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DETERMINATION AND SPECIATION OF MERCURY IN SEDIMENT AND RELATED SAMPLES FROM GREECE AND SCOTLAND

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A thesis presented in fulfilment of the requirements for the degree of

Doctor of Philosophy

Declaration of author's rights

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

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For my parents John and Irene,
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Previously published work from this thesis

Cavoura, O., Aspray, T. R., Cortis, R., Davidson, C. M., Keenan, H. E., Katsiris, N. and Reis, A. T., (2013), Mercury contamination and related microbial community characterisation in sediments of the Union Canal, Scotland, U.K., 11th International Conference on Mercury as a Global Pollutant, Edinburgh, U.K..

Cavoura, O., Keenan, H., Davidson, C. M. and Katsiris, N., (2011), Screening and determination of mobility for mercury in marine sediment, 10th International Conference on Mercury as a Global Pollutant, Halifax, Canada.

Cavoura, O., Campbell, E., Keenan, H. and Davidson, C. M, (2011), A rapid colorimetric paper-based screening method for measuring mercury in marine systems, 10th International Conference on Mercury as a Global Pollutant, Halifax, Canada.

Previously published work related to this thesis

Rodriguez-Gil, C., Cavoura, O., Davidson, C. M., Keenan, H. E. and Aspray, T. J., (2013), Presence, diversity and abundance of the mercuric reductase (*mer*A) gene in sediment along a section of the Union Canal, Scotland, 7th Annual Environmental and Clean Technology conference in Edinburgh, Scottish Environmental Technology Network.

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APPENDICES

Abbreviations

AAS	atomic absorption spectrometry
AFS	atomic fluorescence spectrometry
ASGM	artisanal and small scale gold mining
ASTDR	Agency for Toxic Substances and Disease Registry
BBB	blood-brain barrier
BMDL	bench mark dose lower
CCME	Canadian Council of Ministers of the Environment
COT	Committee on the Toxicity of Chemicals in Food Consumer Products and the Environment
CRM	certified reference material
CV	cold vapour
DI	deionised
EPA	Environmental Protection Agency
EQS	environmental quality standard
EtHg	ethylmercury
EXAFS	extended X-ray absorption fine structure
GC	gas chromatography
GEM	gaseous elemental mercury
GI	gastrointestinal
GOM	gaseous oxidised mercury
HF	hydrofluoric acid
HPLC	high performance liquid chromatography
IARC	International Agency for Research on Cancer
IED	Industrial Emissions Directive
ISQG	interim sediment quality guideline
IUPAC	International Union of Pure and Applied Chemists
LOD	limit of detection
MeHg	methylmercury
MS	mass spectrometry
NHANES	National Health and Nutrition Examination Survey
NOAEL	no observed adverse effect level
OM	organic matter
PB	placental barrier

PEL	probable effect level
PhEt	phenylmercury
psu	practical salinity units
PTFE	polytetrafluoroethylene
PTWI	provisional tolerable weekly intake
RPDC	Redding Park Development Company
SCDS	Seychelle Child Development Study
SEPA	Scottish Environment Protection Agency
SPE	solid phase extraction
SPME	solid phase micro extraction
SQV	sediment quality guideline
TD	thermal desorption
TEL	threshold effect level
TV	target value
UNEP	United Nations Environment Programme
WHO	World Health Organisation
XANES	X-ray absorption near-edge structure
XRF	X-ray fluorescence spectroscopy

Abstract

Mercury is a global pollutant. Research is needed to improve methods for Hg detection and improve understanding of its biogeochemical cycle and risks to human health. In this study, a simple, colorimetric method utilising the reaction of Hg with copper(I)iodide was characterised and adapted for screening marine samples, and the speciation and relationships of Hg with other sediment parameters in contaminated environments was examined. Field application of the colorimetric method indicated Hg concentrations in sediments from West Scotland, U.K., and Kifissos River, Greece, were below Canadian sediment quality guideline values of 0.13 and 0.17 mg/kg for marine and freshwater sediment respectively, whereas sediment from the Falkirk area of the Union Canal, U.K., and Elefsina Bay, Greece, had Hg concentrations above probable effect levels (0.49 and 0.7 mg/kg).

Total sediment Hg concentrations, determined quantitatively using cold-vapour atomic absorption spectrometry (AAS), ranged from 0.220 to 2.96 mg/kg in Elefsina Bay. Concentration was influenced by proximity to nearshore pollution sources but was not found to vary seasonally or be related to sediment organic matter content. Sequential extraction indicated that Hg speciation – hence mobility – varied between sites. No Hg was detected in the fish *Mugil Cephalus* indicating no threat to humans from consumption.

Total sediment Hg concentrations in the Union Canal ranged from 22 to 1200 mg/kg. Both sequential extraction and speciation analysis using thermodesorption AAS indicated that *ca.*>70% of the Hg present was mobile. Analysis by gas chromatography inductively coupled plasma mass spectrometry showed that the methylmercury content was <0.03% of the total Hg content. A negative relationship was found between total Hg concentration and % methylmercury (r^2 = 0.60). Ethylmercury was detected in the samples and weak positive relationships determined between methylmercury and ethylmercury and organic matter content, while low pH appeared to favoured the partitioning of methylmercury into the water column.

CHAPTER 1 MERCURY IN THE ENVIRONMENT

1.1 Introduction

1.1.1 Mercury: a global threat

Mercury (Hg) is a potentially toxic element that can be harmful to humans and wildlife. Distributed throughout the environment in different chemical forms with characteristics ranging from global transfer to neurotoxicity, Hg is ranked third on the priority list of substances that pose a potential threat to human health in the Agency for Toxic Substances and Disease Registry (ASTDR) (ASTDR, 2013). With atmospheric emissions of Hg increasing over the last century (Chen *et al.*, 2012), recent legislation and policies have aimed to reduce Hg emissions and minimise its environment impact. On a European level, in 2010, the Industrial Emissions Directive (IED) (EU, 2010) aimed to minimise emissions of, and transboundary pollution caused by, pollutants released in industrial processes by setting targets for emission levels and outlining preventative measures to reduce or minimise pollution. More recently, in October 2013, the Minamata Convention on Hg was agreed in Kumanamoto, Japan (UN, 2013). An international treaty agreed under the United Nations Environment Programme (UNEP), the objective of the convention is to 'protect human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds' by banning or restricting the manufacture of Hg-containing products and phasing out the use of Hg in industry by the year 2020 (UNEP, 2014).

1.1.2 Identity

Named after the Roman God Mercury, Hg has been used since ancient times. Its chemical symbol comes from the Greek word 'hydrargyrus' meaning liquid silver and Hg is the only metal that is liquid at ambient temperature and pressure. It exists in three valence states, Hg^0, Hg^I (mercurous) and Hg^{II} (mercuric). Mercurous Hg is found only as a dimer and, owing to its chemical instability, is rare in the environment (Ullrich *et al.,* 2001; Lin and Pehkonen, 1999). In addition to inorganic compounds, in the Hg^{II} valence state Hg can form organometallic compounds, or 'organic' Hg compounds, as they are commonly known, containing a Hg-carbon bond. Of these, methylmercury compounds, represented by the general formula

CH_3HgX, and often simply called collectively methymercury (MeHg) are the most common organic forms of Hg in the environment (UNEP, 2002). These different forms of Hg can be described as different species, where chemical species refers to the 'specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure' (IUPAC, 1997).

1.2 Sources of Hg in the environment

1.2.1 Sources of Hg emission to the atmosphere

Estimates of total annual Hg emissions to the atmosphere vary between 5500 and 8900 t/y (AMAP/UNEP, 2013). The release of Hg to the atmosphere can occur from natural sources, anthropogenic sources or from the re-emission of combined past natural and anthropogenic releases (Table 1.1). Current annual releases from natural sources are of geogenic origin such as the weathering of rocks, volcanic activity, underwater vents and geothermal areas, and are estimated to be 80 - 600 t/y (Mason *et al.*, 2012). Over 99% of Hg released to the atmosphere from natural sources is gaseous elemental Hg (GEM), $Hg^0_{(g)}$ (AMAP/UNEP, 2013). While natural emissions have remained roughly constant over the years, it is estimated that anthropogenic activities have resulted in between two and five times as much Hg emission to the atmosphere than before the industrialised period (Sunderland and Mason, 2007). Anthropogenic emissions of Hg to the atmosphere are composed of approximately 60% GEM, 30% gaseous oxidised Hg (GOM) ($Hg^{II}_{(g)}$), and approximately 10% Hg^{II} attached to particulate matter, $Hg_{(p)}$ (Pacyna *et al.*, 2006). Anthropogenic sources of Hg are varied and emissions are estimated to be over 2000 t/y (AMAP/UNEP, 2013; Pirrone *et al.*, 2010). The largest anthropogenic emission today, estimated to be over 800 t/y, comes from coal fired power plants (Pirrone *et al.*, 2010) where Hg impurities in coal are released on combustion at high temperature. Similarly, Hg is released from Hg impurities in ores mined for ferrous and non-ferrous metal production. Such releases can be described as unintentional 'uses' of Hg. The extraction of Hg itself comes from the mining of cinnabar, HgS, the major mineral deposit of Hg (EA, 2009a). Intentional uses of Hg include its use in artisanal and small scale gold mining (ASGM), in certain industrial processes such as vinylchloride production, and in man-made products. In ASGM Hg is used to amalgamate gold following its extraction. The amalgam, which contains

approximately equal parts Hg and gold, is then heated, releasing Hg to the atmosphere (WHO, 2013). Gold obtained in this way accounts for approximately 15% of the world gold production and recent estimates suggest the Hg emitted in this process accounts for approximately 25% of the global anthropogenic emission (400 t) (Pirrone *et al.*, 2010; Telmer and Veiga, 2009). The chlor-alkali industry, in which Hg is used as a cathode in the production of caustic soda and chlorine from brine, has also been, until recently, a major industry utilising Hg. New technologies, such as the substitution of the Hg cell with the membrane cell, have reduced use in this area. The disposal or breakage of Hg-containing products such as electrical devices, lamps and batteries and formerly Hg thermometers, is also a source of Hg release. Cremation also results in the release of Hg contained in dental amalgams. Re-emission of Hg from oceans and streams, from soil and vegetation, and from the burning of biomass contributes approximately 60% of total Hg atmospheric emissions (AMAP/UNEP, 2013).

Table 1.1 Global Hg emissions from natural sources after Mason *et al.* (2012), anthropogenic sources after Pirrone *et al.* (2010) and re-emission after Mason *et al.* (2012)

Source		**Releases to atmosphere (t/yr)**
Natural	Geogenic sources	80 - 600
Anthropogenic	Coal and oil combustion	810
	Artisanal and small scale gold mining	400
	Non-ferrous metal production	310 (USGS, 2004)
	Cement production	236
	Waste disposal	187
	Chlor-alkali industry	163
	Hg production	50
	Vinylchloride production	24
	Other	140
Re-emissions of natural and anthropogenic origin from oceans and other surface water		2000 - 2900
Re-emissions of natural and anthropogenic origin from soil/vegetation		1700 - 2800
Re-emissions of natural and anthropogenic origin from biomass burning		300 - 600

A record of sources of emissions over 270 years has been obtained by extracting ice cores from Wyoming which were undisturbed for hundreds of years and determining Hg concentrations. Schuster *et al.* (2002) were able to attribute peaks in Hg concentration from atmospheric deposition to specific anthropogenic and natural emission sources (Figure1.1).

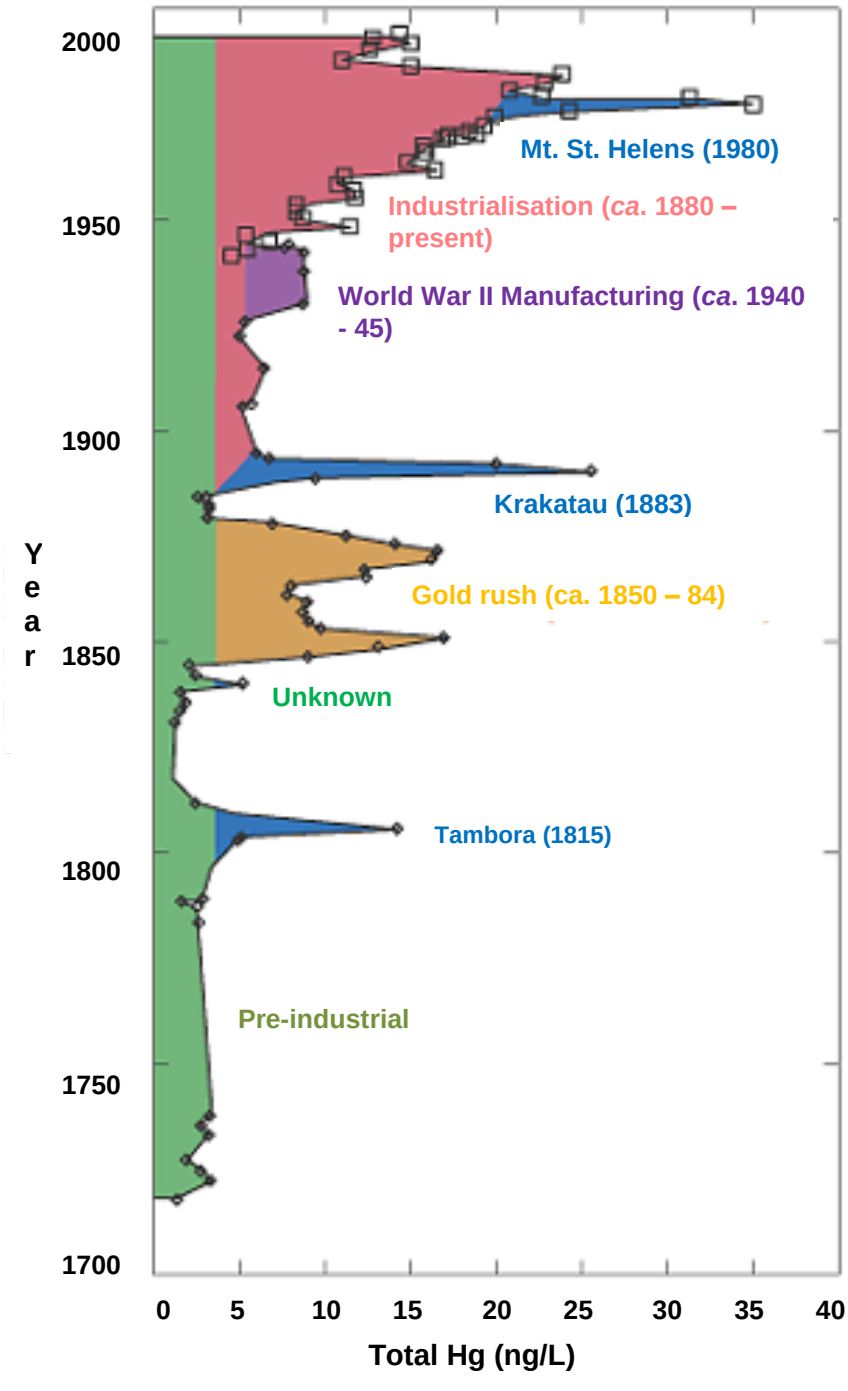


Figure 1.1 Atmospheric Hg depositions from natural and anthropogenic events over the past 270 years (after Schuster *et al.*, 2002).

Specifically, peaks in the years 1815, 1883 and 1980 were attributed to volcanic eruptions; the increased concentrations of Hg at the depth corresponding to the period 1850 - 1884 was attributed to the US gold rush; and the gradual increase from the 1950s until the present day was deemed a result of recent industrial expansion.

1.2.2 Sources of Hg release to the aquatic environment and land

Atmospheric deposition, surface water run-off, industrial effluent and natural weathering all contribute to Hg in the aquatic environment, with atmospheric deposition estimated to account for over 80% of the total Hg deposition to water (AMAP/UNEP, 2013). As in the case of emissions to the atmosphere, releases may be from natural (geogenic) sources or of anthropogenic origin such as direct discharge from industrial processes. It is estimated that ASGM releases over 880 t/y Hg to both land and water and that point source emissions from other industries and from waste disposal are 185 t/y (AMAP/UNEP, 2013). In additional to industry, agricultural practises also contribute to Hg release to land and water. Although from the 1980s onwards the use of pesticides containing Hg has been prohibited in an increasing number of countries (UN, 2002), the use of sewage sludge is estimated to contribute 4.4 t/y Hg to agricultural soils (BIO intelligence Service, 2012). However these figures are estimates and no global inventories exist. The release of Hg to the aquatic environment and to land remains an area where many gaps in knowledge exist; Pacyna *et al.* (2008) suggest that releases of Hg to land from solid waste may be greater than emissions to atmosphere and to water.

1.3 Environmental cycling of Hg

1.3.1 Factors influencing the fate of Hg in the environment

The environmental cycling of Hg involves atmospheric transport, deposition to land and water bodies, and re-emission, before, after an estimated cycling time of 3000 years, final burial in deep sea sediment (Selin, 2009) (Figure 1.2). During this time, Hg undergoes redox and methylation/demethylation reactions that determine its transportation and fate. Some of the main transportation and transformation mechanisms are discussed below.

