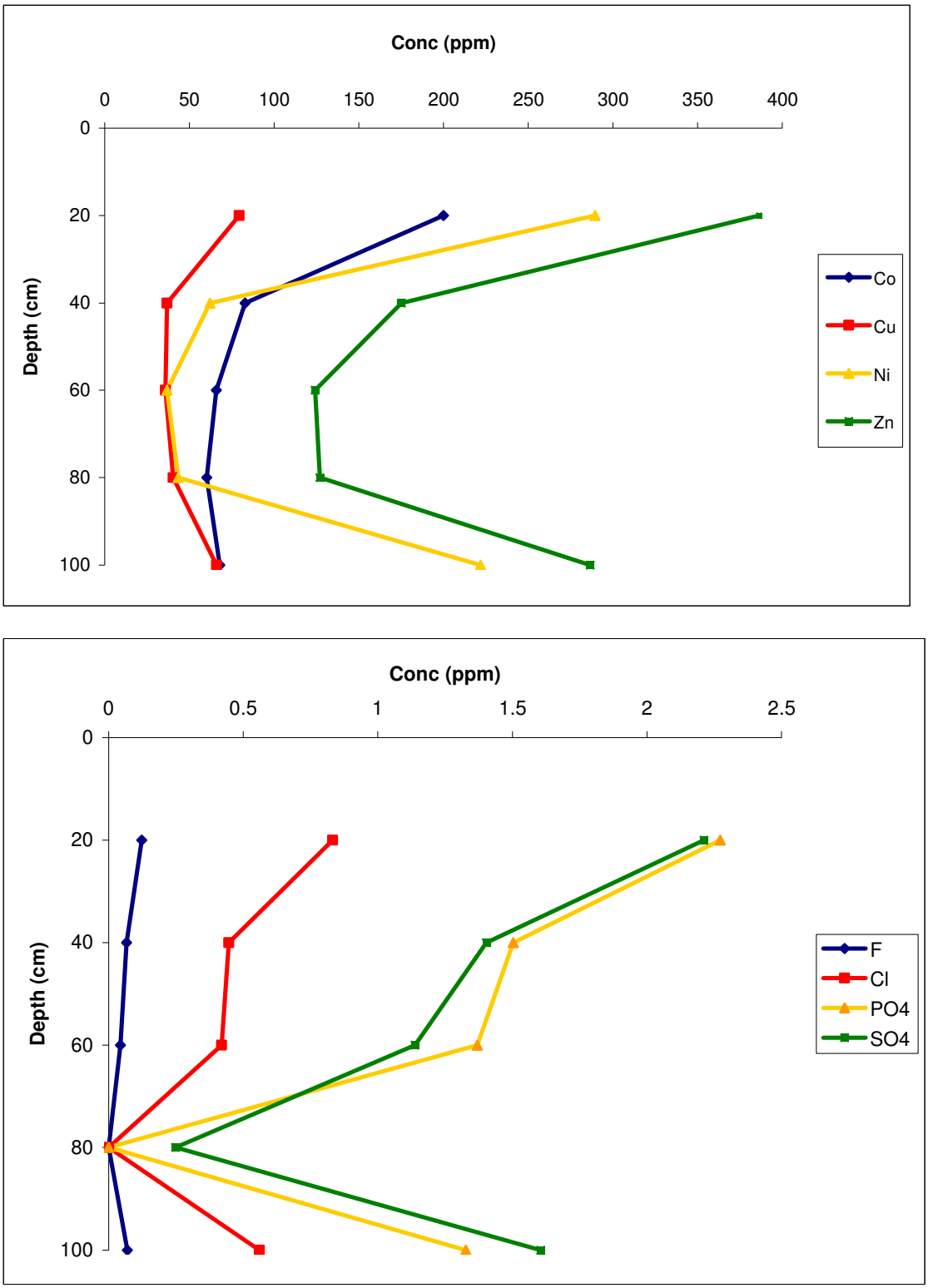


Figure 6.30: Examples of metals and anions concentrations with depth for the sediment profile B.

Most of the analyzed metals have shown the same trend (Figure 6.30) as described above with the highest concentration at the near surface and enrichment at about the same depth in the profile (80-100 cm below surface). McCarthy and Venter (2006) have reported the same trend on metal concentrations at the surface of peat profiles from the same site with enrichment of some of them at about 120 cm depth. As mentioned by these authors, the relatively high pH of the sediment would tend to immobilize metals. Therefore, mobilization of metals can be ruled out as the cause of the near-surface enrichment. Complex mix of sources has probably contributed to the metal loads and, therefore, it is not possible to quantitatively apportioning sources for most of the metals. It has to be noticed that F^-, Cl^-, PO_4^{3-} and SO_4^{2-} also have shown the same trend.

The wetlands serve as a sink for pollutants where polluting metals are trapped in sediments and peatlands. This is because of the wetlands' ability to absorb and remove toxins and pollutants from water by reduction and in raising the pH (Tutu, 2005).

Finally, the % carbon varied from 0.844 for the uppermost sediment to 1.013 at 80-100 cm with a minimum of 0.545 at 40- 60 cm.

6.4.3.1 Mercury in water samples

Concentrations of total mercury in water samples were low, ranging between 0.69 -1.93 µg L^{-1}, increasing following the direction of the flow. But when compared to those reported for natural water systems (up to 0.02 µg L^{-1}) (Harris et al., 2007) and also to the result reported by Nsengimana (2007) for the same study site, with total mercury concentrations ranging from 0.007 to 1.00 µg L^{-1}, the current results show a drastic increase of the mercury concentration in the studied water. However, it has been shown that streams draining areas with large accumulations of mercury-contaminated tailings from mercury or gold mining can exceed 0.1 or

even 1 µg L^{-1} (Ganguli et al., 2000; Gray et al., 2000). This confirms the negative impact of the tailings dams of the Wits basin to the surrounding water system.

It has to be noticed that neither IHg nor MeHg was quantified during the speciation analysis of water samples. This is due to the fact that mercury concentration in water samples was below the detection limit of the developed method for speciation, which was about 1.4 µg L^{-1} for both species.

6.4.3.2 Mercury in sediment samples

Total mercury concentration ranged from 139.0 to 541.7 µg kg^{-1} for the sediment profile B and was higher at 0-20 cm depth. Enrichment in Hg_{TOT} was observed at 60-80 cm depth with a concentration of 331.8 µg kg^{-1}. The speciation analysis of mercury (Figure 6.31) has shown the same trend for the inorganic mercury species with concentrations ranging from 132.4 to 498.4 µg kg^{-1} and with concentration of 314.7 µg kg^{-1} at 60-80 cm.

Many published studies also have reported similar trends (Hintelmann et al., 2002; De Lacerda et al., 1991; Hines et al., 2004).

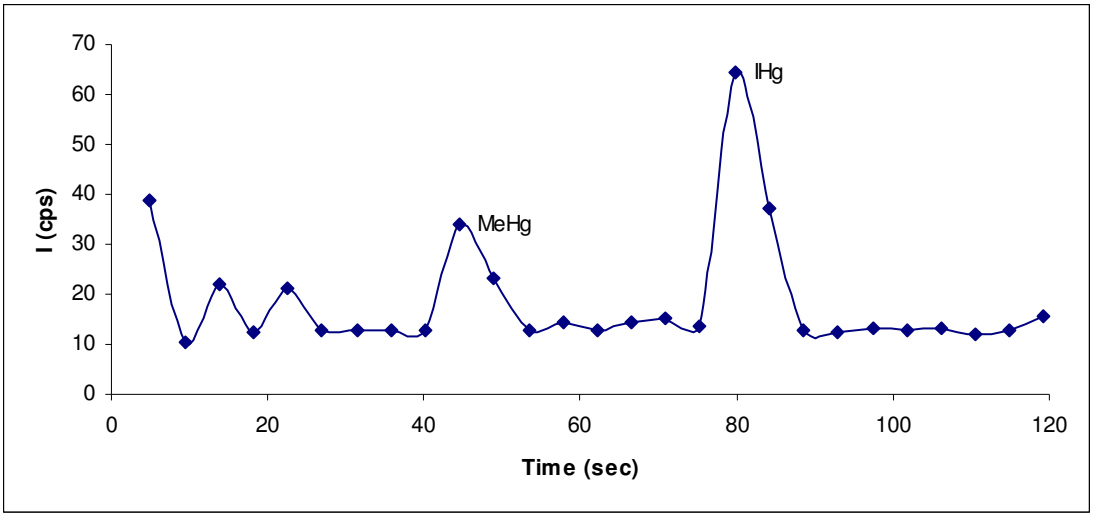


Figure 6.31: Example of chromatogram obtained on a sediment sample.