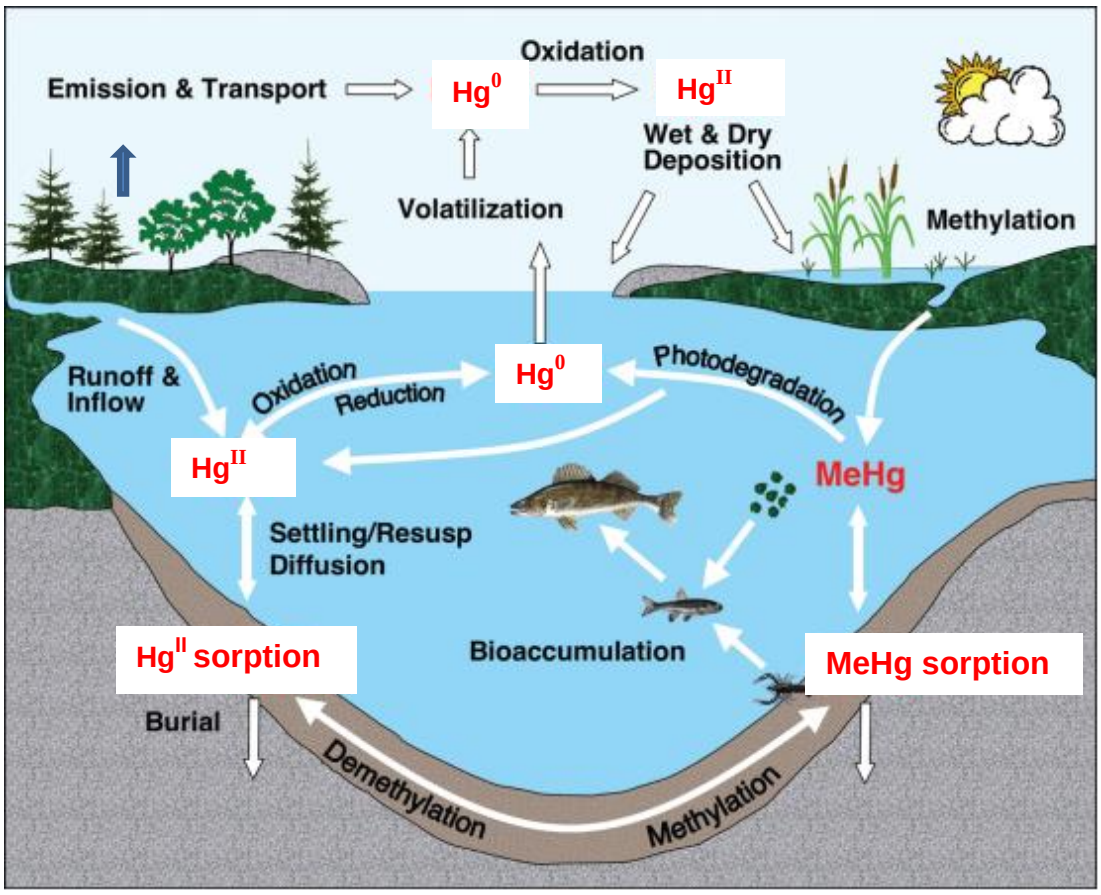


Figure 1.2 Biogeochemical Hg cycle. (Adapted from Selin, 2009).

1.3.2 Hg in the atmosphere

In the atmosphere Hg partitions between the gas, aqueous (cloud, rain, fog) and solid phases, primarily in three species, $Hg^0_{(g)}$, $Hg^{II}_{(g)}$ and $Hg_{(p)}$. The exact composition of neither $Hg^{II}_{(g)}$ nor $Hg_{(p)}$ is known; $Hg^{II}_{(g)}$ has been proposed to be mercuric halides (such as $HgCl_2$, $HgBr_2$) and mercury hydroxide ($Hg(OH)_2$) (Lin and Pehkonen, 1999) and $Hg_{(p)}$ mostly divalent species bound to particulate matter. Over 95% of Hg in the atmosphere is $Hg^0_{(g)}$. With a vapour pressure of 0.18 Pa at 20 ℃ (NRC, 2001), Hg^0 has a high volatility. This, coupled with a low solubility (5.6×10^{-5} g/L at 25 ℃) (NRC, 2001) and slow reactivity with common atmospheric oxidants (AMAP/UNEP, 2013), results in very little Hg deposition on the earth's surface in this form. The main mechanism for the removal of $Hg^0_{(g)}$ is oxidation to $Hg^{II}_{(g)}$. Generally however this occurs slowly and $Hg^0_{(g)}$ has an atmospheric residence time of 0.5 - 1 year (Selin, 2009; Slemr *et al.,* 1985) allowing its global transportation before oxidation and dry or wet deposition on land or water. As an exception, in the polar regions $Hg^0_{(g)}$ is rapidly converted to $Hg^{II}_{(g)}$ during the spring polar sunrise in a

process known as Hg depletion (Edinghaus *et al*., 2002). There is on-going debate over which compounds oxidise $Hg^0_{(g)}$. The most important are thought to be ozone (O_3), the hydroxyl radical and reactive bromine species (AMAP/UNEP, 2013). A review is given in Subir *et al.* (2011). Oxidised Hg species have a much shorter atmospheric residence time than Hg^0; $Hg^{II}_{(g)}$ species have an atmospheric residence time of hours to days after which they are removed as a result of their greater solubility relative to $Hg^0_{(g)}$ (for example the solubility $HgCl_2$ is 2.0 x 10^{-5} g/L at 25 ℃) (NRC, 2001), and the removal times of $Hg_{(p)}$ species in the lower atmosphere ranges from days to weeks. In contrast to $Hg^0_{(g)}$, these species contribute to local Hg deposition around point sources.

1.3.3 Hg in soil

In soil Hg is found mainly as inorganic Hg^{II} with total average Hg concentrations ranging from 0.050 to 0.200 mg/kg (Davis *et al.*, 1997). After atmospheric deposition the majority of Hg becomes bound to soil and vegetation although rapid volatilisation back to the atmosphere may also occur. By spraying 7.7 mg of Hg^{II} 99.2% enriched with stable isotope ^{202}Hg in a boreal forest and monitoring its fate, Hintelmann *et al.* (2002) determined that over one season, 8% was volatilised back to the atmosphere, less than 1% was lost in run-off and the remaining ^{202}Hg was associated with vegetation and soil. The residence time for Hg in soil has been estimated at > 100 years since it is tightly bound mainly to organic matter (OM) (Tipping, 2011). In addition to loss from soil run-off, Hg bound to soil may be slowly evaded back to the atmosphere, a process dependent on surface sorption, temperature, moisture, light and microbial activity (Tipping, 2011) estimated to occur over years to centuries (Driscoll *et al*., 2013). Methylation can also occur in the soil environment converting inorganic Hg^{II} to MeHg (ASTDR, 1999). The processes of sorption and methylation in soil are common to Hg^{II} in sediment and will be discussed in Section 1.3.4. Concentrations of MeHg in soil are estimated to range from 1 to 3% of total Hg concentration (Kabata-Pendias and Mukherjee, 2007) with typical MeHg levels in background soils ranging from 0.01 to 2 *μ*g/kg (Davis *et al.*, 1997). Certain plants can accumulate Hg compounds which can lead to increased concentrations in the edible parts in plants growing in Hg-contaminated soils (EA, 2009a).

1.3.4 Hg in the aquatic environment

1.3.4.1 The partitioning of Hg in the aquatic environment

In the aquatic environment Hg will partition between the dissolved, colloidal and sediment phases in forms largely controlled by its affinity for reduced sulfur groups such as sulfides and thiols. The principal pathways for the removal of Hg from the aquatic environment are deep burial in sediment and volatilisation to the atmosphere.

1.3.4.2 Interactions with sediment

The main environmental sink for Hg is sediment and it is estimated that 90 - 95% of the total Hg in the environment is associated with sediment (Faust and Osman, 1981). Mercury concentrations in sediment vary. Generally concentrations < 0.1 mg/kg are considered unpolluted (Di Leonardo *et al.*, 2006) although Boszke *et al*. (2003) cite natural mercury concentrations in the range 0.010 - 0.2 mg/kg. The most common type of mercury species in sediment are inorganic Hg^{II} compounds (Beldowski and Pempkowiak, 2003). Organic species, primarily MeHg, typically contribute < 2% of the total mercury concentration (Boszke *et al.,* 2003). For example, Bloom *et al.* (1999) found concentrations of MeHg in the range 0.1 - 10.3 *μ*g/kg in Lavaca Bay surface sediments, corresponding to a maximum of 0.65% of the total mercury concentration while Hammerschmidt *et al*. (2008) determined a greater contribution of MeHg to total Hg concentration in New Jersey harbour sediments; 1.65% maximum MeHg concentration was determined where total Hg concentrations ranged from 1.9 to 8.5 *μ*g/kg.

Sediment formed *in situ* comprises both an inorganic and an organic component. The inorganic component, or mineral matrix, is derived from primary silica and aluminium (Al) oxide minerals such as quartz and feldspars, their weathering products (also called secondary minerals and which includes clay minerals), and metal (hydr)oxides formed from the weathering of primary minerals high in metals such as iron (Fe), manganese (Mn) and Al. Living or decaying plant and animal biomass and their excretion products comprise the organic component of soil, that is composed of large ill-defined molecules, rich in oxygen (O), nitrogen (N) and S ligands. External inorganic and organic particulates from surface water run-off,

storm waters, and urban and industrial waste waters also contribute the sediment composition.

The interactions of Hg in sediment are governed almost exclusively by its affinity for reduced S ligands as indicated by formation constant (log K) values found by Dyrssen and Wedborg (1991) for the Hg complexes in the reactions shown in Equations 1.1 to 1.3.

$Hg^{2+} + SH^- + Cl^- \rightleftharpoons$	HgClSH	log K = 25.8	**Equation 1.1**
$Hg^{2+} + SH^- + OH^- \rightleftharpoons$	HgOHSH	log K = 30.3	**Equation 1.2**
$Hg^{2+} + SR^- + OH^- \rightleftharpoons$	HgOHSR	log K = 32.2	**Equation 1.3**

Much of the Hg^{II} exists as HgS, an insoluble sulfide with very limited mobility except under certain conditions (Section 6.2). Reduced S ligands in OM also contribute significantly the sediment adsorption of Hg. By spiking an organic peat soil with Hg^{II} to produce soil Hg concentrations ranging from 100 to 4000 mg/kg and using EXAFS to determine Hg associations, Skyllberg *et al.* (2006) proposed that Hg formed a two-coordinated linear complex with two reduced S groups assumed to be thiols at a distance of 2.33 Å, and a weaker bond with a third reduced S at a distance of 2.95 Å (Figure 1.3).

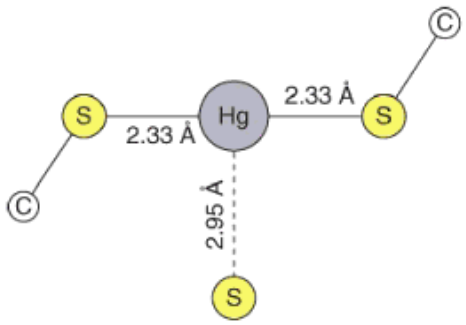


Figure 1.3 Proposed structure of Hg complexed by organic matter (after Skyllberg *et al.*, 2006).

Rates of Hg^{II} adsorption are fast. By spiking lake sediment with 55 pM (14.9 μg/L) isotopically labelled $^{200}HgCl_2$ and monitoring adsorption Hintelmann and Harris (2004) determined equilibrium was reached between an hour and a day. The strong

binding of Hg^{II} to reduced S groups in OM and the stability of HgS limits the release of Hg in these forms and subsequent potential methylation (Ravichandran, 2004) (Section 1.3.4.4). After saturation of the reduced S ligands, O and N ligand associations are formed with OM (Skyllberg *et al*., 2006).

In heavily polluted areas Hg adsorption to the inorganic matrix may occur if binding sites on OM become saturated (Hissier and Probst, 2006). Inorganic minerals are well recognised as important surfaces for adsorption of metal cations. It is thought that for Hg, hydroxylated species are adsorbed to the mineral surface rather than Hg^{II}. Clay minerals, formed from layers of silica tetrahedra (SiO_4) and alumina octahedra (AlO_6), can adsorb cations at both the Si and Al surfaces. Possible surface complexes of Hg with the clay mineral kaolinate for example are indicated in Table 1.2.

Table 1.2 Possible surface complexes of Hg^{II} with kaolinite (after Senevirathna *et al*., 2011)

	SiO_4 layer	AlO_6 layer
Surface site	$\rangle SiOH$	$\rangle AlOH$
Hg^{II}-kaolinite surface complexes		
Inner sphere	$\rangle SiOHg(OH)_2^-$	$\rangle AlOHg(OH)_2^-$
Outer sphere	$\rangle SiO^- \text{-} HgOH^+$	$\rangle AlO^- \text{-} HgOH^+$

$\rangle$ = mineral surface; $Hg^{II} = HgOH^+$ or $Hg(OH)_2$

Hydroxyl function groups on metal (hydr)oxide surfaces also provide sites for the adsorption of Hg. Using Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy Kim *et al.* (2004) observed that Hg^{II} formed bidentate innersphere complexes with goethite (α-FeOOH) and bayerite (β-$Al(OH)_3$, and a monodentate inner sphere complex with γ-alumina when no OM was present.

1.3.4.3 Colloidal Hg

Colloids include both organic and inorganic particles ranging from 1 nm to 1 *μ*m in size. Whereas adsorption of Hg onto these particles prevents sedimentation and may promote colloidal-facilitated transport, it also effectively reduces the potential for Hg^{II} methylation since only truly dissolved species can be methylated (Section 1.3.4.4).

1.3.4.4 Dissolved Hg species and methylation

In oxic waters in the absence of reduced sulfur ligands, the predominant aqueous species of Hg will be chloro and hydroxy species (Ullrich *et al*., 2001). At high sulfide concentrations soluble bi and polysulfide complexes are formed such as $HgSH^+$, $Hg(SH)_2$ and $Hg(S_x)_2^{2-}$ and in the presence of reduced sulfur ligands, such as organic thiols, whose concentrations increase under anoxic conditions in bottom waters, $Hg(SR)_2$ complexes dominate. These soluble species are mobile in the aquatic environment allowing their transportation, but more significantly, depending on their properties, dissolved uncharged species may be methylated.

Methylation of Hg^{II} to MeHg can be achieved through both biotic and abiotic mechanisms (Ullrich *et al.*, 2001). Biotic methylation, occurring primarily under anoxic conditions at the sediment-water interface where microbial activity is high by certain species of sulfate and iron reducing bacteria (Copeau and Barth, 1985; Fleming *et al*., 2006), is considered the predominant process. Methylation without microorganisms, for example through photochemical reactions with methyl sources in sewage effluent and industrial wastewater has also been observed (Ullrich *et al.*, 2001). Demethylation of MeHg to Hg^{II} through both biotic and abiotic process also occurs (Ullrich *et al*., 2001) and the overall or net methylation of Hg is the difference between the two processes. The concentrations of MeHg represent approximately 5% of the total Hg concentration in marine water and can reach up to 30% in freshwater (Kudo *et al*., 1982).

1.4 Bioaccumulation and biomagnification of Hg

The International Union of Pure and Applied Chemists (IUPAC) definition of bioaccumulation is a 'Progressive increase in the amount of a substance in an organism or part of an organism which occurs because the rate of intake exceeds the organism's ability to remove the substance from the body' (IUPAC, 2007). The degree of bioaccumulation depends on factors such as the route by which the substance enters the organism, the duration of exposure, the rate of uptake and elimination from the organism and the nature of the substance involved. Generally, the more hydrophobic a substance, the more likely it is bioaccumulate. Microbial uptake of Hg species is believed to occur by diffusion of lipid soluble species through the lipid membrane (passive diffusion) although active transport is also

possible (Schaefer and Morel, 2009). Lipid solubility can be estimated experimentally using the octanol-water partition ratio, K_{ow}, determined as the ratio of the concentration of a chemical in n-octanol (a non-polar solvent) to its concentration in water (a polar solvent) at equilibrium and at a specified temperature, where octanol is used to represent lipids. The K_{ow} values of soluble Hg species are given in Table 1.3.

Table 1.3 Partition ratios for Hg^0, $HgCl_2$, MeHgCl, HgClOH and $Hg(OH)_2$

Species	**K_{ow}**
Hg^0	4.2 (Schroeder and Munthe, 1998)
$HgCl_2$	3.33 (Mason et al., 1996)
MeHgCl	1.7 (Mason *et al*., 1996)
HgClOH	1.2 (Mason *et al*., 1996)
$Hg(OH)_2$	0.05 (Mason *et al*., 1996)

As indicated by a low K_{ow} value, $Hg(OH)_2$ diffuses very slowly through cellular membranes. Likewise the passive diffusion of HgClOH is slow. Being the most lipid soluble based on K_{ow} values, Hg^0 should bioaccumulate inside organisms, however, since diffusion out of cells is also rapid, no bioaccumulation occurs (Morel *et al*., 1998). The higher K_{ow} value of $HgCl_2$ and MeHgCl imply that they would be bioaccumulated, and that $HgCl_2$ would be accumulated to a greater degree. In laboratory experiments however increased bioaccumulation of MeHg relative to Hg^{II} was observed: using burrowing mayfly nymphs (*Hexagenia rigida)* in freshwater sediment as a biological model, the uptake of $HgCl_2$ and MeHgCl was considered by incubating nymphs for four weeks in two different sediment samples, one spiked with $HgCl_2$ at 10 mg/kg and one with MeHgCl at 1 mg/kg. Based on whole organism Hg determinations after clearing the digestive tract, the ratio of Hg accumulated from ingestion of sediment with MeHgCl to that accumulated from ingestion of sediment with $HgCl_2$ was 6.3, indicating over 60 fold greater accumulation of MeHgCl under the same spiking concentrations (Saouter *et al.*, 2001). In fact mean bioaccumulation factors calculated by averaging reported values for Hg^{II} and MeHg at a steady state are 5000 and 9000 respectively (McGeer *et al*., 2003) indicating a higher bioaccumulation for MeHg. Additionally, biomagnification, where biomagnification is defined as the ‘sequence of processes in an ecosystem by which

higher concentrations are attained in organisms at higher trophic levels (at higher levels in the food web)' (IUPAC, 2007), is only observed for MeHg.

In order for biomagnification to occur, a chemical must not only be bioaccumulated inside an organism, but it must also be transferred to its predator at higher levels of the food chain, resulting in a high concentration of Hg in the predator relative to its prey. To assess biomagnification of Hg^{II} and MeHg from phytoplankton to zooplankton, Mason *et al*. (1995) fed marine diatoms, which had been exposed to Hg^{II} and MeHg, to marine copepods. The calculated assimilation efficiency in the copepods was found to be four times greater for MeHg relative to Hg^{II}. This was attributed to the different ways in which the Hg species had been stored in the phytoplankton: by separating the phytoplankton cells into membrane and cytoplasm it was found that MeHg had been accumulated in the soluble cytoplasm of the diatom that was in turn assimilated by the copepod, whereas Hg^{II} had been accumulated in the membrane of the diatom that was excreted by the copepod. Field experiments confirm the biomagnification of MeHg, with concentrations increasing as trophic levels increase in natural freshwater lakes (Watras *et al.*, 1998) and in marine waters (Campbell *et al*., 2005) while concentrations of Hg^{II} decrease (Watras *et al.*, 1998). Although the concentration of MeHg as a percentage of total Hg concentration varies from study to study, and even within the same study (for example %MeHg in zooplankton ranged from 11 to 83% of the total Hg concentration in one study (Watras *et al.*, 1998)), on average 15% of zooplankton total Hg concentration is MeHg, increasing to 95% in predatory fish (Leopold *et al*., 2009) (Figure 1.4).

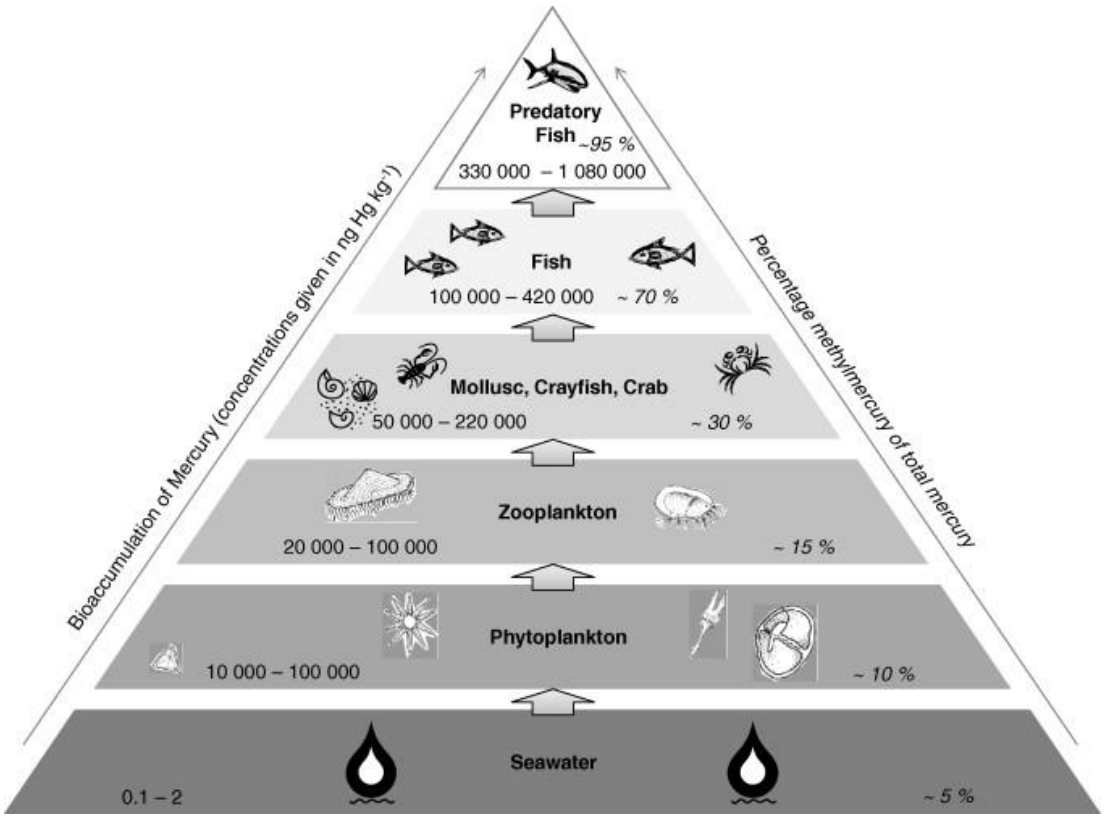


Figure 1.4 Biomagnification of Hg through the food web (after Leopold *et al.,* 2009).

In fish MeHg is found primarily in muscle tissue associated with S ligands on proteins (Harris *et al*., 2003). Concentrations of Hg found in various fish types in recent studies are listed in Djedjibegovic *et al*. (2012) and Mahaffey *et al*. (2011). Concentrations are influenced by physiological aspects of the fish such as the size, age, species, sex and diet (Verdouw *et al.,* 2011) and also by environmental conditions such as level of contamination and temperature (Carvalho *et al.*, 2005). Top predators such as pike and trout in freshwaters and whale, shark, tuna and swordfish in marine environments have the largest MeHg concentrations, frequently over 1 mg/kg (Mahaffey *et al*., 2011). The maximum allowable concentration of Hg in fish in the European Union is 0.5 mg/kg and 1 mg/kg (wet weight) depending on fish type (EC, 2006). Body burdens in this range, indeed even as low as 0.1 mg/kg, however have been shown to affect fish behaviour and reproduction (Crump and Trudeau, 2009). For example a body burden of 0.68 mg/kg Hg (wet weight) has been shown to cause widespread damage to the brain of Atlantic Salmon and in Walleye body burdens between 0.25 and 2.37 mg/kg (wet weight) significantly reduced gonadosomatic index (Crump and Trudeau, 2009). Piscivorous birds and

aquatic mammals such as seals and polar bears accumulate MeHg through their diet and the eggs of sea birds have also been found to contain Hg (Braune *et al.,* 2005). Studies on arctic sea birds and mammals however did not indicate adverse health effects as a result of Hg exposure (Fisk *et al.*, 2005). Biomagnification in terrestrial food chains is not expected due to short food chains and low concentrations in terrestrial herbivores. For example in a study carried out in the Czech republic assessing metal concentrations in terrestrial food chains, Hg concentrations in kidneys of cattle, fallow deer and pheasant were found to be 18.3 ± 2.3 *μ*g/kg, 27 ± 24 *μ*g/kg and 1.5 ± 0.9 *μ*g/kg respectively (Celechovska *et al*., 2008).

1.5 Population exposure and health effects

1.5.1 Health effects of human exposure and toxicological data

As in the environment, inside the human body Hg can be oxidised, methylated and demethylated and the health effects of Hg exposure vary depending on the species, with different species targeting different organs (Table 1.4) (NRC, 2001; Clarkson *et al.*, 2007; EA, 2009b).

Table 1.4 Toxicology of elemental, inorganic and organic Hg (NRC, 2001; Clarkson *et al.*, 2007; EA, 2009b)

Toxicological data	Hg^0	Hg^{II}	MeHg
Inhalation	• Approximately 80% of dose readily absorbed	• No human studies on Hg^{II} compounds	• Vapours of MeHg absorbed
Oral	• GI tract absorption poor	• Proportional to compound solubility • 7-15% of ingested $HgCl_2$ absorbed from GI tract • Greater uptake by neonates than adults	• Approximately 95% of MeHg fish content readily absorbed by GI tract
Dermal	• On exposure to Hg^0 vapour, 2.6% is absorbed dermally	• 2-3% of applied dose ($HgCl_2$) absorbed by guinea pigs	• 3-5% of applied dose absorbed by guinea pigs
Distribution	• Due to lipophilicity rapid distribution occurs throughout body	• Highest accumulation in kidney • In neonates distribution is wider • Fraction retained is dose dependent	• Distribution throughout body • MeHg-thiol complex involved in the transport of MeHg into cells • Accumulated into scalp hair
Barrier crossing	• Readily crosses BBB and PB	• Does not readily cross BBB or PB	• Readily crosses BBB and PB
Bio-transformation	• Oxidised slowly to Hg^{II} in tissue and blood • Most crosses BBB and PB before oxidation	• Hg^{II} not methylated in body tissues • Can be methylated by GI microorganisms	• Slow demethylated to Hg^{II} in GI tract
Excretion	• Mainly with faeces and urine (90%) as Hg^{II} • Breath, sweat and saliva as Hg^0	• 1% body burden daily • Mainly as faeces, urine, breast milk as Hg^{II} • Breath, sweat and saliva (as Hg^0)	• 1% of body burden daily • Mainly with faeces (90%) and urine (10%) as Hg^{II} • Breast milk as Hg^{II} and MeHg
Target organ	• Brain and kidney	• Kidney	• Brain
Causes of toxicity	• Oxidation to Hg^{II}	• Hg^{II} binds to thiol groups in enzymes and interferes with enzymic function	• Intrinsic toxicity of MeHg that interferes with neural migration and neural cell division • Demethylation to Hg^{II}

GI: gastrointestinal; BBB: blood-brain barrier; PB: placental barrier

Although oral absorption is poor, Hg^0 is readily absorbed by inhalation and distributed to all parts of the body due to its lipophilicity, easily crossing the blood-brain and placental barriers. In the brain it is oxidised to Hg^{II}. Although the exact mechanism leading to damage is not fully understood, it has been proposed that the binding of Hg^{II} to thiol groups in proteins and enzymes and interference with their function is a possible mechanism. Symptoms observed from exposure include tremors, mood swings, memory loss, muscular weakness and headaches. In the brain Hg^{II} accumulates in an insoluble form believed to be Hg selenide (HgSe) (Clarkson *et al*., 2007). Modelling, animal studies and human case studies indicate a half life in the brain of several years to several decades (Rooney, 2014). Kidney and thyroid function are also negatively affected following exposure to Hg^0, again believed to be a consequence of damage caused by Hg^{II} (UNEP, 2002; NRC, 2001). Lack of data prevents the classification of Hg^0 as to its carcinogenicity to humans (IARC, 1993).

The absorption of Hg^{II} is dependent on the water solubility of the Hg compound in question. On absorption Hg^{II} binds to proteins and enzymes and targets the kidney, causing immune-mediated kidney toxicity. In rat kidneys, Hg^{II} is found almost exclusively bound to metallothionine, a low molecular weight protein consisting of 25% cysteine (Wisniewska *et al.*, 1970). As a result of its charge it does not readily cross the blood-brain or placental barriers. Excretion occurs at a rate of 1% of body burden per day in faeces. Lack of data prevents the classification of Hg^{II} as to its carcinogenicity to humans (IARC, 1993).

Approximately 95% of MeHg in the diet is absorbed by the gastrointestinal tract (Clarkson *et al.*, 2007). It is highly mobile in the body due to formation of small molecular weight thiol complexes that allow its transportation into cells, and distribution to all tissues is complete in approximately 30 hr (Clarkson, 2002). In hair, where MeHg rapidly accumulates, levels are approximately 250 times greater than in blood. Excretion occurs mainly as Hg^{II} in faeces, following demethylation by intestinal flora capable of breaking the Me-Hg bond, at a rate of 1% of body burden per day. The target organ is the brain where approximately 10% of the total body burden of MeHg is found (NRC, 2001). The mode of toxicity is an interference with the migration of neurons that results in altered brain structure and reduced brain size (EA, 2009b). Once inside the brain, it is slowly demethylated to Hg^{II} where it

accumulates probably as HgSe (Clarkson, 2002; Clarkson *et al*., 2007). The latency period before the onset of symptoms ranges from weeks to over a year (NRC, 2001). Symptoms of exposure range from paresthesia (numbness), tremors, dysarthria (articulation disorders), hearing loss and loss of coordination, caused by loss of neuronal cells in specific regions of the brain, to lower cognitive responses, coma and death at high levels of exposure. Since MeHg can cross the placental barrier, the foetus is exposed if MeHg is ingested by pregnant women, affecting the developing babies' brain and nervous system. Cord blood Hg concentrations are up to 25% higher than maternal blood Hg concentrations, and the concentration in the brain of the growing foetus is reported to be as much as five times greater than Hg concentration in maternal blood (COT, 2003; Clarkson, 2002). Consequently the central nervous system of the developing foetus can be negatively affected without the mother having any symptoms. Unlike in adults, damage to the brain is widespread and cerebral palsy-like symptoms have been observed following prenatal exposure. Exposure of babies to MeHg in breast milk can also depress their neurological function and development. More recently exposure to MeHg has been associated with cardiovascular effects such as carotid atherosclerosis (Salonen *et al*., 2000) and myocardial infarction (Wennberg *et al*., 2012). The International Agency for Research on Cancer classifies MeHg as a possible carcinogen (IARC, 1993).

1.5.2 Population exposure and epidemiological studies

There have been two widely-reported incidents of Hg poisoning that have highlighted the neurological effects of MeHg exposure. The incident following which acute Hg poisoning was named 'Minamata disease', occurred in Minamata Bay, Japan, in the 1950s. The largest chemical manufacturer in Japan at the time, Chisso, operated an aldehyde production plant between 1921 and 1968 in the area that used Hg as a catalyst. Effluent discharged in the bay over those years contained MeHg formed during the aldehyde production process (EEA, 2013)). Initial symptoms of MeHg exposure were observed in the 1940s in fish that were 'swimming around as though crazy', in birds unable to fly, in cats unable to balance, until, in the 1950s, the first symptoms appeared in residents of the bay that were exposed to high levels of MeHg from the consumption of sea food (EEA, 2013). Those who developed the disease between March and October 1959 had hair MeHg concentrations of between 97 and 705 mg/kg (Kitamura *et al*., 1960).

Concentrations of MeHg in fish from the bay in 1969 were found to be over 50 mg/kg (Ui, 1971). Neurological symptoms of exposure ranged from tremors to death (NRC, 2001), while foetal exposure resulted in increased foetal deaths (Itai *et al*., 2004), mental retardation and severe cerebral palsy type symptoms at birth named congenital Minamata disease (Harada, 2010). This incident was the first to illustrate the transfer of toxic compounds though the placenta to the developing foetus, previously thought to be protected in the womb. Over 2200 people in the area have been diagnosed with Minamata disease.

The second incident of Hg poisoning occurred in Iraq in 1971, where population exposure resulted from the consumption of grain which had been treated with a MeHg fungicide. Over 6500 people were affected with symptoms including blurred vision, impairment of hearing, ataxia and slurred speech (Clarkson, 2002) and 459 deaths were recorded (Bakir *et al.*, 1973). From the determination of Hg concentration in the blood of affected individuals, body burdens of Hg were associated with symptoms observed. Specifically, the threshold Hg body burden for the onset of ataxia, dysarthria, deafness and death were calculated to be 55, 90, 170 and 200 mg (Bakir *et al.*, 1973). A dose response association was also established between foetal exposure dose during pregnancy and neurological effects such as delay in walking and talking after birth (Marsh *et al.*, 1987). The World Health Organisation (WHO) suggested negative effects in children were observed when Hg concentrations in maternal hair were between 10 and 20 *μ*g/g during pregnancy (IPCS, 1990).

Background population exposure to Hg occurs primarily as a result of the consumption of MeHg-contaminated fish. The specific level of exposure depends on factors such as the type of fish, the frequency of consumption and the population group. No method of cooking or preparing fish can remove MeHg, in fact boiling fish can increase concentrations due to moisture loss from the fish meat (Oudraogo and Amyot, 2011). Estimates of weekly Hg (assumed to be the MeHg) intake from consumption of fish with high Hg content for the UK population is given in Table 1.5 (COT, 2003).

Table 1.5 Weekly Hg intake for specific age groups from consumption of shark, swordfish, marlin and tuna

Age group (years)	Body weight (kg)	Av. Portion size (g)	Weekly Hg intake assuming one fish meal/week (*μ*g/kg bw/week) where bw = body weight				
			Shark	Sword fish	Marlin	Fresh tuna	Canned tuna
1.5 - 4.5	14.5	50	5.24	4.62	3.79	1.38	0.66
4 - 6	20.5	60	4.44	3.90	3.22	1.17	0.56
7 - 10	30.9	85	4.17	3.69	3.04	1.10	0.52
11 - 14	48.0	140	4.44	3.92	3.21	1.17	0.55
15 - 18	63.8	105	2.51	2.21	1.82	0.66	0.31
Adults	70.1	140	3.04	2.68	2.20	0.80	0.38

Source: COT, 2003

In other parts of the world, in addition to fish, large birds and mammals such as whales and seals can also be a source of MeHg exposure (ATSDR, 1999). Other sources include dietary intake from food products such vegetables and cereal (FSA, 2009), occupational exposure, inhalation of elemental Hg^0 vapour in ambient air, and from Hg^0 contained in dental amalgam fillings (WHO, 1990). In some cultures, the use of creams, ointments or medicines containing Hg could result in substantial exposure (ATSDR, 1999). Results from the 1999-2000 National Health and Nutrition Examination Survey (NHANES) study indicated geometric hair Hg concentrations in a cross section of US women was 0.2 *μ*g/g, which increased to 0.38 *μ*g/g in frequent fish eaters (McDowell *et al*., 2004).

To assess the effect of long-term background exposure to Hg on the developing foetus, epidemiological studies have been carried out on communities with high fish consumption. A summary of these studies with their neurological outcomes can be found in Julvez *et al*. (2012). The two largest epidemiological studies, the Seychelle Child Development Study (SCDS) and the Faroe Islands Study had different outcomes and are discussed below.

The SCDS commenced in 1980 in the Seychelles Islands in the Indian Ocean where population exposure to Hg occurs primarily due to a diet of ocean fish. The main cohort involved 740 mother-child pairs. Maternal hair Hg concentration measured

immediately after birth was used as the index of foetal exposure, taking nine cm of hair to reflect average concentration throughout pregnancy (NRC, 2001). Concentrations ranged from 0.5 to 27 *μ*g/g with a mean of 6.8 *μ*g/g (NRC, 2001). Children's hair samples were analysed at birth and children were tested against developmental milestones at different ages throughout 19 years of follow up. No adverse effects were noted in any of the developmental tests even in a subgroup of highest exposure. In fact, at 66 months, children who had been exposed prenatally to higher concentrations were found to perform better. This was attributed to the nutritional value of fish (COT, 2003).

In contrast to the SCDC, the Faroe Islands study identified adverse effects of prenatal exposure to Hg. The Faroe Islands are located in the North Atlantic Ocean and population exposure to Hg is due to eating whale meat and ocean fish. The study commenced in 1986 and involved over 900 mother-child pairs. Prenatal exposure was determined using umbilical cord blood and maternal hair where geometric averages of Hg were 22.9 *μ*g/L and 4.3 *μ*g/g respectively (Grandjean *et al.,* 1997). Physical and neurological examination including hand-eye coordination, reaction times, verbal and naming tests, at the ages of seven and 14 indicated an association between higher exposure levels and poorer language skills, poorer memory and poorer attention skills, while motor skills were also negatively affected.

Differences in assessing MeHg exposure, in the tests performed, in ages at testing, in sources of exposure and the effect of covarients are possible explanations for the differences in results (NRC, 2001).

1.5.3 Acceptable levels of exposure

Data on exposure levels and corresponding health effects from the Iraqi incident and from the SCDC and Faroe Island epidemiological studies have been used by international organisations to derive acceptable levels of exposure and critical doses. However, there is disagreement about which study and which endpoints should be used, on the conversion of the concentrations found to cause negative effects to exposure levels and whether hair or cord blood are more appropriate for an estimation of exposure levels. Thus organisations use data in different ways, resulting in different estimates of 'safe' exposure as illustrated below.

The US Environmental Protection Agency (EPA) use a reference dose (R_fD) to quantify an acceptable level of exposure, where R_fD is an estimate of the level of population daily exposure unlikely to cause an appreciable risk during a life time of exposure (EPA, 2001). In 1995, based on results from the Iraqi incident, the EPA established a R_fD of 0.1 *µ*g/kg bw/day based on a hair MeHg level of 11 *µ*g/g associated with neurological effects, where bw is body weight. The data used to establish a R_fD was changed in 2001 to the data obtained from the Faroe Island study. Based on bench mark dose lower confidence limit (BMDL) values between 46 and 79 *µ*g/L Hg in cord blood associated with adverse neurological effects, a R_fD of 0.1 *µ*g/kg bw/day was calculated which is in fact the same as the R_fD obtained when using data from the Iraqi incident (EPA, 2001). This dose could be exceeded with the consumption of one meal of fresh tuna per week, depending on the body weight of the consumer (Table 1.5).