It was already mentioned in chapter 2 that Schwesig *et al.* (1999) has reported a similar pattern in mercury concentrations in forest soils and wetland sediments, with maximum concentrations Hg_{TOT} of >400 µg kg^{-1}. Evans (1986) and Rekolainen et al. (1986) have reported Hg_{TOT} in surface sediments of 560 µg kg^{-1} and 550 µg kg^{-1}, respectively. McCarthy and Venter (2006) also have reported mercury concentrations on peat profiles close to 500 µg kg^{-1}, except for one profile which showed a concentration of more than 1000 µg kg^{-1}. This latest was likely to be a consequence of analytical errors.

Methylmercury concentrations ranged from 2.19 µg kg^{-1} at the top level (0-20 cm depth) to a maximum of 4.45 µg kg^{-1} at 20-40 cm depth. It was also observed an increase in MeHg concentration at the bottom level (4.09 µg kg^{-1} at 80-100 cm depth). The percentage of MeHg in Hg_{TOT} ranged from 0.4 to 3.2%. Harris et al. (2007) have reported that MeHg usually represent less than 5% of the Hg_{TOT} in sediment. These authors have also stated that, although there are many factors controlling net formation of MeHg in the environment, two important factors are the abundance and availability of inorganic mercury (IHg); which in turn is related to the atmospheric deposition rate. Thus, MeHg will respond positively to changes in mercury loading.

As it was mentioned above, a previous study was carried out on the same site by Nsengimana (2007) in April 2006 i.e. at the end of the wet season in SA. The study reported concentrations ranging between 0.2-1.2 µg kg^{-1} for MeHg and 20-200 µg kg^{-1} for IHg. The concentration of mercury compounds was expected to be higher in winter (dry season) since the absence of rain should enhance the preconcentration of pollutants.

When compared the maximum mercury concentrations reported by Nsengimana and those of the current study, the latest shows an increase on mercury concentration of about 4 times for MeHg and 2.5 times for IHg. This shows that accumulation of mercury has occurred in the studied sediment. But, in order to

confirm this observation and eventually to determine the accumulation rate for MeHg, a long term study on the site is required.

MeHg in sediment is a useful indicator to assess changes in mercury load, changes to the net bioaccessibility of IHg and changes in bacterial activity. Hintelmann et al. (2002) and Krabbenhoft et al. (2005) have shown a positive relationship between the amount of IHg added in sediments and the amount of MeHg produced. Thus, positive correlations in sediments are expected between MeHg levels and changes in mercury load. Thus, the increase of both IHg and MeHg in the studied sediment agrees with the above statement.

The sediment profile B has shown high concentration of MeHg at 20-40 cm depth and lower concentration of IHg (Figure 6.32). At 60-80 cm depth the opposite trend was observed. It is well known that methylation of mercury occurs at reducive conditions, lowering the concentration of IHg. This might explain the relatively high concentration of MeHg at 20-40 cm from the surface. This trend was also observed by Nsengimana (2007) with increase in MeHg concentration at 30 cm depth. Hines et al. (2004) also have observed the increase in MeHg concentration at depth sediments. They concluded that redox potential, advective transport, or higher temperatures, stimulating microbial sulfate reduction should explain this trend. It has to be mentioned that 60-80 cm is also the layer where the concentration of SO_4^{2-} was the lowest, suggesting the occurrence of sulfate reduction at this layer.