The US Agency for Toxic Substances and Disease Registry (ATSDR) derived a minimum risk level (MRL), where an MRL is defined as 'an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure' (ATSDR, 2012). This was based on results of the SCDS, using the mean concentration of Hg in hair in the subgroup with highest exposure level (15.3 *µ*g/g) to represent the level at which no neurological effects were observed. This corresponds to a maternal intake of 1.3 *µ*g/kg bw/day as a no observed adverse effect level (NOAEL) (DEFRA-EA, 2002a) and results in a MRL of 0.3 *µ*g/kg bw/day (ASTDR, 2013), three times higher than the EPA reference dose.

In 2003, the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives (JECFA, 2003) reduced its previous recommendation of 3.3 *µ*g/kg bw/week to a provisional tolerable weekly intake (PTWI) for MeHg of 1.6 *µ*g Hg/kg bw. The new recommendation was based on a mean maternal Hg hair concentration of 14 *µ*g/g calculated by averaging maternal hair NOAEL concentrations from the SCDS (15.3 *µ*g/g) and a hair BMDL from the Faroe Island study (12 *µ*g/g).

In the United Kingdom, the Committee on the Toxicity of Chemicals in Food Consumer Products and the Environment (COT) 'consider that a methylHg intake of

3.3 *μ*g/kg bw/week may be used as guideline to protect against non-developmental adverse effects' and 'the 2003 JECFA PTWI of 1.6 *μ*g/kg bw/week is sufficient to protect against neurodevelopmental effects in the fetus' while noting that 'consuming one weekly 140 g portion of either shark, swordfish or marlin would result in dietary methylHg exposure close to or above 3.3 μg/kg bw/week in all age groups' (COT, 2003) (Table 1.5).

While acceptable levels of exposure provide guidelines for the protection of human health, environmental quality standards, discussed below, set permissible concentrations of pollutants in environmental samples.

1.6 Environmental quality standards

Environmental quality standard (EQS) values specify the concentration of a potentially toxic compound that should not be exceeded in order to protect organisms that are exposed to the compound and/or human health. They can apply to air, water, soil and sediment. Sediment contamination was traditionally assessed by comparing total contaminant concentrations with background levels. Such assessments however provided little information on the possible effects on organisms that live in, or are in contact with, sediment. Consequently, more recently, sediment quality guideline (SQG) values have been derived taking into consideration the toxicity of contaminants to aquatic organisms and/or contaminant bioavailability.

Adverse effects on organisms from exposure to Hg in sediment as a function of concentration can be assessed using both field and laboratory data and range from impaired development and reduced fertilisation to mortality and lethality (CCME, 1999). Field results for example have indicated that in marine sediment Hg concentrations of 0.18 mg/kg have been associated with significant mortality of the anthropod *Leptocheirus plumulosus* (McGee *et al*., 1993), and, in fresh water sediments, decreased abundance of Gastropoda and Chironomidae has been reported at mean total Hg concentrations of 0.987 mg/kg and 1.09 mg/kg respectively (Jaagumagi, 1988; Jaagumagi *et al*., 1989). In toxicity tests, lethal concentrations are generally found to be higher, probably since exposure is only to one compound, whereas in the environment exposure is to mixture of contaminants.

For example the concentration of Hg lethal to 50% of the freshwater amphipod *Hyalella Azteca* in 10 days (10d-LC_{50}) was calculated to be 15.2 mg/kg (Swartz *et al*., 1988).

In the derivation of SQGs, two levels are generally determined; a lower level at which adverse effects rarely occur, and a higher level above which effects are likely. As an example, the Canadian Council of Ministers of the Environment (CCME) has SQGs for total Hg concentration in marine and freshwater sediment (CCME, 1999) expressed as interim sediment quality guideline (ISQG) values and probable effect levels (PELs) values. Derived primarily from field data which measured Hg concentration and the occurrence (or absence) of adverse effects in organisms, values below ISQGs (or a threshold effect level, (TEL)) indicate adverse effects are rarely seen. Between ISQGs and PELs adverse effects are occasionally observed and above PELs adverse effects are frequently observed. In freshwater sediment the ISQG value for total Hg concentration is 0.17 mg/kg and the PEL is 0.486 mg/kg, and in marine sediment the ISQG value is 0.13 mg/kg and the PEL is 0.7 mg/kg. The Dutch SQG values are calculated by considering background levels in the Netherlands, toxic effects from laboratory tests and also the partitioning of the chemical between the water and sediment (Crommentuijn *et al*., 2000). A target value (TV) (also called negligible concentration, NC) is defined as the concentration at or below which the contaminant should remain in the long term and the maximum permissible concentration (MPC) is the concentration that should not be exceeded for the protection of the ecosystem. The MPC values are calculated by determining a Maximum Permissible Addition (MPA) multiplied by a sediment/water partition coefficient relevant to Dutch sediment, and adding it to the background level of the contaminant. The TV values are calculated from the background concentrations plus a negligible addition, defined as MPA/100. By assuming background concentrations for Dutch sediment of 0.3 mg/kg for both total and MeHg concentration in sediment, TVs have been established at 0.56 mg/kg for total Hg and 0.31 mg/kg for MeHg, and MPCs at 26 mg/kg for total Hg and 1.4 mg/kg for MeHg (Crommentuijn *et al*., 2000).

While there are no international or European standards, some countries including Australia and New Zealand, North America, Hong Kong and Norway have their own sediment guideline values for total Hg concentration in sediment, reviews of which can be found in Burton (2002) and Hubner *et al*. (2009). The Dutch SQGs also set

values for MeHg concentrations (Crommentuijn *et al*., 2000). Although SQGs are a helpful tool for assessing sediment contamination and toxicity, there are some limitations. While values that are derived solely on toxicity levels are comparable - four set of SQGs give the same values for TEL and PEL (or their equivalent) (Huber *et al*., 2009) - when background levels and partitioning are considered SQGs can vary considerably due to different sediment types and geochemical parameters as indicated by the Dutch SQGs. Since guidelines are chemical specific, cumulative effects from mixtures cannot be assessed. Despite limitations, if correct methods of sampling and determination are followed, SQGs can help identify areas of contamination, gauge potential for adverse effects on benthic organisms, evaluate sediment dredging and remediation options and allow monitoring.

1.7 Methods for the sampling and determination of Hg species in sediment

1.7.1 Importance of species determination

The environmental implications of Hg in sediment depend not only on the total Hg concentration, but also its complexation and speciation. While the determination of total Hg concentration allows an assessment of the overall level of contamination and can provide information on potential toxicity to organisms living in or coming in to contact with sediment, the mobility, or ease of extractability, of Hg from sediment into the water column influences transportation and thus the spread of contamination, and also influences methylation, biomagnification and potential for human exposure.

1.7.2 Sampling procedures

Preserving the original distribution of the target analytes during sampling and storage is particularly important in speciation studies (Abranko *et al*., 2005). Various transformations can occur so that the species after sampling and storage are different in form and concentration than the original species in the environment (Gardiner, 1988). Since the concentrations of MeHg and inorganic Hg^{II} can be very low any losses or transformations result in significant errors (Yu and Yan, 2003). Consequently it is necessary to store samples in such a way so as to minimise transformations and maximise stability. Stability during storage is affected by factors such as container material, sample composition, storage temperature, oxidation and

light exposure. With regard to container material, for aqueous samples it has been reported that MeHg degrades faster in polyethylene bottles (Parker and Bloom, 2005) and that the best storage material is polytetrafluoroethylene (PTFE) (Lansens *et al.*, 1990). For speciation analysis of solid samples, amber glass bottles with PTFE caps are recommended (EPA, 2005). Following sampling, if samples are to be stored they should be frozen immediately to retard microbial activity (Wells, 1992).

1.7.3 Determination of Hg species in sediment

1.7.3.1 Determination of total Hg concentration, Hg speciation and mobility and Hg bonding environment

A range of techniques are available for the determination of total Hg concentration, the determination of specific Hg species, Hg mobility and for the determination of the bonding environment of Hg, examples of which are given in Table 1.6.

Table 1.6 Examples of techniques used for the determination of total Hg concentration, Hg speciation, mobility and bonding environment in sediment

Technique	**Reagents/ Conditions**	**Hg species determined/ Hg bonding**	**Reference**
Digestion	HF	Total Hg	Remy *et al*., 2006
	HNO_3 (conc.)	*Pseudo*-total Hg	Sloan *et al*., 2001
	HNO_3 (conc.) + HCl (conc.) (*aqua regia*)	*Pseudo*-total Hg	Hammerschmidt and Fitzgerald, 2004
Extraction	DI water	Water soluble Hg	Rodrigues *et al*., 2010
	DI water 0.01 M HNO_3 1 M KOH Na_2S HNO_3 (conc.)	Water soluble Hg Exchangeable Hg Hg bound to humic and fulvic acids HgS Residual Hg	Wallschlager *et al*., 1998
	10% KCl 0.1 N NaOH 1 N HCl 30% H_2O_2, 0.02 N HNO_3, 2 M NH_4Cl	Exchangeable Hg Hg bound to humic and fulvic acids Hg bound to Fe and Mn oxides Hg bound to OM and sulfides	Ram *et al*., 2009
	DI water 0.1 M CH_3COOH + 0.01 M HCl 1 M KOH 12 M HNO_3 *aqua regia*	Water soluble Hg Weak acid soluble Hg Organo-complexed Hg Strongly complexed Hg Mineral bound Hg	Bloom *et al*., 2003

	2% HCl and 10% CH_3CH_2OH 1:2 HNO_3 1:6:7 HCl:HNO_3: reagent water	Mobile Hg Semimobile Hg Nonmobile Hg	Han *et al*., 2003
	4 M HNO_3	Mobile Hg	Rahman and Kingston, 2005
Thermal desorption	T = 750 ℃	Total Hg	Reis *et al*., 2012
	125 ≤ T ≤ 225 ℃ 100 ≤ T ≤ 250 ℃ 225 ≤ T ≤ 325 ℃	$HgCl_2$ Hg bound to humic acids HgS	Reis *et al*., 2012
	150 ≤ T ≤ 250 ℃ 200 ≤ T ≤ 280 ℃ 250 ≤ T ≤ 350 ℃ T ≥ 400 ℃	Hg bound to matrix components Hg bound to humic acids HgS $HgSO_4$, HgO	Biester *et al*., 1999
	60 ≤ T ≤ 120 ℃ 100 ≤ T ≤ 190 ℃ 110 ≤ T ≤ 400 ℃ 165 ≤ T ≤ 450 ℃ 270 ≤ T ≤ 340 ℃ 250 ≤ T ≤ 435 ℃	Hg^0 $HgCl_2$ HgO $HgSO_4$ Hg bound to humic acids HgS	Coufalik *et al*., 2014
X-ray absorption (XAS)	L_{III}-edge	Identification of inner and outer sphere Hg bonding	Kim *et al*., 2004
X-ray fluorescence (XRF)	Bulk soil analysis	Total Hg concentration	Brumbaugh *et al*., 2013
Colorimetric paper-based sensors	Detecting papers coated with copper(I)iodide	Semi-quantitative Hg determination	Yallouz *et al*., 2008

1.7.3.2 Digestion and extraction procedures

1.7.3.2.1 Total and *pseudo*-total digestion

The determination of total Hg concentration in sediment is more straightforward than the determination of different species, since no attention has to be paid to the possibility of species interconversion. Typically, acid digestion is used to remove all Hg-containing compounds from the sample. If geogenic metal concentration is required, hydrofluoric acid (HF) is used to dissolve the silicate inorganic matrix and solubilise the sample releasing all metals (Remy *et al*., 2006). Such harsh conditions would not occur in the environment. Anthropogenic inputs are usually determined using a '*pseudo*-total digest', reflecting the maximum metal concentration that could hypothetically be released to the environment (Davidson, 2013) using either one or a mixture of strong acids, such as nitric acid, sulfuric and hydrochloric acid, or strong acids with other oxidising agents (Sloan *et al*., 2001; Hammerschmidt and Fitzgerald, 2004). The different reagents, their concentrations and digestion times for determining total Hg concentration in soils and sediments have been reviewed in recent papers (Stoichev *et al*., 2006; Issaro *et al*., 2009). The extracted Hg^{II} can then be reduced by tin chloride or sodium borohydride to Hg^{0} which is purged from the sample and carried into an atomic absorption spectrometry (AAS) system (Appendix A), or atomic fluorescence spectrometry (AFS) system for determination (Sanchez-Rodas *et al*., 2010).

1.7.3.2.2 Extraction procedures

As with other potentially toxic metals, in order to remove Hg bound to particular sediment phases, single or sequential extraction procedures can be used to remove only the species of interest while at the same time maintaining its form. These extractions remove Hg from the sample into operationally defined groups of more, or less, soluble species. If desired, organic Hg species (e.g. MeHg, ethylmercury (EtHg), phenylmercury (PhHg)), can be extracted alone or together with mobile inorganic Hg^{II} species and can then can be further separated if required.

The mobility of metals in sediment can be assessed using sequential extraction procedures. Based on the stepwise use of increasingly harsh reagents to remove the metals from increasingly stronger metal complexes, extraction gives an indication of metal mobility and availability, since metals removed from sediment

under less harsh conditions could more readily be released in the environment, whereas those which require harsh reagents to be removed from the matrix are considered less mobile (Wallschlager *et al*., 1998). In the case of Hg, availability relates not only to mobility and transport of the species, but also to methylation and bioaccumulation potential. Reviews on operational speciation by sequential extraction were presented by Issaro *et al.* (2009) and Bacon and Davidson (2008). Some of these approaches, shown in Table 1.6, are discussed below with results from their implementation in sediment.

A one step extraction procedure to remove 'highly available water-soluble' species of Hg (such as $Hg(OH)_2$ and $HgCl_2$) implemented by Rodrigues *et al*. (2010) involved mechanical shaking of sediment with DI water for two hours. The procedure was applied to sediment from the Aveiro Lagoon, Portugal, contamined from a chlor-alkali plant in the area. Total Hg concentrations ranged from 0.15 to 3180 mg/kg. The water-soluble Hg fraction was found to be very low in comparison, ranging from 0.17 to 108 *µ*g/kg and was not related to total Hg concentration.

Wallschlager *et al.* (1998) proposed a sequential extraction procedure based on five steps. The extraction removed free Hg ions and water-soluble inorganic and organic Hg compounds in the first step, and exchangeable Hg in the second step. Less mobile species, including Hg bound to medium molecular weight OM were removed in the third step; species considered nonmobile such as HgS and high molecular weight Hg-OM complexes were removed in the fourth step. Residual Hg was determined in the last step. Beldowski and Pempkowiak (2003) implemented this extraction in sediments of the Gdansk Basin, Southern Baltic Sea, where total Hg concentration ranged from 29 to 844 *µ*g/kg. The largest fraction of the total Hg concentration was extracted in steps 3 and 4, that is associated with OM and sulfides.

Ram *et al.* (2009) implemented a four step sequential extraction procedure developed by Strunk (1991) to examine Hg species in sediment from Ulhas Estuary, India. The extraction involved removal of exchangeable Hg species in the first step; base-soluble OM-bound Hg species in the second step; acid-soluble (Fe and Mn oxide bound) Hg species in the third step; and oxidisable (organic and sulfide

bound) species in the fourth step. Ram determined highest Hg content in the organics and sulfides phase in agreement with Beldowski and Pempkowiak (2003).

In assessing the forms of Hg in contaminated sediment from Guanabara Bay, Brazil, Covelli *et al.* (2012) carried out a five step sequential extraction procedure based on an extraction scheme proposed by Bloom *et al*. (2003). Total Hg concentration ranged from < 0.1 to 3.22 mg/kg, of which < 0.1% was in more soluble and exchangeable forms; ≤ 1.5% was found in the least mobile fraction (HgS); and 2.6% was bound to OM. The largest contribution to the total Hg content (> 96%) was from elemental Hg strongly bound to the mineral lattice as is common where contamination is a result of pollution from a chlor-alkali plant, as in this case.

A speciation process proposed by Han *et al.* (2003) used acidic ethanol solution to extract the 'mobile and toxic' forms of Hg, which contained mobile inorganic species and included Hg weakly bound to acid-soluble OM complexes, and the organic species MeHg and EtHg in the first step. Semimobile species such as Hg^0 and Hg strongly complexed with OM, and nonmobile species such as HgS were then extracted separately using increasing acid strengths. Rahman and Kingston (2005) documented the use of a closed vessel microwave extraction for the removal of the same mobile and toxic Hg species as Han *et al*. (2003). It had the advantage, however, of being faster and more straightforward: extraction was accomplished by an irradiation time of 10 minutes, whereas the extraction propsed by Han involved several steps such as vortex shaking, pH adjustment, sonication and centrifugation. Both methods have been adopted by the EPA for speciation of Hg in sediment (EPA, 2005).

Since Hg concentrations are generally low following extraction, pre-concentration is frequently required prior to quantification. For example liquid-liquid extractions can be used to concentrate organic Hg species from a large volume of an aqueous matrix into a small volume of an organic solvent (De Smaele *et al*., 1998; Uria and Sanz-Medel, 1998). Solid phase extraction (SPE) and solid phase micro extraction (SPME) are also commonly used to concentrate both MeHg and Hg^{II} fractions (De Smaele *et al*., 1998; Fernandez-Martinez *et al*., 2005). Techniques such as gas chromatography (GC) with various detectors such as atomic fluorescence (Carrasco *et al*., 2009; Mao *et al*., 2008) and mass spectrometry (MS) (Avramescu *et al*., 2010;

Mao *et al*., 2008; Mishra *et al*., 2005; Park *et al*., 2010), can then be used to determine the individual species that have been extracted together (Appendix A). Application of high performance liquid chromatography (HPLC) can also be used to separate Hg species however poorer limits of detection are achievable and the technique is best suited to use in biological samples. With advances in suitable pre-concentration methods for use with HPLC, its application is also gaining popularity in water samples (Leopold *et al*., 2009; Chen *et al*., 2009).

1.7.3.3 Thermal desorption techniques for total Hg determination and Hg speciation

As an alternative to the use of acid digestion and/or extraction, thermal desorption (TD) techniques can be used to provide information on both total Hg content and on the types of species adsorbed to the matrix (Bollen *et al*., 2008; Reis *et al*., 2012) (Table 1.6). The principle of total Hg determination is based on the use of a suitably high temperature to desorb all Hg species from the matrix. The sample is decomposed in a combustion tube or decomposition furnace and the desorbed species are trapped on the surface of a gold amalgamator. The amalgamator is heated to quantitatively release the Hg, the concentration of which is then determined by AAS or AFS. The TD technique can also be used for Hg speciation (Biester *et al.,* 1999; Reis *et al.,* 2012). Utilising the propery that different species of an element are adsorbed to solid particles with different strengths, their stepwise removal can be achieved by a gradual increase in temperature, in a process that can be compared to sequential extraction where species are released by increasing harshness of extracting reagents. Species are continuously desorbed from the matrix, trapped on and desorbed from the gold trap, and detected resulting in a separate thermal desoprtion curve for each individual species. By comparing thermosdesorption curves to thermosdesorption curves of Hg standard materials such as $HgCl_2$, Hg associated with organic matter and HgS, the Hg species can be identified.

1.7.3.4 X-ray absorption spectroscopy

The use of X-ray absorption spectroscopy (XAS) can provide details on the immediate bonding environment of Hg (Table 1.6). Information on the oxidation state and geometry of Hg atoms can be obtained from X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) can

provide details on the type and number of neighbouring atoms allowing elucidation of direct surface sorption (Kim *et al*., 2004a). Concentrations over 100 mg/kg are necessary for use of these techniques limiting their application to very contaminated environments.

1.7.3.5 Hg screening

1.7.3.5.1 The need for screening methods

While in many cases quantitative determination of Hg is desirable or necessary, cost often prevents the use of the sophisticated equipment required. As the need for Hg determination increases, so does the need for rapid, portable, inexpensive methods of analysis that can be performed in the field, thus reducing not only cost but also time. Over the last decade there has been much interest in developing portable sensors for rapid, on-site identification of Hg contamination. These sensors work by registering a response such as light emission (fluorescence) (Singh *et al*., 2014), electrochemical (Zhou *et al*., 2013) or potentiometric response, or are based on colorimetry (Choi *et al*., 2014; Yallouz *et al*., 2008). Reviews can be found in Nolan and Lippard (2008) and Kim *et al*. (2012). Although some of these techniques merely provide information on the presence or absence of Hg, others, can give a semi-quantitative determination. Most sensors are limited to use in aqueous media (Choi *et al*., 2014; Deng *et al.*, 2013; Bazzicalupi *et al.*, 2013) and as such cannot be applied to sediment. Two screening methods that can easily be applied to sediment are the use of portable X-ray fluorescence spectroscopy (pXRF) and colorimetric paper-based sensors using copper(I)iodide (Table 1.6). These are discussed below.

1.7.3.5.2 X-ray fluorescence spectroscopy

Used for the determination of elemental composition, X-ray fluorescence spectroscopy (XRF) is a non-destructive technique that can be applied to a range of samples including sediments. The technique involves irradiating the material to be analysed with X-rays. When the energy of the X-rays is greater than the energy binding an electron to the nucleus of the atom, the electron is emitted from one of the atom's inner orbital shells. To regain stability, an electron from a shell with higher energy drops to the lower energy level emitting the energy difference as an X-ray (Kenkel, 2003). Energy levels have a specific energy and the energy of the fluorescent X-ray is specific to the difference in energy levels of each atom. Thus

each atom can emit a unique set of X-rays from which it can be identified. Simultaneous determination of the fluorescence X-rays of the elements in a sample allows their simultaneous identification. Fluorescent X-rays are sorted either by their energies (energy dispersive anaylsis) or on their wavelengths (wavelength dispersive analysis) (Dean, 2003). The number of X-rays counted is proportional to the mass of the element. The technique is rapid and involves no extraction or digestion. The use of portable instruments allows the technique to be implemented in the field giving rapid screening results (Brumbaugh *et al*., 2013).

1.7.3.5.3 Colorimetric paper-based sensors

Paper-based sensors are analogous to litmus paper in that they are impregnated with reagent(s) that change colour on exposure to a specific pollutant, the intensity of which can be estimated 'by-eye' or read out by an electronic device, and relates to the pollutant concentration present. Key analytical challenges in the development of such sensors include ensuring that they are selective, sensitive and stable. Such a method has recently been reported for screening Hg in water involving reduction of inorganic Hg^{II} to Hg^{0} and trapping on detecting papers with a copper(I)iodide coating to form a complex the characteristic colour of which is proportional to the amount of Hg in the original sample (Yallouz *et al*., 2008). A preliminary sample digestion procedure is added for the determination of Hg in soils and mine tailings. Concentrations are semi-quantitatively determined by comparing the colour obtained from samples with colours obtained from standards, giving results in the form of a concentration range.

1.8 Research needs

It is accepted that human exposure to Hg arises mainly from the consumption of contaminated fish. However there is no simple technique for the screening of Hg in marine sediment to allow the identification of contaminated sites where Hg biomagnification in fish could result in human exposure. Furthermore, while Hg speciation in sediment is largely controlled by its affinity for S, the role of other factors on overall mobility and methylation are less clear.

1.9 Aims

The aim of this thesis was to consider the mobility and speciation of Hg in contaminated sediment, through screening and quantitative analysis, as influenced by pollution sources and the sediment environment, and to assess the potential for human exposure. Specific objectives were

1) The characterisation of a colorimetric paper-based sensor technique for the determination of total Hg concentration in sediment and its application in a marine environment (Chapter 2).
2) Testing of the colorimetric method in the field by application to freshwater and marine sediment with different characteristics, and the comparison of the results with quantitative determination using CVAAS (Chapter 3).
3) The investigation of Hg mobility in sediments using sequential extraction, the influence of pollution sources, OM, and seasonal variation, and the bioaccumulation of Hg in the fish species *Mugil cephalus* (Chapter 4).
4) The comparison of CVAAS, TDAAS and portable XRF as methods for the determination of total Hg contamination in sediment, assessment of species mobility using sequential extraction and TD techniques and the influence of OM (Chapter 5).
5) The influence of total Hg concentration, OM and pH on Hg methylation in sediment (Chapter 6).

CHAPTER 2 CHARACTERISATION AND APPLICATION OF A SEMI-QUANTITATIVE METHOD FOR TOTAL MERCURY DETERMINATION IN MARINE SEDIMENT

2.1 Introduction

With the adoption of the Minamata Convention aimed at protecting human health from the harmful effects of Hg through reduction of emissions (UNEP, 2013), screening methods for Hg have gained significance (Section 1.7.8). Rapid, inexpensive screening is necessary not only to save time and lower cost, but also to allow assessment of Hg contamination to be made in areas where the threat posed by Hg exposure can be great but resources are limited. In ASGM communities in South Africa, Asia and Africa for example, neurological symptoms of Hg exposure are observed not only in those directly working in ASGM, but in the community as a whole as a result of the consumption of Hg-contaminated fish (WHO, 2013). However, as a result of limited laboratory instrumentation and the high cost of testing, Hg monitoring is not regularly carried out. Such areas require a test that is inexpensive, portable and easily implemented. Screening methods vary in accuracy, cost and speed and ease of sample analysis (Section 1.7.3.5). One of the least expensive and most simple in terms of implementation and equipment that can be applied to sediment is the colorimetric screening test based on the reaction between Hg and copper(I)iodide, Cu_2I_2.

First described as a colorimetric method for the identification of Hg by Gettler (1937), the reaction of Hg with Cu_2I_2 to produce a coloured Hg complex has been used for monitoring Hg^0 in air and in the workplace atmosphere (Crisp *et al.*, 1981). The reaction, which is specific for Hg (Gettler and Kaye, 1950), produces cuprous mercury iodide, $Cu_2[HgI_4]$, an Hg complex with a charactertic orange colour (Equation 2.1).

$$Hg^o + 2Cu_2I_2 \rightarrow Cu_2[HgI_4] + 2Cu^o \qquad \textbf{Equation 2.1}$$

Not only can Hg be identified colorimetrically on reaction with Cu_2I_2, but also, since the intensity of the colour produced is proportional to the Hg concentration, by comparing the colour obtained from a sample with colours obtained using standards

of known concentration, a semi-quantitative determination of Hg content can be made, expressed as a concentration range. Recently methods for the screening of fish (Yallouz *et al*., 2000), gold mining residues and fluvial sediments (Yallouz *et al*., 2008) have been proposed based on the reaction. The method proposed for gold mining residues and fluvial sediments is discussed below.

2.2 Principle of colorimetric screening method

In the colorimetric method for screening fluvial sediment and mine tailings described by Yallouz *et al*. (2008) solid samples were digested and, following reduction, Hg^0 species were trapped on a detecting paper coated with Cu_2I_2. Digestion of solids was carried out by heating samples with *aqua regia* in a conical flask fitted with a cold finger to prevent Hg loss. Following digestion, tin(II)chloride was added to reduce Hg^{II} to Hg^0 (Equation 2.2).

$$Sn^{2+} + Hg^{2+} \rightarrow Sn^{4+} + Hg^0 \qquad \textbf{Equation 2.2}$$

A two-armed aeration duct (bubbler) was then inserted into the conical flask. One arm of the aeration duct was connected to an aquarium pump and the other to condenser (Figure 2.1). Using the aquarium pump to bubble air through the sample, Hg^o vapour was removed via the aeration duct through the condenser and onto a preconditioned detecting paper positioned inside a holder (Figure 2.2).

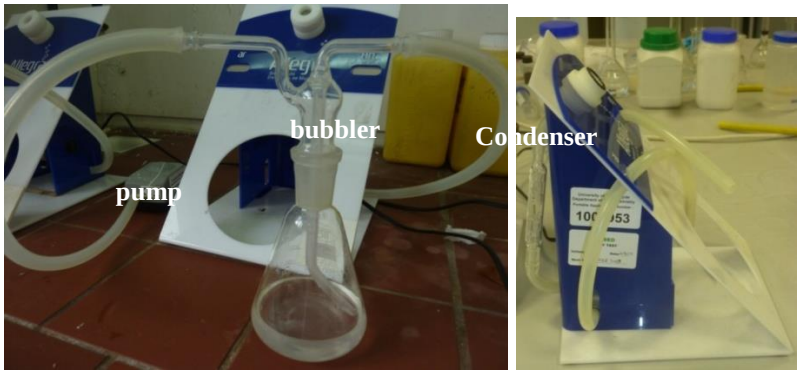


Figure 2.1 Set up of apparatus for Hg reduction and determination as described by Yallouz *et al*. (2008). Conical flask with aeration duct (bubbler) connected to pump (left). The condenser (right) is attached through tubing to the aeration duct and the paper holder.

Figure 2.2 Empty holder (left) and holder with detecting paper (right) used in colorimetric Hg screening method as described by Yallouz *et al*. (2008).

On reaction with the coating, $Cu_2[HgI_4]$ was formed. Since the intensity of the colour was proportional to the amount of Hg in the sample, a semi-quantitative determination of Hg concentration was obtained by comparison of the colour intensity produced from Hg in standard solutions, analysed in the same manner (without digestion) (Figure 2.3). Standards and samples should be determined simultaneously since the colour developed on the detecting paper slowly fades (Yallouz *et al*., 2008). To optimise colour discrimination, the authors suggested using standard lighting conditions to view the detecting papers (Yallouz *et al*., 2008).

Figure 2.3 Increasing colour intensity of Hg complex formed on Cu_2I_2-coated detecting papers obtained following analysis of standards with increasing Hg concentration (blank is shown on 1st paper from left).

In addition to common laboratory chemicals and glassware, an electrical supply, a heating device, such as a water bath or hot plate for sample digestion, and a pump for the transfer of Hg^o to the detecting paper are required. While method implementation is straight forward, there are several considerations that are not addressed in the method description given by Yallouz *et al*. (2008). For example, the flow rate of the aquarium pump is not stated and may be important with regard to

the transfer of Hg^0 to the detecting paper. While a lower determination limit of 100 *μ*g/kg total Hg is reported, i.e. with a sample Hg concentration of 100 *μ*g/kg, a discernible colour is produced, no upper limit is stated. Additionally, there is no indication as to the concentration difference required between standards to produce a difference in intensity visible to the eye. While the method has been applied to real samples and produced results consistent with quantitative determination, other than type of sediment (fluvial, mine tailings), no additional sample information, such as for example the OM content, which if present may interfere with the process, was given.

2.3 Aim

The aim of Chapter 2 was to more fully characterise and assess the applicability of the screening method described by Yallouz *et al*. (2008) to marine samples. Specifically:

- the pump flow rate required to give reproducible results was considered
- the concentration difference between standards required to produce a difference in intensity visible to the eye was assessed
- the method range was determined
- the effect of OM on digestion efficiency and method performance was assessed
- method performance under salinities ranging from freshwater to marine water (APHA, 1989) was tested
- method performance was compared in deionised and sea water standard solutions
- interferences from the marine sediment matrix were assessed
- overall method efficiency in marine sediment was tested using a reference material certified as to its total Hg concentration.

2.4 Materials and methods

2.4.1 Procedures

2.4.1.1 Preparation of glassware

All glassware was soaked overnight in 10% (v/v) nitric acid (HNO_3), prepared from concentrated HNO_3 (> 65%, for trace analysis, Sigma-Aldrich Company Ltd. Dorset, U.K.), and rinsed with distilled water before use.

2.4.1.2 Solution preparation

Tin(II)chloride solution, 50% $SnCl_2$ (w/v), was prepared by weighing $SnCl_2.2H_2O$ (50 g) (AnalaR NORMAPUR, BDH, VWR International BVBA, Leuven, Belgium) into a 500 mL beaker, followed by slow addition of concentrated hydrochloric acid (HCl) (50 mL) (30% for trace analysis, Sigma-Aldrich Company Ltd. Dorset, U.K.) and distilled water (50 mL). The solution was stored in an amber storage bottle containing grains of metallic tin (Analar, BDH Laboratory Chemicals Division, Poole, England) to maintain stability.

A stock standard Hg solution (10 mg/L in 10% (v/v) HNO_3) was prepared by pipetting 1000 μL from a 1000 mg/L Hg standard solution ($Hg(NO_3)_2$, Certipur, Merck, Leicester, U.K.) into a 100 mL volumetric flask containing approximately 50 mL distilled water, following which HNO_3 (10 mL) was added. The solution was made up to 100 mL with distilled water, stored at 4 ℃ and replaced monthly. Standard solutions of various Hg concentrations were prepared daily as required from the 10 mg/L stock solution in distilled water.

2.4.1.3 Conditioning of detecting papers

Detecting papers coated with Cu_2I_2 emulsion (Cu_2I_2 (5 g); 3% carboxymethylcellose (10 g); $MgCl_2$ moistening agent (1.5 g), Cetem, Centro de Tecnologia Mineral, Rio de Janeiro, Brazil) were conditioned for 48 hours by positioning on an acrylic support (Cetem, Centro de Tecnologia Mineral, Rio de Janeiro, Brazil) in a desiccator containing a saturated solution of magnesium sulfate ($MgSO_4$, dried, GPR, BDH VWR International Ltd., Poole, England) to provide an atmosphere with 90 - 95% humidity (Figure 2.4) since paper humidity directly affects Hg^0 capture (Crisp *et al*., 1981; Yallouz *et al.*, 2008).

Figure 2.4 Sheet containing six detecting papers conditioned in a saturated solution of magnesium sulfate ($MgSO_4$).

2.4.1.4 Digestion of solid samples

Solid samples were digested by accurately weighing (4 decimal places (d.p)) (Kern balance 870, KERN & SOHN GmbH, Balinger, Germany) approximately 10 g of sample into a 250 mL conical flask. To prevent excessive foaming, five drops of anti-foaming solution (Bristol-Myers Squibb, Cetem, Centro de Tecnologia Mineral, Rio de Janeiro, Brazil) was added followed by the slow addition of a*qua regia* (25 mL), prepared immediately before use by mixing HCl with HNO_3 in a ratio of 3:1 to produce the amount required. A cold finger (University of Strathclyde, Scotland, U.K.) filled with water was fitted on top and the flask was then heated in a water bath (30 min., 80 ^oC) (PD Group, P-D Industriegesellschaft mbH, Dresden, Germany). After cooling to room temperature, distilled water (50 mL) was added before proceeding to the determination step. No filtration step was necessary (Yallouz *et al*., 2008).

2.4.1.5 Determination step/ sample reduction

Sample reduction was carried out by addition of reductant ($SnCl_2$, 5 mL) to the sample. An aeration duct (Cetem, Centro de Tecnologia Mineral, Rio de Janeiro, Brazil) was inserted in place of the cold finger. The long arm of the duct (immersed in the solution) was connected to the pump and the short arm of the duct was connected to the condenser at the back of the unit via appropriate tubing (Figure 2.1). Once connection was completed, the detecting paper was removed from the desiccator and cut to the appropriate size by placing it on top of a mould (Cetem, Centro de Tecnologia Mineral, Rio de Janeiro, Brazil) and cutting around it (Figure 2.5).

Figure 2.5 Sheet with six detecting papers and plastic mould.

The detecting paper was then fitted inside the holder (Cetem, Centro de Tecnologia Mineral, Rio de Janeiro, Brazil) (Figure 2.2). The pump was turned on and allowed to run for 20 minutes during which time any Hg^0 vapour was transferred from the digest onto the paper where it reacted with the coating to produce $Cu_2[HgI_4]$. Following this, the paper holder was opened and the paper removed before turning off the pump to avoid pump damage. Standards were screened in an identical manner, using 75 mL of standard solution.

2.4.2 Method characterisation

2.4.2.1 Pump flow rate and reproducibility

Colour reproducibility using two different flow rates was tested; a flow rate of 2.5 L/min (Elite Pro, Brazil) and a lower flow rate of 1 L/min. (AquaAir Mini, Interpet, Surrey, U.K.). Reproducibility was checked for 3 replicates of a 40 *µ*g/L Hg standard with both flow rates.

2.4.2.2 Standard concentration differences for colour discrimination

Standard solutions of Hg differing in concentration by 25 *µ*g/L and by 20 *µ*g/L were tested to determine if colour discriminations could be made at both levels.

2.4.2.3 Method range

Standard solutions of Hg ranging from 5 to 300 *µ*g/L were tested to assess the method range.

2.4.2.4 Effect of OM on digestion efficiency and method performance

The adequate release of Hg from different matrices containing no OM and 10% OM was tested. This selection covered the typical soil OM content of 5% (CTAHR,

2014). Soil with 10% OM content (sand: 75%, silt: 10%, clay: 5%, humus (OM): 10%, University of Strathclyde, Scotland, U.K.) and sand samples without OM (sand pit sand, B&Q, Glasgow, Scotland, U.K.) were spiked with 3 *μ*g Hg (by addition of 300 *μ*L 10 mg/L Hg standard solution per 10 g sample), mixing, covering and leaving overnight before digestion and determination. Soil and sand blanks (without a spike) were also analysed.