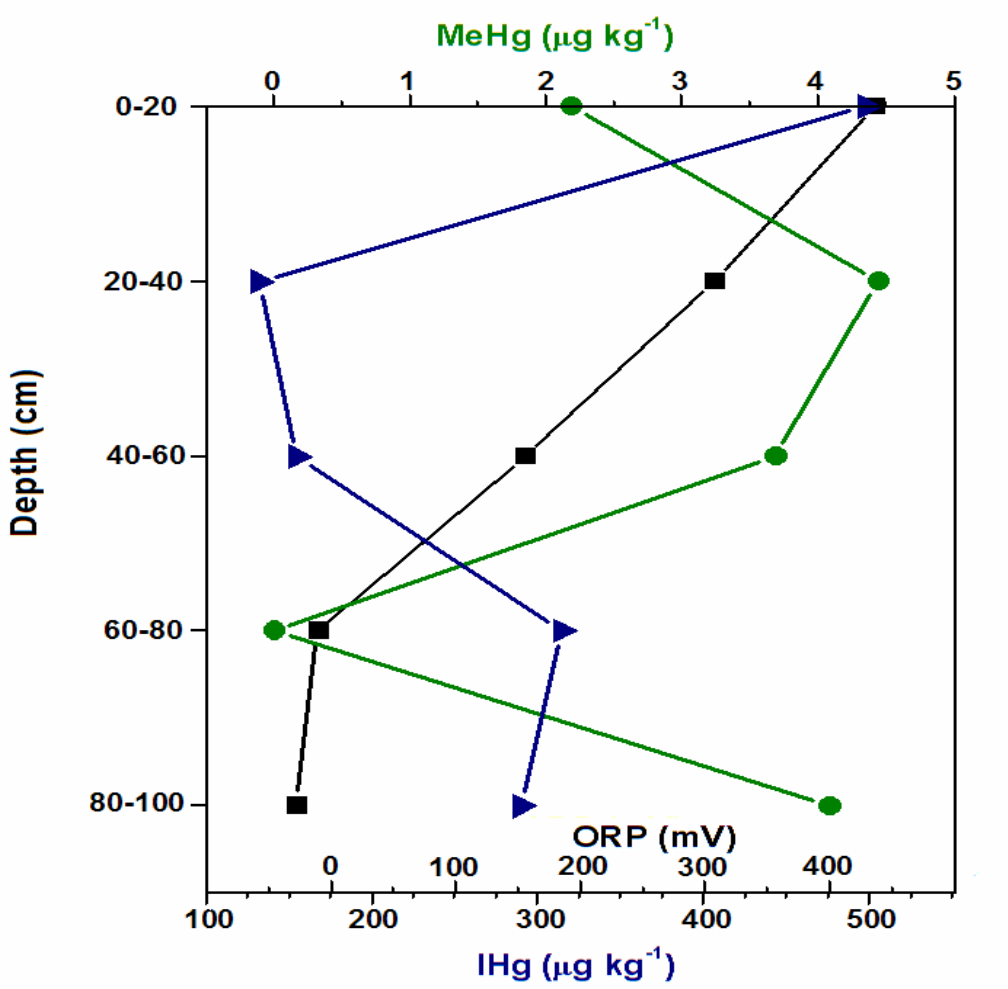


Figure 6.32: Speciation of mercury compounds in the sediment profile B.

Furthermore, Harris et al. (2007) reported that results from the Aquatic Cycling of Mercury in the Everglades (ACME) showed the maximum of %MeHg at the location where the inorganic pool of mercury in sediment was the most bioavailable to the methylation process. This confirms that changes in sediment MeHg concentration are, in part, driven by Hg_{TOT} or, specifically, by IHg concentration.

Studies of MeHg levels in bulk surface sediment (e.g. Gilmour et al., 1998; Krabbenhoft et al., 1999; Mason and Lawrence, 1999) have shown dependencies on inorganic mercury, organic matter, and sulfide. The determination of the percentage of carbon, using Leco CHNS Analyser, has shown the highest

percentage at the bottom level (80-100 cm) suggesting an important concentration of organic matter. This might explain the high concentration of MeHg observed at this level.