2.4.3 Applicability of method to a marine environment

2.4.3.1 Effect of increasing salinity on method

Standard solutions of Hg (40 *μ*g/L) with salinities ranging from 0.01 to 41 practical sailinty units (psu) were prepared by the addition of potassium chloride (KCl, GPR, BDH Laboratory Supplies, Poole, England). The salinity was determined based on an *in situ* electrical conductivity measurement (conductivity meter, PCM3 Jenway, Bibby-Scientific, Staffordshire, U.K.) at 20 ℃ and P = 10 kPa. The conductivity was converted to salinity using flinders conductivity converter (http://www.es.flinders.edu.au) (Table 2.1).

Table 2.1 Conductivity measurements and related salinity at T = 20 ℃ and P = 10 kPa

Conductivity (mmho/cm)	Salinity (psu)
0	0.01
15	9.76
30	20.8
42	30.2
45	32.6
55	40.9

2.4.3.2 Comparison of method performance in deionised and sea water standard solutions

Sea water (collected in a 2 L glass bottle from the intertidal zone at Loutsa, Greece, (latitude 37.968833 and longitude 24.0075)) and deionised water were spiked with appropriate volumes of 10 mg/L Hg stock solution to produce pairs of spiked samples in the range from 5 to 200 *μ*g/L. The colours obtained for pairs of the same concentration were compared to assess potential interferences from sea water.

2.4.3.3 Screening for Hg in spiked marine sediment

Dried and sieved (2 mm stainless steel sieve, Fisherbrand, Fisher Scientific UK Ltd., Loughborough, U.K.) marine sediment (10 g) (from the intertidal zone at Lochgilphead, West of Scotland, U.K. (grid reference NR 86200 87900)) was spiked with 1.5 and 6 *µ*g Hg, mixed thoroughly, covered and left overnight. Samples were then digested, screened for Hg concentration and semi-quantitative determination made by comparison with colours obtained for standards containing 0.75, 3.75 and 7.5 *µ*g Hg.

2.4.3.4 Overall method efficiency in marine sediment

To assess the overall method efficiency reference material CRM BCR 580 estuarine sediment with 132 ± 3 mg/kg Hg (IRMM, Institute for Reference Materials and Measurements, Geel, Belgium) was analysed in duplicate.

2.5 Results and discussion

2.5.1 Method implementation

2.5.1.1 Pump flow rate and reproducibility

The colour intensities obtained for replicate analysis of 40 *µ*g/L standards were unsatisfactory using a flow rate of 2.5 L/min. This was indicated by the lack of a distinct edge on the detecting paper and was probably a result of too fast a pump flow rate resulting in losses from the paper holder (Figure 2.6). Using mini pumps with a flow rate of 1 L/min. reproducible results were obtained, giving circles with distinct edges (Figure 2.7).

Figure 2.6 Leaks resulted when using pump flow rate of 2.5 L/min. indicated by a smudged circle edge.

Figure 2.7 The same colour intensity was obtained for 3 standards (40 µg/L) when using a 1 L/min pump flow rate. Distinct edges indicated no leaks.

2.5.1.2 Standard concentration differences for colour discrimination

A difference in colour intensity could be seen between standards with a 25 µg/L concentration (Figure 2.8) and a 20 µg/L concentration difference (Figure 2.9) over the ranges tested. Greater differences in standard concentrations were required for colour discriminations to be made at higher concentrations (Section 2.5.1.3).

Figure 2.8 Increase in colour as standard solutions increase in concentration by 25 µg/L (blank, 15, 40, 65, 90 µg/L).

Figure 2.9 Increase in colour intensity as standard solutions increase in concentration by 20 µg/L (blank, 15, 35, 55 µg/L).

2.5.1.3 Method range

Colour intensity increased for standard solutions from 15 to 120 µg/L although between 90 and 120 µg/L the change was very slight (Figure 2.10). Above 100 µg/L colour discriminations became harder to discern and bleeding of the coloured complex was sometimes observed under the holder (Figure 2.11). A slight increase

in colour intensity was observed between 100 and 200 *µ*g/L, however there was no obvious increase between 200 and 300 *µ*g/L (Figure 2.11). Thus, 200 *µ*g/L was determined to be the upper level of screening. Concentrations of 5 *µ*g/L were discernible from the blank making this the lower level of screening (Figure 2.13).

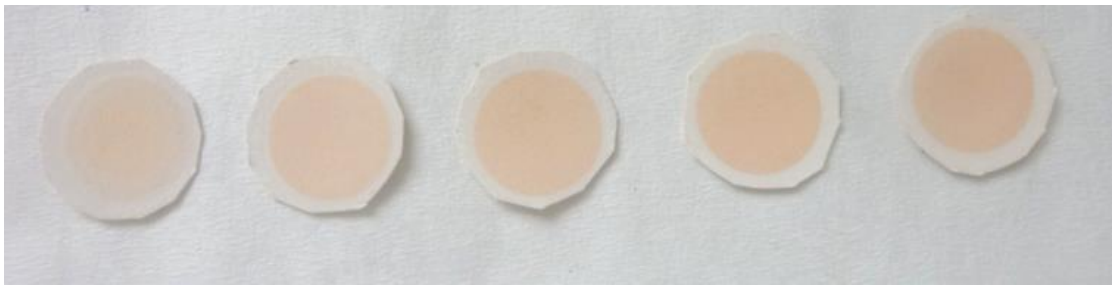


Figure 2.10 Increase in colour intensities for standard solutions between 15 and 120 *µ*g/L (15, 40, 65, 90, 120 *µ*g/L).

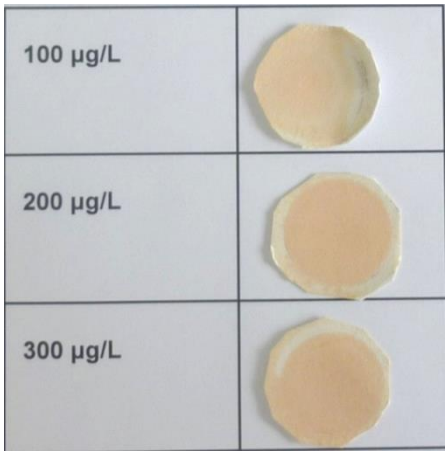


Figure 2.11 Colour intensities produced for 100, 200 and 300 *µ*g/L standard solutions.

2.5.1.4 Effect of OM on digestion efficiency and method performance

The effect of OM on digestion efficiency and method performance was tested using samples with, and without, OM. Colours obtained on screening a sand sample without OM and a soil sample with 10% OM, both of which had been spiked with 3 *µ*g Hg, were compared to colour intensities obtained from standard solutions (75 mL) of concentration 15, 35 and 55 *µ*g/L corresponding to 1.1, 2.6, and 4.1 *µ*g Hg. A reagent (acid) blank and unspiked sand and soil samples were also tested. No Hg was detected in the reagent blank, unspiked soil and unspiked sand sample. Sand and soil spiked with 3 *µ*g Hg indicated Hg content in the range 2.6 - 4.1 *µ*g, as expected (Figure 2.12) (Table 2.2), indicating that for the specific soil sample 10% OM did not affect the digestion or method performance. If samples contained higher

OM, further testing would need to be carried out to ensure that, at higher concentrations, OM does not influence the release of Hg from the matrix.

Table 2.2 Results obtained on screening blank samples and samples spiked with 3 *µ*g Hg

Sample	Results as compared to standard solutions containing 1.1, 2.6 and 4.1 *µ*g Hg
acid blank	< 1.1 *µ*g
Unspiked soil	< 1.1 *µ*g
Unspiked sand	< 1.1 *µ*g
Acid spiked with 3 *µ*g Hg	2.6 - 4.1 *µ*g
Soil spiked with 3 *µ*g Hg (rep. 1)	2.6 - 4.1 *µ*g
Soil spiked with 3 *µ*g Hg (rep. 2)	2.6 - 4.1 *µ*g
Sand spiked with 3 *µ*g Hg (rep. 1)	2.6 - 4.1 *µ*g
Sand spiked with 3 *µ*g Hg (rep. 2)	2.6 - 4.1 *µ*g

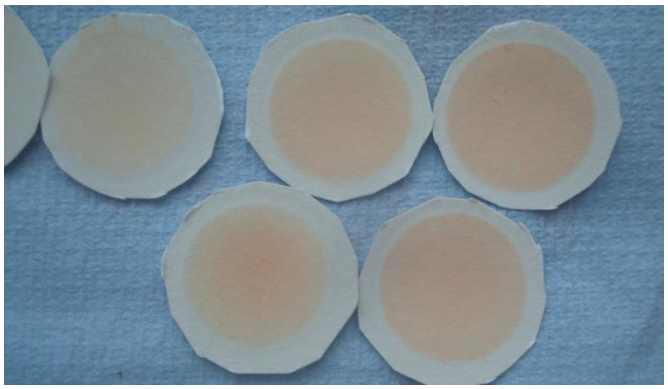


Figure 2.12 Colour intensities produced on screening standard solutions and samples. Upper row: colours obtained for standard solutions containing 1.1, 2.6 and 4.1 *µ*g Hg. Lower row: colours obtained for sand (left) and soil (right) spiked with 3 *µ*g Hg.

2.5.2 Applicability of method to a marine environment

2.5.2.1 Effect of increasing salinity

Increasing the salinity from 0.01 to 41 psu at a solution concentration of 40 *µ*g/L did not affect the intensity of the colour produced.

2.5.2.2 Comparison of method performance in deionised and sea water standard solutions

For each increase in standard concentration, an increase of similar intensity was observed in the colour obtained for both deionised water and sea water standard solutions (Figure 2.13).

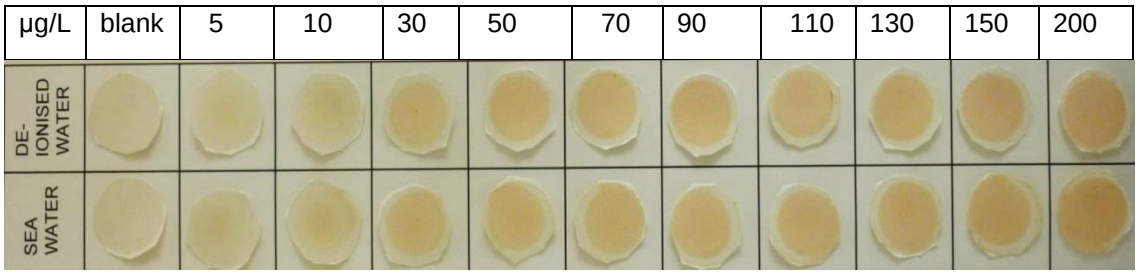


Figure 2.13 Comparable increasing colour intensity obtained from screening sea water and deionised water standard solutions as standard solution concentrations were increased (5, 10, 30, 50, 70, 90, 110, 130, 150 and 200 *µ*g/L Hg).

2.5.2.3 Screening for Hg in spiked marine sediment

The colours obtained on detecting papers following the screening of marine sediment spiked with 1.5 *µ*g and 6 *µ*g Hg were in the expected colour range when compared to the colours obtained from standard solutions containing 0.75, 3.75 and 7.5 *µ*g Hg (Figure 2.14).

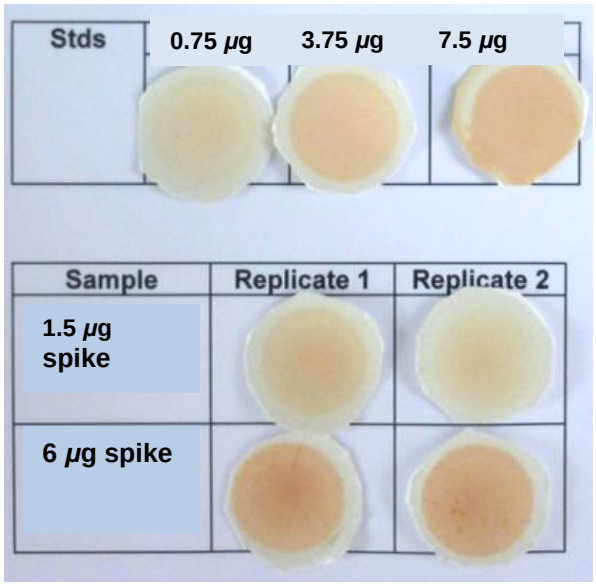


Figure 2.14 Colour intensities obtained from screening for Hg in spiked sediment compared with colours obtained on screening Hg standard solutions.

2.5.2.4 Overall method efficiency

A screening result in the correct range was obtained from the determination of Hg in CRM BCR 580 estuarine sediment that contained 132 ± 3 mg/kg Hg (Table 2.3).

Table 2.3 Screening results for Hg concentration in CRM BCR 580 with 132 ± 3 mg/kg Hg

CRM weight (g)	*μ*g Hg	Results as compared to standard solutions containing 1.1, 2.6 and 4.1 *μ*g Hg
0.0210	2.81	2.6 - 4.1 *μ*g
0.0412	5.48	> 4.1 *μ*g

2.6 Conclusions

The screening method was successfully implemented using a pump flow rate of 1 L/min to produce reproducible results over a range of standard concentrations from 5 to 100 *μ*g/L. Differences in standard concentrations of 20 *μ*g/L resulted in changes in colour intensities visible to the eye over this range. Above 100 *μ*g/L changes in intensity were observed, however larger differences in concentrations were needed to be discernible to the human eye. Screening of spiked sand samples and soil samples with 10% OM content gave a semi-quantitative determination of Hg in the correct range. Further testing would be required to ensure higher OM content did not influence the method if samples contained higher OM. Increasing salinity did not affect method response and the screening of spiked marine sediment gave a semi-quantitative determination of Hg in the expected range as did the screening of CRM BCR 580. Thus initial results indicate that the semi-quantitative screening method can be extended from use in fluvial sediments and mine tailings to use in a marine environment, either for water or for sediment. The lack of analytical equipment needed, coupled with the large number of matrices in which it can be used indicated that the method could be widely implemented for the identification of Hg-contaminated sites. The method was therefore implemented in freshwater and marine sediments with different expected Hg content in both Scotland and Greece (Chapter 3).

CHAPTER 3 TEST APPLICATIONS OF SEMI-QUANTITATIVE SCREENING AND ASSESSMENT OF SEDIMENT CONTAMINATION

3.1 Introduction

Techniques employed to assess Hg contamination in sediment range from simple screening tests to the comparison of quantitative determinations with SQGs. It is also possible to classify the level of pollution in sediment through the comparison of the Hg content with background levels, where background levels can refer to a pre-industrial reference value such as the concentration in continental crust or shale, or to the concentration in unpolluted sediment in a particular area. Two commonly used methods for assigning a contamination level, discussed below, are the use of the contamination factor, C_f, and the use of the geoaccumulation index, $I_{geo.}$

3.2 Classifying levels of contamination

Hakanson (1980) first proposed the use of the contamination factor C_f (Equation 3.1) in an ecological risk approach for contamination in a basin where C_0 was the concentration of the metal in the sample and C_b was the average background concentration of the metal.

$$C_f = C_0/C_b \qquad \textbf{Equation 3.1}$$

Depending on the calculated C_f value sediment contamination levels were defined as shown in Table 3.1. Hakanson proposed that an average C_f value should be calculated from C_f values from at least five locations within a basin (Hakanson, 1980), although more recently C_f values have been used to assess levels of contamination in open systems such as bays based only on one sampling position (Valdes *et al*., 2010; Balkis *et al.,* 2010).

Table 3.1 Classification of sediment contamination based on contamination factor (C_f)

C_f	Contamination level
$C_f < 1$	Low contamination
$1 \leq C_f < 3$	Moderate contamination
$3 \leq C_f < 6$	Considerable contamination
$C_f \geq 6$	Very high contamination

Another classification system for describing metal enrichment in sediment compared to background levels is the geoaccumulation index (I_{geo}) (Equation 3.2) where C_n represents concentration of metal *n*; B_n represents the background concentration of metal *n* and the factor of 1.5 is to account for possible variations in the background concentrations (Martinez and Poleto, 2014; Wu *et al.*, 2011a; Valdes *et al.*, 2010). Classification of the level of sediment pollution based on I_{geo} values are shown in Table 3.2.

$$I_{geo} = \log_2(C_n/1.5B_n) \qquad \textbf{Equation 3.2}$$

Table 3.2 Classification of sediment contamination based on geoaccumulation index (I_{geo})

I_{geo}	**Description of sediment quality**
$I_{geo} > 5$	extremely contaminated
$4 \leq I_{geo} < 5$	strongly to extremely contaminated
$3 \leq I_{geo} < 4$	strongly contaminated
$2 \leq I_{geo} < 3$	moderately to strongly contaminated
$1 \leq I_{geo} < 2$	moderately contaminated
$0 \leq I_{geo} < 1$	uncontaminated to moderately contaminated
< 0	uncontaminated

Pre-industrial reference values for Hg based on rock type vary depending on whether shale or continental crust values are used. In shale, background concentrations of Hg are reported to be in the range 0.3 - 0.4 mg/kg (Krauskopf and Bird, 1995; Turekian and Wedepohl, 1961). In continental crust, the value of Hg is reported to be 0.08 mg/kg (Taylor, 1964; Krauskopf and Bird, 1995). Sediment cannot be classified as either type of 'rock' and therefore neither value is entirely accurate. However, Turekian and Wedepohl (1961) assign an 'order of magnitude' value for background Hg levels in deep sea sediments of 10^{-2} mg/kg for carbonates and 10^{-1} mg/kg for clays. Sometimes Hg concentrations in unpolluted sediment cores or soils from a particular study area are used as background levels. For example in assessing contamination in surface sediment of the Miyan Reservoir, Beijing, Zhu *et al.* (2013) used a background Hg value in Beijing soil of 0.03 mg/kg to calculate $I_{geo.}$ Both values of C_f and I_{geo} and the associated classification of

contamination level can be affected by the background value chosen. Using three different background values, Valdes *et al.* (2010) obtained three different I_{geo} values corresponding to three different contamination classifications for sediment in the San Jorge Bay, Chile. Using a background value of 1.4 mg/kg representing the background level in average shale, I_{geo} values indicated sediment from the San Jorge Bay was uncontaminated ($I_{geo} < 0$). This background value however is extremely high. The authors quote Turekian and Wedepohl (1961) as the source of this value, although either the value or the reference have been used in error since Turekian and Wedepohl (1961) quote a value of 0.4 mg/kg for Hg in shale. Using a background reference value of Hg in continental crust of 0.08 mg/kg, I_{geo} values indicated moderately to strongly contaminated sediment ($2 \leq I_{geo} < 3$) while based on a background value of 0.3 mg/kg, the Hg concentration in a sediment core from Mejillones Bay, 50 km from the study area, the I_{geo} value indicated uncontaminated to moderately contaminated sediment ($0 \leq I_{geo} < 1$).

3.3 Aim

The aim of Chapter 3 was to test a semi-quantitative screening method characterised and evaluated for use in marine sediment (Chapter 2) in real marine and freshwater sediment samples from locations with different characteristics and to determine sediment contamination levels based on C_f and I_{geo} values.

3.4 Materials and methods

3.4.1 Locations and sampling procedures

3.4.1.1 Selection of locations

Locations chosen were a marine environment considered unpolluted, a freshwater river receiving industrial inputs and surface water run-off, a marine environment receiving both industrial effluents and wastewater and a freshwater canal with a history of Hg contamination. The locations differed not only in salinity, but were also likely to vary in Hg concentration and sediment matrix composition such as OM content which, due to strong associations with Hg (Section 1.3.4.2), could affect the method response.

3.4.1.2 West of Scotland, U.K.

Marine sediment, assumed to be uncontaminated, was taken from the coastal areas around the Kintyre Peninsula in the West of Scotland, U.K. (Figure 3.1).

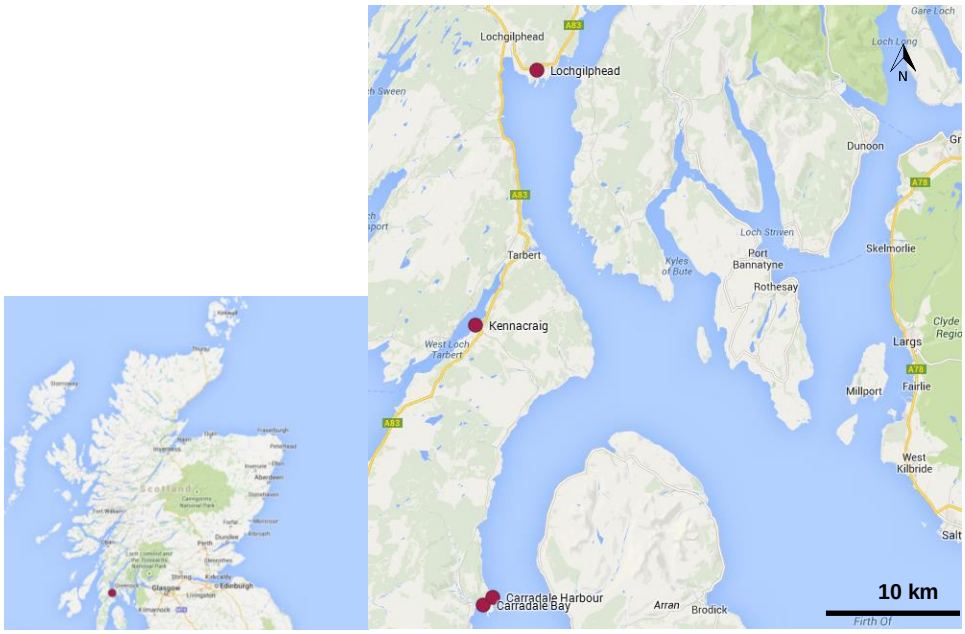


Figure 3.1 West of Scotland, U.K., showing sampling locations.

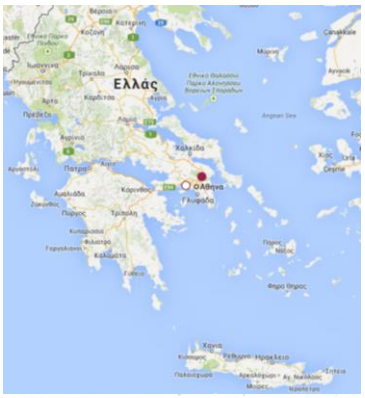
Specifically samples were taken from Lochgilphead and Carradale (bay and harbour) within the lower estuary of the River Clyde, where there are no industrial inputs, and one sample was taken from Kennacraig on the west coast of Scotland (Table 3.3). Samples were taken using a stainless steel trowel from the intertidal zone and places in widemouth glass bottles for transport to the laboratory.

Table 3.3 Locations and positions (OSGB36) for sediment samples taken from the West of Scotland, U.K.

Location (N to S)	Grid Reference (OS)	Latitude (approx.)	Longitude (approx.)
Lochgilphead	NR 86200 87900	56.03612	-5.4335
Kennacraig	NR 81500 61900	55.80083	-5.48776
Carradale Harbour	NR 81900 38600	55.59204	-5.46285
Carradale Bay	NR 81100 37400	55.58092	-5.47457

3.4.1.3 Kifissos River, Greece

Freshwater sediment receiving industrial inputs and surface water run-off was taken from Kifissos River, the largest river in the Attica region of Greece. Running from Krioneri, North Attica, to Faliro, South Attica, where it empties into the Saronic Gulf, the river has a length of 30 km (Figure 3.2).



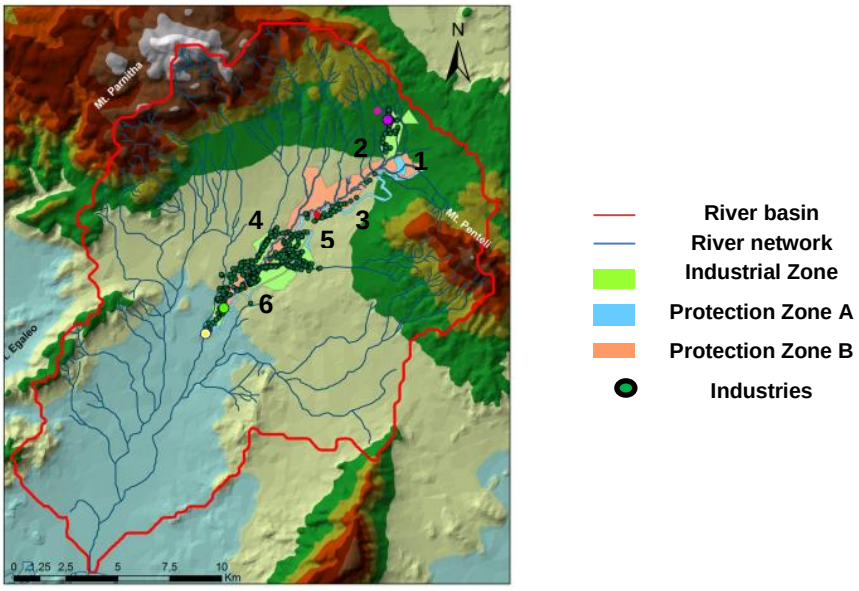


Figure 3.2 Kifissos River basin, Greece (adapted from Evrenoglou *et al.*, 2013) showing river network, protection and industrial zones, industries and sampling points.

Run-off from an area of approximately 361 km^2 drains into the river which is the most important drainage system in the region. In the absence of regulatory legislation, industries operating in the area until the 1990s including fabric and stone works, chemical, pharmaceutical and steel works, discharged untreated effluent into the river. In 1994, legislation establishing river protection zones was passed: in protection zone A, any kind of building was prohibited, as was the discharge of any forms of industrial effluent, whereas in protection zone B, construction was limited to small buildings (FEK 632, 1994). These protection zones cover an area of 12 km^2 (Figure 3.2). The legislation was not well implemented and in 2002, the 'Organisation for the Management and Regeneration of the Kifissos River and its Tributaries' was established, whose aim was to coordinate all relevant bodies involved in monitoring and protection of the river (FEK 287, 2002). Despite these measures, a recent report has indicated that metal concentrations in the river may be linked to adverse effects in children living in the area (Evrenoglou *et al*., 2013). For the present study, six sampling locations along the river were selected. Coordinates were obtained using a Garwin eTrex 10 GPS unit (Table 3.4). Locations 1, 2 and 4 were streams located inside the industrial zones that feed into Kifissos River and locations 3, 5 and 6 were located in protection zone A on the main river. Sediment samples were taken using a steel trowel at the edge of the river and placed in wide mouth glass bottles for transport to the laboratory.

Table 3.4 Locations and coordinates (WGS84) for samples taken from Kifissos River, Greece

Sample	Coordinates (Degrees Minutes)	Lattitude	Longitude
1 (Krioneri)	38°08.33500', 023°50.15000'	38.1389167	023.8358333
2 (Rema Fasideri)	38°07.48333', 023°50.43400'	38.1247222	023.8405667
3 (Adames)	38°05.71200', 023°47.01700'	38.0952000	023.7836167
4 (Rema Souna)	38°04.83900', 023°45.81900'	38.0806500	023.7636500
5 (Metamorfosi)	38°04.47700', 023°45.50500'	38.0746167	023.7584167
6 (Nea Philadefia)	38°03.27500', 023°44.50500'	38.0545833	023.7417500

3.4.1.4 Elefsina Bay, Greece

Sediment from a marine environment receiving both industrial effluents and wastewater was taken from Elefsina Bay (also known as Elefsis Bay and the Gulf of Elefsina), 20 km west of Athens. With the island of Salamina to the south, the bay is sheltered and water mixing is poor. Two narrow channels of water connect the bay to the Saronic Gulf (Figure 3.3).

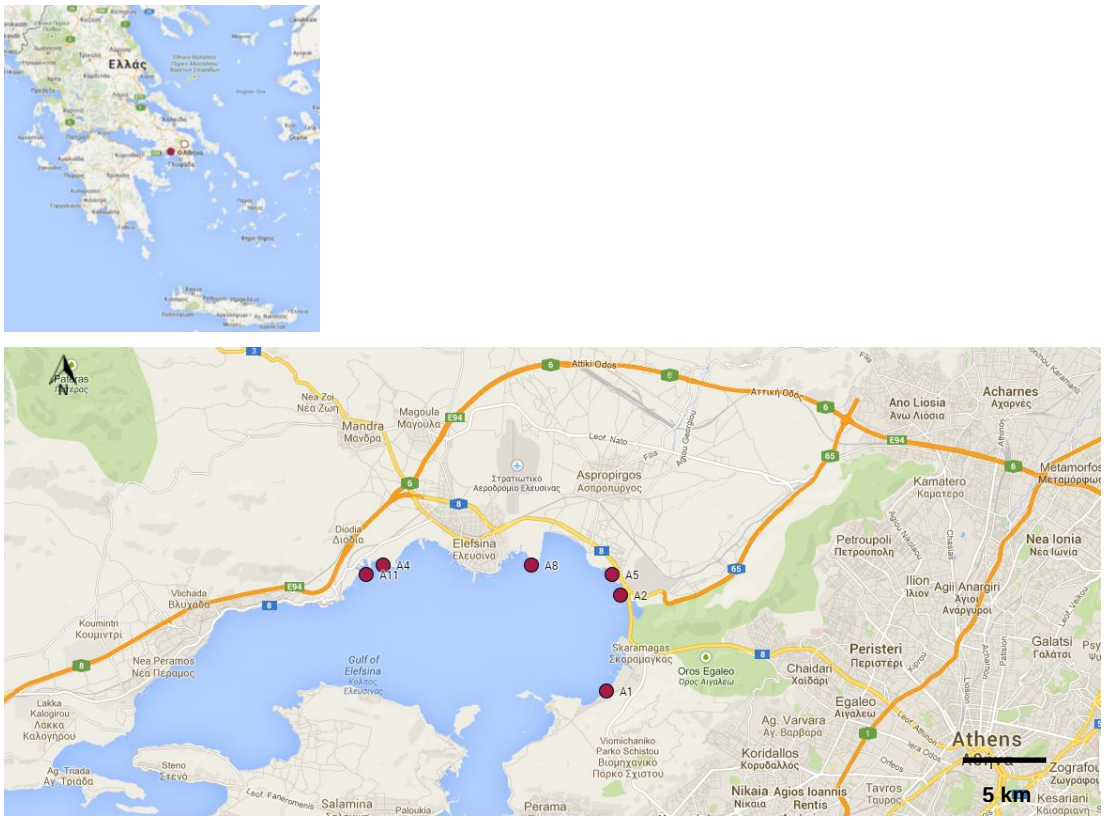


Figure 3.3 Elefsina Bay, Greece, showing sampling locations.

The bay has a surface area of 67 km^2, and a maximum depth of 33 m and receives effluent from some of the largest industrial plants in Greece, such as shipyards, oil refineries, paper and cement industries and metal extraction facilities. The main municipal effluent from Athens, which until 1995 was without treatment, is also discharged into the bay. Consequently the bay is polluted both with metals and organic substances (Pantazidou *et al.*, 2010). The Organisation for the Development of Thriasion Plain was established in 1986 in order to monitor the environmental condition of the bay, and concentrations of pollutants (not including Hg) have been recorded since that time. In 2005, the bay was recognised as an area of major environmental concern in a European Environment Agency (EEA) and UNEP report for the Mediterranean environment (EEA, 2005). For the present study sampling

was carried out in co-operation with the Organisation for the Development of the Thriasino Plain. Permission to sample was received from the Elefsina port authorites. Nearshore locations were selected along the bay (Figure 3.3) in the vicinity of specific industries. Location names already in use by the Organisation for the Development of the Thriasino Plain were maintained to facilitate comparisons. Specifically locations A1 and A11 were in the vicinity of ship yards, A2 and A4 were in the area of oil refineries, A8 was near a ship disassembly unit (Figure 3.4), and A5 was the location receiving the outfall from the Agios Georgios stream that contains effluent from many industries. Using a naval boat to approach locations (Appendix B), samples were taken at approximately 500 - 1000 m from the shore using a grab sampler (Figure 3.5), and placed in glass wide mouth bottles for transport to the laboratory. Sample coordinates (Table 3.5) were obtained using a Garwin eTrex 10 GPS unit.

Figure 3.4 Elefsina Bay, Greece, approaching sampling location A8.

Figure 3.5 Sediment collected from Elefsina Bay, Greece, using a grab sampler.

Table 3.5 Locations and coordinates (WGS84) for samples taken from Elefsina Bay, Greece

Locations (E to W) and potential pollution source	Coordinates (Degrees Minutes)	Latitude	Longitude
A1 (shipyard)	38°00.64500', 023°35.29100'	38.0107500	023.5881833
A2 (oil refinery)	38°01.79000', 023°35.72400'	38.0298333	023.5954000
A5 (Agios Georgios stream)	38°01.80900', 023°35.65000'	38.0301500	023.5941667
A8 (dissassembly unit)	38°02.31600', 023°33.26700'	38.0386000	023.5544500
A4 (oil refinery)	38°02.27200', 023°30.43200'	38.0378667	023.5072000
A11 (shipyard)	38°01.71100', 023°29.72500'	38.0285167	023.4954167

3.4.1.5 Union Canal, Scotland, U.K.

Freshwater sediment was taken from the Falkirk to Polmont section of the Union Canal, Scotland, U.K. (Figure 3.6), a 50 km engineered waterway running from Lochrin Basin, Edinburgh, in the East, to Falkirk in the West.

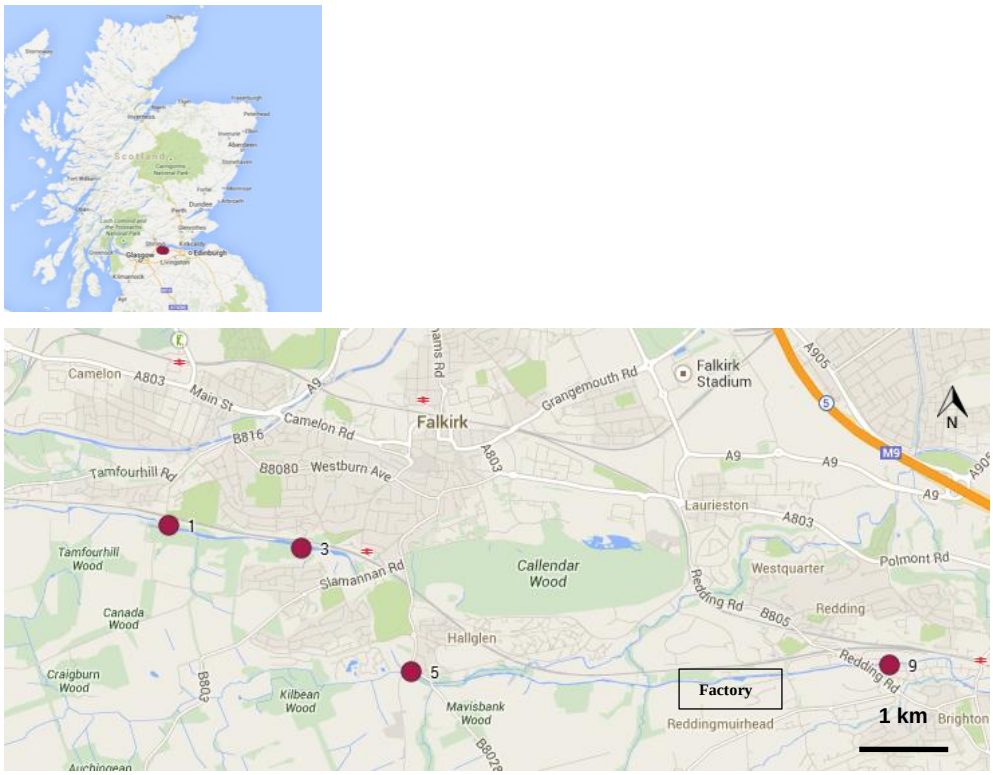


Figure 3.6 Falkirk to Polmont stretch of the Union Canal, Scotland, U.K., showing sampling locations and location of munitions factory.

During a national canal sediment sampling scheme, carried out in 1992 by British Waterways, concentrations of total Hg exceeding 1570 mg/kg were determined in

sediments from the Falkirk to Polmont area of the canal (BW, 1992). The extremely high levels were attributed to historic contamination from a munitions factory in Reddingmuirhead that operated in the area between 1876 and 1968 producing detonators, the main constituent of which was mercury fulminate (Smith and Lassiere, 2000). A major canal dredging operation was undertaken in the year 2000 to remove the Hg-contaminated sediment and dispose of it to landfill. For the present study, four locations between Falkirk and Polmont were sampled, which included the stretch of the canal in front of the mutitions factory (Table 3.6, Figure 3.6). Permission to sample was received from British Waterways, U.K.. Sample coordinates (Table 3.6) were obtained using a Garwin eTrex 10 GPS unit. Sediment samples were collected by throwing a stainless steel bucket attached to a rope across the width of the canal and slowly pulling it back along the canal bottom (Figure 3.7). The sediment was placed in wide mouth glass bottles for transport to the laboratory.

Table 3.6 Locations and coordinates (WGS84) for samples taken from the Union Canal, Scotland, U.K.

Location (W to E)	Coordinates (Degrees Minutes)	Latitude	Longitude
1	55°59.75100', -003°49.91100'	55.99585	-3.83139
3	55°59.61400', -003°48.58200'	55.99357	-3.80970
5	55°59.02700', -003°47.24600'	55.98378	-3.78743
9	55°59.06700', -003°43.68100'	55.98445	-3.72802

Figure 3.7 Sediment collected from the Union Canal, Scotland, U.K., using a stainless steel bucket.