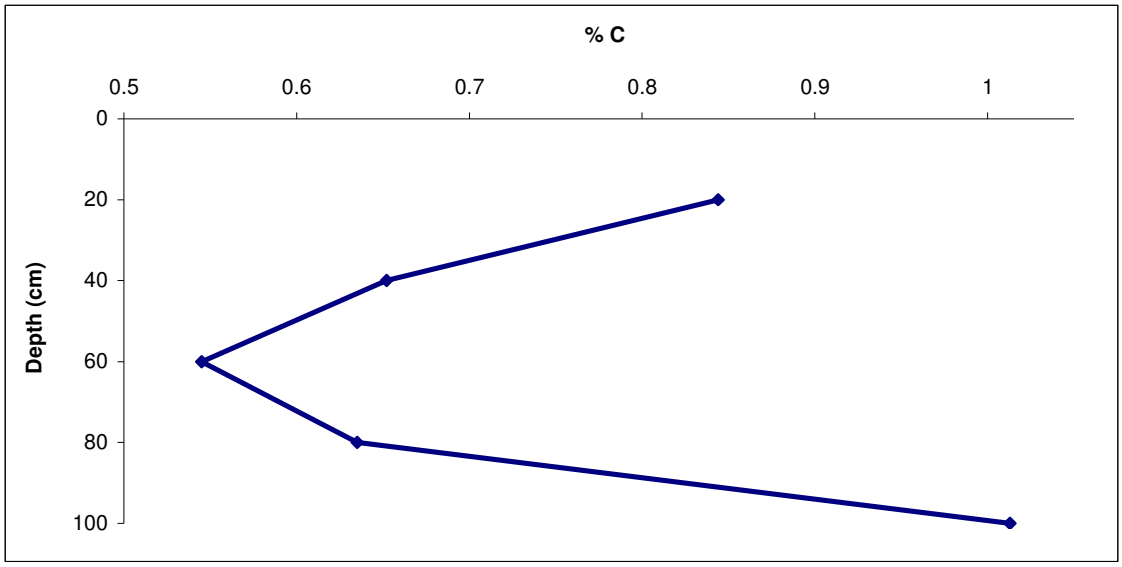


Figure 6.33: % carbon in the sediment profile B

6.4.4 Conclusion

This study was an attempt on determining the speciation of mercury in an environment affected by mining activities, especially gold mining. The analysis has revealed a rise in the concentration of total mercury in the stream. Obtained values were comparable to those reported in the literature for water systems affected by mining activities. Accumulation of the total, and therefore of the inorganic mercury, was observed accompanied by a large increase in methylmercury concentration in the sediment suggesting an increase in the methylation rate during the dry season. Anthropogenic activities, especially the establishment of mine tailings, are the main likely source of mercury load in the study site.

This study, together with the one done by Nsengimana (2007), provides a useful basis to indicate the level of mercury pollution in the Klip River wetland and has revealed that sediment and stream-water is a useful barometer of changes in mercury load in the Klip River catchment.

A deeper understanding of the mercury pollution record and distribution based on more widespread sampling as well as long-term study in selected localities is needed.

CHAPTER 7

GENERAL CONCLUSION

The development and optimization of a hyphenated system for mercury speciation analysis was achieved. The system provides species separation by capillary column gas chromatography and detection by inductively coupled plasma mass spectrometry. Construction of the transfer line was achieved by means of a relatively simple coupling procedure.

General procedures for the analysis of both solid (Soil, sediment, biotissues) and liquid (water) samples were presented and validated, for some, by analysis of certified material. The obtained IHg and MeHg concentrations are in agreement with the certified values.

The developed GC-ICP-MS technique has been shown to be an attractive tool for mercury species determination with a fast data acquisition time of two minutes, good linearity, precision and accuracy with detection limits below 5 ng ml^{-1} for both inorganic and methylmercury species in all analyzed matrices.

Simple, rapid and efficient sample leaching/digestion protocols based on microwave-assisted technique also have been developed for the determination of total and mercury species in environmental solid samples. The use of a microwave system offers reproducible and quantitative recovery of the analytes. Optimum extraction conditions of 3-4 minutes irradiation and 40-60 W power were selected for mercury speciation analysis leading to a drastic reduction of the sample preparation time compared, for example, to the overnight cold digestion technique. Therefore, microwave-assisted techniques for total and mercury speciation analysis offer advantages in terms of simplicity, reliability and analysis time.

The optimized methods were successfully applied in real environmental samples collected from different South African compartments affected by mercury pollution.

The assessment of mercury distribution in soil and water samples collected from a dismantled chlor-alkali facility revealed moderately to extremely contaminated soil and water. The $MeHg/Hg_{TOT}$ ratio in soil profiles were in the same proportions than those reported in the literature.

Total mercury analysis and mercury speciation in human hair collected from inhabitants living along side the Inanda Dam and in fish collected from the dam indicated that the Inanda Dam is affected by mercury pollution and could be a source of bio-accumulation and mobilisation of mercury into the ecosystem.
Methylmercury was found to be the predominant form of mercury in hair samples and 50% of the analyzed fish have shown MeHg concentrations above the SA limits for mercury in edible fish tissues.

Finally, the speciation of mercury in Klip River water and sediment has revealed a rise in the concentration of total mercury in the stream. Accumulation of the total mercury concentration in sediment also was observed. This was accompanied by a large increase in methylmercury concentration suggesting an increase in the methylation rate during the dry season.

This study was, in our knowledge, a first attempt in the development of a GC-ICP-MS technique for the speciation of mercury done within a SA laboratory. Still many have to be done in order to enlarge the use of the developed technique to other matrices such as coal, fly ashes and air samples. Therefore, the development of methodologies for very low mercury concentrations is needed.

Furthermore, the use of an internal standard is advisable for a better monitoring of the instrument signal and for mass bias correction during analysis. Recent techniques such as isotope dilution, which helps in monitoring eventual artifact methylation that may occur during sample preparation, will contribute in improving the precision and accuracy of the analysis.

Although the developed GC-ICP-MS system has been specifically optimized for analysis of mercury compounds, it can be extended to other metals in environmental matrices.

PUBLICATION

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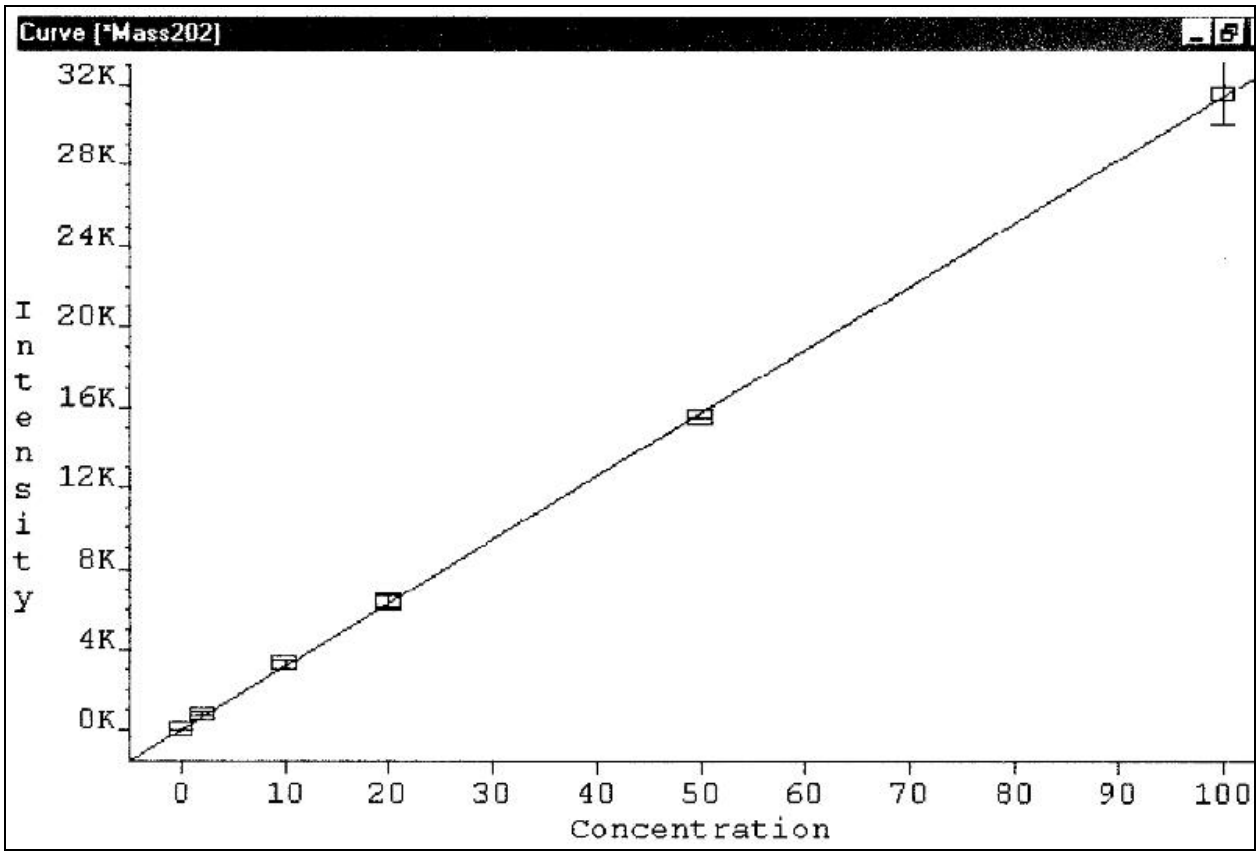
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APPENDIX 1 ICP-MS CALIBRATION GRAPH

Figure 1A: Hg^{202} standard calibration (R^2= 0.999).



APPENDIX 2 GC-ICP-MS CHROMATOGRAMS

Figure 2A1: Chromatogram of standard IHg (5 ppb) and MeHg (15 ppb).

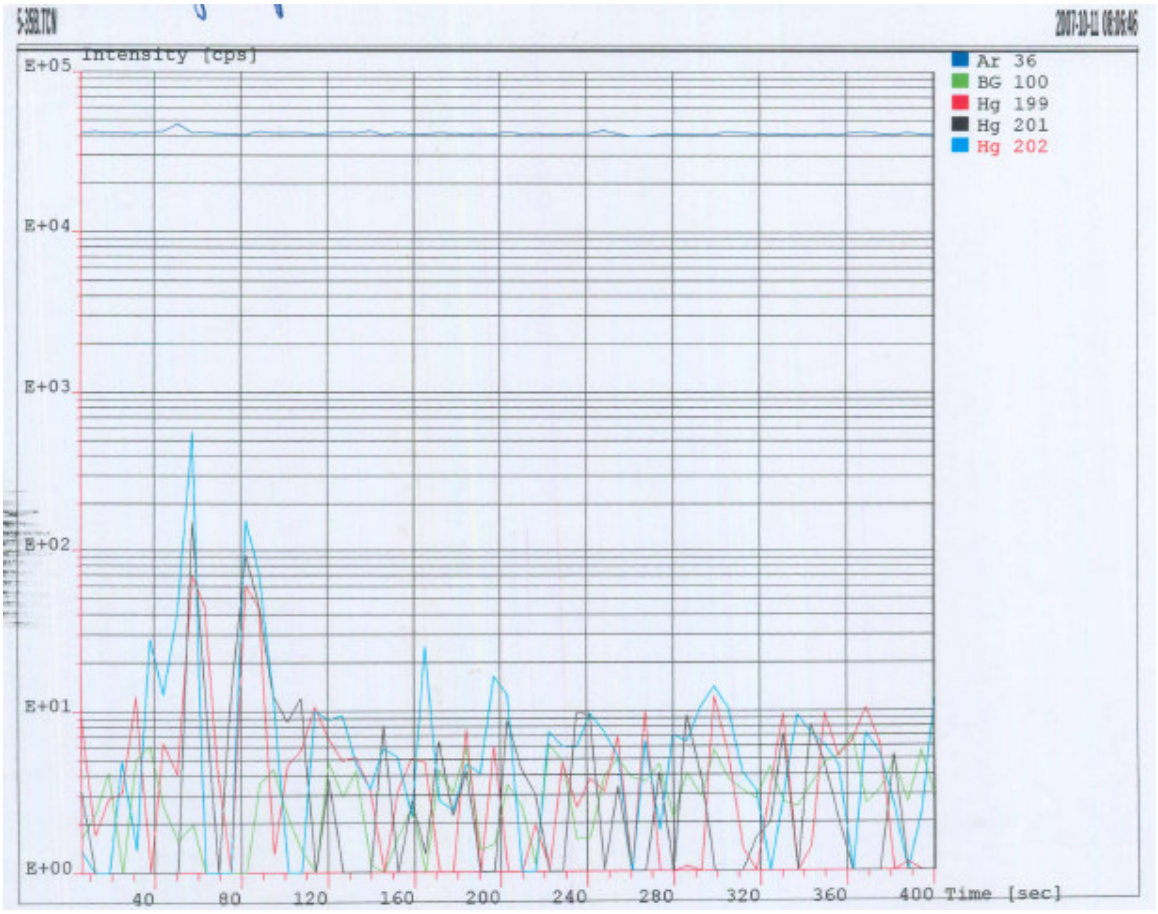
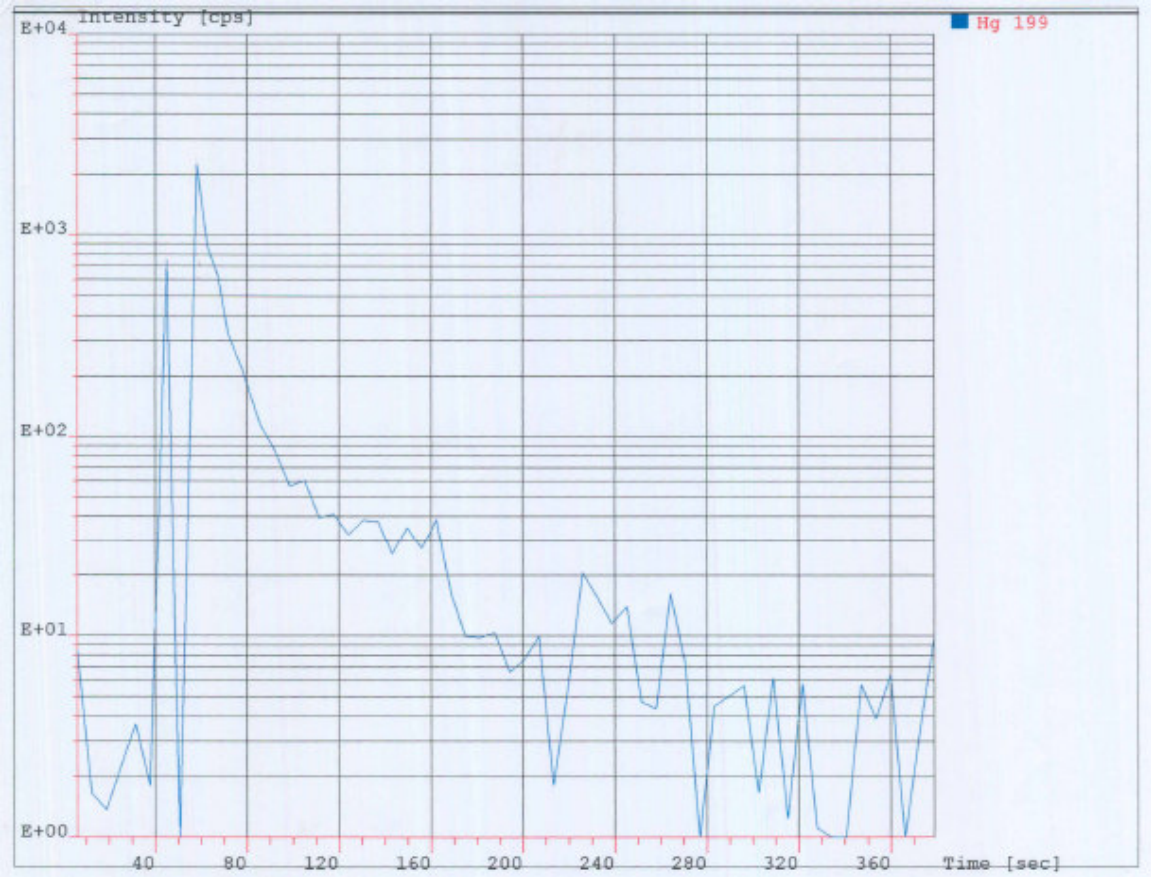


Figure 2A2: Chromatogram of IHg (5 ppb) and MeHg (35 ppb).



APPENDIX 3 Photographs of features in the study area (Klip River, Johannesburg).

(a)



(b)



Figure 3A1 (a) and (b): A view of the study water system



Figure 3A.2: Dump (at the back) from the brick factory.