3.4.2 Procedures

3.4.2.1 General Procedures

All glassware was soaked in 10% (v/v) HNO_3 (> 65%, for trace analysis, Sigma-Aldrich Company Ltd. Dorset, U.K.) overnight and rinsed with deionised (DI) water (prepared using water deionisation system, Barnstead EASYPureII RF ultrapure water system, Thermo Fisher Scientific Inc. Waltham, USA, following water distillation (water distillation system Autostill 4000X, Jencons (Scientific) Ltd., Franklin, USA) before use. Glass containers were used for storing Hg samples, standard solutions and reagents.

3.4.2.2 Solution preparation

Sodium borohydride reductant, 3% $NaBH_4$ in 1% sodium hydroxide (NaOH) solution

Reductant was prepared daily by addition of NaOH (2.5 g) (NaOH pellets, AR, Mallinckrodt, Dublin, Ireland) to a 250 mL volumetric flask containing approx. 100 mL DI water, followed by addition of sodium borohydride (7.5 g) ($NaBH_4$) (GR for analysis, Merck KGaA, Darmstadt, Germany) and addition of DI water to a final volume of 250 mL. The solution was filtered (glass fibre filters, Pall A/E Glass fibre filters 1.0 μm, 110 mm, Pall GmbH, Dreieich, Germany) into the MHS-10 reductant vessel (Perkin Elmer, Massachusetts, USA) before use. Any unused reductant was diluted and reacted with dilute acid (1.5% (v/v) HNO_3) before disposal.

Potassium permanganate ($KMnO_4$) solution, 5%

A 5% solution was prepared by addition of $KMnO_4$ (5 g) (≤ 0.000005% Hg, Sigma-Aldrich Company Ltd. Dorset, U.K.) to DI water to a final volume of 100 mL.

Mercury standard solutions

Reagent-matched standard solutions with Hg concentration < 10 mg/L were prepared daily from a 1000 mg/L Hg standard solution ($Hg(NO_3)_2$, Certipur, Merck, Leicester, U.K.).

3.4.2.3 Screening

The experimental procedure used for screening is detailed in Section 2.4.1. Where necessary, screening was repeated with a smaller sample size.

3.4.2.4 Sediment preparation

As soon as possible after sampling and always within the same day, sediment samples were dried (drying oven, natural convection, Binder E28, VWR International GmbH, Leuven, Germany) in foil containers at 30 ℃ and sieved (2 mm sieve, stainless steel, Fisherbrand, Fisher Scientific UK Ltd., Loughborough, U.K.) before storage in glass bottles. Each dried, sieved sample was coned and quartered to obtain a representative sample for analysis.

3.4.2.5 Determination of moisture content

In order to allow reporting on a dry weight basis, the moisture content of each dried sample was calculated (APHA, 1989). To allow moisture content and OM (Section 3.4.2.6) to be determined on the same portion of sediment, porcelain crucibles were conditioned by heating at 440 ℃ (muffle furnace Select-Horn, J. P. Selecta, s.a.) for 2 hours and were cooled in a dessicator before use. Approximately 0.5 g of each dried sample was accurately weighed in a pre-weighed crucible and heated at 103 ℃ overnight. After cooling moisture content was calculated using Equation 3.3. Determinations were carried out in duplicate.

$$\%\ \text{moisture content} = \frac{\text{dried weight} - \text{oven dried weight (103 ℃)}}{\text{dried weight}} \times 100 \qquad \textbf{Equation 3.3}$$

3.4.2.6 Determination of organic matter

The OM of the samples was calculated using loss of ignition (LOI) at 440 ℃. At this temperature the OM in the sample is destroyed and inorganic matter remains (Shumacher, 2002). After calculation of moisture content, the crucible (containing the sample) was place in a muffle furnace at 440 ℃ for 2 hours. After cooling in a dessicator, the sample was reweighed and %OM calculated using Equation 3.4. Determinations were carried out in duplicate.

$$\%\text{OM} = \frac{\text{weight (103 ℃)} - \text{weight (440 ℃)}}{\text{weight (103 ℃)}} \times 100 \qquad \textbf{Equation 3.4}$$

3.4.2.7 Microwave digestion procedure for total Hg determination

Approximately 0.5 g of sediment was accurately weighed (4 d.p.) (analytical balance Kern 870, KERN & SOHN GmbH, Balinger, Germany) into a microwave digestion vessel (microwave digestion vessels with pressure release valves, Berghof Products + Instruments GmbH, Eningen, Germany). After addition of HNO_3 (10 mL), samples were digested using microwave digestion (microwave, Berghoff Speedwave MWS-2 microwave system, Berghof Products + Instruments GmbH, Eningen, Germany) following the programme in Table 3.7 (Berghoff, 2006).

Table 3.7 Microwave programme for sediment digestion for Hg determination by cold vapour atomic spectrometry (CVAAS)

Step	**1**	**2**	**3**
T(℃)	140	160	175
Power (%)	80	90	90
Time (min.)	5	5	20

After digestion and cooling of vessels overnight, DI water (10 mL) was added. The samples were then filtered into a 50 mL volumetric flask, the filtered washings (using DI water) of the microwave vessel added and the volume made up to 50 mL with DI water. Samples were digested in duplicate.

3.4.2.8 Standard solutions for calibration

Calibration was carried out using reagent-matched standard solutions with concentrations of 0, 1, 2, 3 and 5 μg/L, prepared daily.

3.4.2.9 Determination of total Hg concentration with CVAAS

Determination of total Hg concentration using CVAAS was carried out using MHS-10 Hg/ Hydride system (Perkin Elmer, Massachusetts, USA) followed by AAS (AAS system, AAnalyst 100 Perkin Elmer, Massachusetts, USA). The AAS instrument conditions and method parameters given in Table 3.8 for standard solutions and digested samples. A sample aloquot of digest or standard solution (10 mL) was added to a MHS-10 reaction vessel (Perkin Elmer, Massachusetts, USA) followed by addition of $KMnO_4$ solution (2 drops) (PE, 1985). After attachment of the vessel to the MHS-10 system, $NaBH_4$ was added dropwise to the sealed unit for 20 s.

Samples were analysed in duplicate. Appropriate dilutions were carried out where necessary using 1.5% HNO_3.

Table 3.8 Instrument and method parameters used for determination of Hg concentration by cold vapour atomic absorption spectrometry (CVAAS)

AAS instrument and method parameters	
Light Source	Hollow cathode lamp (Hg) (Perkin Elmer, Massachusetts, USA)
	Current: 6 mA
	Wavelength: 253.6 nm
	Slit: 0.7 nm
Transfer Gas	Argon
	Pressure: 3.5 bar
Method parameters	Reading: Background corrected AA
	Measurement: Peak Height
	Read time: 20 s

Conversions from mg/L for digests to mg/kg for sediment samples were made using Equations 3.5 and 3.6.

$$\mathrm{Hg(mg/kg)} = \frac{analyte\ concentration\ in\ solution\ (mg/L) \times\ volume\ of\ solution\ \ (L) X\ dilution\ factor}{mass\ of\ sample(kg)\ (dry\ weight)} \quad \textbf{Equation 3.5}$$

where

$$\text{mass of sample (dry weight)} = \text{weight used X} \frac{(100 - \%\ moisture\ content)}{100} \quad \textbf{Equation 3.6}$$

3.4.2.10 Procedure efficiency

To assess the procedure efficiency, digestion of CRM BCR 320R (channel sediment containing 0.85 ± 0.09 mg/kg Hg, Institute for Reference Materials and Measurements, Geel, Belgium) was carried out using approximately 0.1 g of sample, followed by determination of total Hg concentration. Digestion was carried out in triplicate and each digest was analysed twice.

3.5 Results and discussion

3.5.1 Calibration, LOD and procedure efficiency

Calibration was carried out at 5 levels. The instrument LOD was 0.668 *µ*g/L corresponding to a procedural LOD of 0.067 mg/kg (Appendix C). Recovery of the CRM was 116 ± 20.3% (n=3) indicating that the overall procedure was adequate (Appendix D).

3.5.2 Hg concentration and OM content

The results for semi-quantitative Hg determination using the screening method, quantitative Hg determination using CVAAS and sediment OM content for the areas studied are given in Table 3.9. Results from quantitative Hg determination in the study areas were compared to Canadian sediment quality guideline values (CCME, 1999) since these are the only guidelines that provide TEL and ISQG values for Hg in both freshwater and marine sediment (GESAMP, 2014).

Table 3.9 Screening results for Hg content, Hg concentration as determined by cold vapour atomic spectrometry (CVAAS) and organic matter (OM) content for sediment from selected locations (CVAAS and OM results given as mean of two values (in brackets))

West of Scotland, U.K.			
Sampling point (N to S)	Screening result (mg/kg) (10 g sample)	Total Hg by CVAAS (mg/kg)	OM (%)
Lochgilphead	< 0.075	< 0.067	2.29 (1.93, 2.66)
Kennacraig	< 0.075	< 0.067	1.7 (1.30, 2.09)
Carradale Harbour	< 0.075	< 0.067	1.03 (0.82, 1.25)
Carradale Bay	< 0.075	< 0.067	0.11 (0.09, 0.13)
Kifissos River, Greece			
Sampling point (N to S)	Screening result (mg/kg) (10 g sample)	Total Hg by CVAAS (mg/kg)	OM (%)
1	< 0.075	< 0.067	2.05 (0.89, 2.20)
2	< 0.075	< 0.067	1.46 (1.26, 1.65)
3	< 0.075	< 0.067	3.38 (3.17, 3.58)
4	< 0.075	< 0.067	1.99 (1.91, 2.07)
5	< 0.075	< 0.067	10.1 (9.95, 10.3)
6	< 0.075	< 0.067	1.09 (0.85, 1.34)

Elefsina Bay, Greece				
Sampling point (E to W)	Screening result (mg/kg) (10 g sample)	Screening result (mg/kg) (1 g sample)	Total Hg by CVAAS (mg/kg)	OM (%)
A1	0.075 - 0.300	< 0.75	1.93 (1.77, 2.08)	8.36 (8.06, 8.66)
A2	> 0.525	0.75 - 3.75	1.88 (1.73, 2.03)	8.96 (7.51, 10.4)
A5	> 0.525	0.75 - 3.75	2.96 (2.19, 3.73)	7.23 (6.76, 7.71)
A8	Green colour on detecting paper	< 0.75	1.40 (1.15, 1.66)	8.09 (7.36, 8.87)
A4	0.075 - 0.300	< 0.75	1.50 (1.38, 1.61)	11.8 (11.0, 12.6)
A11	0.075 - 0.300	< 0.75	2.86 (2.20, 3.51)	9.08 (8.07, 10.1)

Union Canal, Scotland, U.K.					
Sampling point (W to E)	Screening result (mg/kg) (10 g sample)	Screening result (mg/kg) (1 g sample)	Screening result (mg/kg) (0.1 g sample)	Total Hg by CVAAS (mg/kg)	OM (%)
1	> 0.525	3 - 5.25, > 5.25	< 7.5	4.56 (4.43, 4.69)	6.70 (6.6, 6.7)
3	> 0.525	> 5.25	75 - 150	122 (107, 136)	13.9 (13.3, 14.6)
5	> 0.525	> 5.25	> 150	452 (444, 460)	16.4 (14.6, 18.3)
9	> 0.525	> 5.25	> 150	161 (154, 167)	13.0 (12.7, 13.2)

Screening of sediment samples from the West of Scotland (10 g sample size, standard solution concentrations of 10, 40 and 70 *μ*g/L) produced no colour on the detecting papers indicating Hg levels below detectable concentrations (Table 3.9). Quantitative determinations were consistent with the screening results; total Hg concentration as determined by CVAAS was below the LOD of 0.067 mg/kg for all samples (Table 3.9). The concentration of Hg at all locations was below the ISQG value (CCME, 1999) for Hg in marine sediment of 0.13 mg/kg indicating adverse effects on marine organisms were unlikely. The OM content of the sediment was low, ranging from 0.11 to 2.29%.

Screening of sediment samples from Kifissos River (10 g sample size, standard solution concentrations of 10, 40 and 70 *μ*g/L) did not indicate Hg contamination. Screening results were consistent with quantitative results where total Hg concentration determined by CVAAS was below the LOD of 0.067 mg/kg for all samples (Table 3.9). The concentration of Hg at all locations was below the ISQG value (CCME, 1999) for Hg in freshwater sediment of 0.17 mg/kg indicating adverse effects on aquatic organisms were unlikely. Values of OM were generally low (< 3.4%) with the exception of location 5, Metamorfosi, where a maximum OM of 10.1% was found. Before this location, Pirnas stream joins the river, carrying effluent from the Metamorfosi wastewater and septic sewage treamtment plant that could contribute to the higher OM at this location (EYDAP, 2012). Sewage effluent can also be associated with Hg contamination (Covelli *et al*., 2012) however this was not observed in this case.

Screening of samples from Elefsina Bay (10 g sample size and standard solution concentrations of 10, 40 and 70 *μ*g/L) indicated Hg at five of the six locations (Figure 3.8). Positions A5 and A2 gave the most intense colour on the detecting paper, darker than the intensity obtained from the highest standard (70 *μ*g/L). Since 75 mL of standard solution were used (Section 2.4.1.5), this volume of standard contained 5.25 *μ*g Hg, which in a 10 g sample size was equivalent to 0.525 mg/kg (Equation 3.5). Therefore screening indicated that positions A2 and A5 contained > 0.525 mg/kg Hg. Position A5 receives the waters from the Agios Georgios stream containing industrial effluent and position A2 is in the vicinity of an oil refinery. Location A8 produced a green colour on the detecting paper which did not allow any

screening assessment to be made. For the remaining three samples screening indicated a Hg concentration of between 0.075 and 0.300 mg/kg (Figure 3.8).

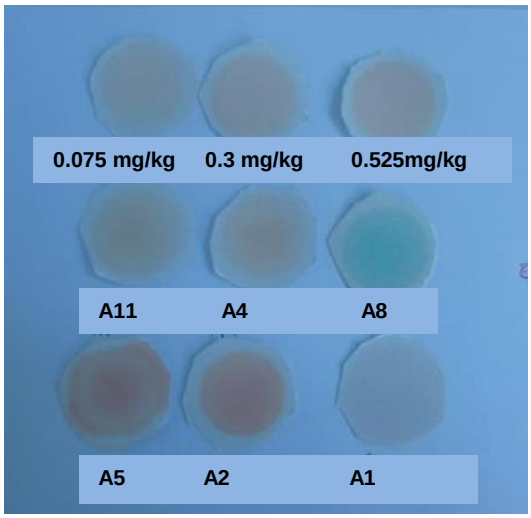
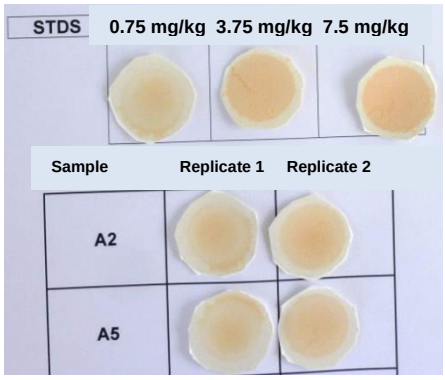


Figure 3.8 Screening results for Hg in Elefsina Bay sediment using 10 g of sediment and standard solution concentrations of 10, 40 and 70 *μ*g/L (top row), equivalent to concentrations of 0.075, 0.300, 0.525 mg/kg for this mass of sample.

Screening was repeated for all locations using standard solutions of concentration 10, 50 and 100 *μ*g/L and 1 g samples (equivalent to 0.75 3.75 and 7.5 mg/kg) (Figure 3.9). Results for locations A2 and A5 gave Hg in the range 0.75 - 3.75 mg/kg, while A8, which had produced the green colour, gave an Hg concentration < 0.75 mg/kg. The results for A1, A4 and A11 were below 0.75 mg/kg as expected from the screening results obtained using 10 g sample mass (0.075 - 0.300 mg/kg).



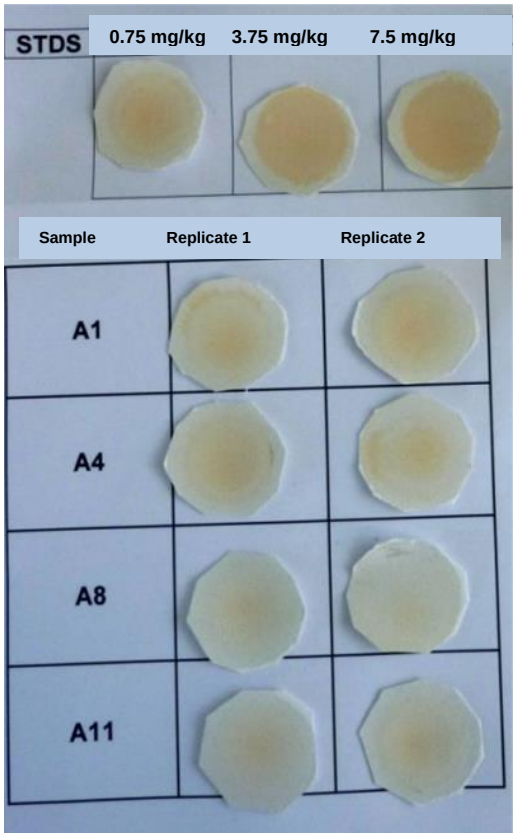


Figure 3.9 Screening results for Hg in Elefsina Bay sediments using 1 g of sample and standard solution concentrations of 10, 50 and 100 *μ*g/L, equivalent to concentrations of 0.75, 3.75 and 7.5 mg/kg for this mass of sample.

Total Hg concentrations in sediments from Elefsina Bay ranged from 1.4 to 2.96 mg/kg, with highest concentrations found in locations A5 (outfall of Agios Georgios stream) and A11 (shipyard) (Table 3.9). High Hg concentration at position A5 is probably a result of effluent discharge from industries known to release Hg such as cement manufacture and metal extraction while the use of phenylmercury acetate as an anitfouling agent in ship paints, which was common until the 1990s, may explain the higher results at locations A11 (Stathopoulou *et al*., 2001). Despite variations between results for duplicate analyses, the concentration of Hg at all locations for duplicates exceeded the PEL for Hg in marine sediment of 0.7 mg/kg (CCME, 1999), the concentration above which adverse effects are frequently observed in aquatic organisms. The OM content of the samples ranged from 7.23 to 11.8%, with highest OM content at position A4. The proximity of this location to an oil refinery could explain the higher OM observed in this area.

Screening and quantitative Hg determination gave consistent results for samples A2 and A5. Although screening identified the presence of Hg for samples A1, A4, A8 and A11, the screening results were lower than the results obtained from quantification (Table 3.9). Lower results are not thought to be a consequence of OM content, since samples for which screening gave a result in the correct range did not differ markedly in OM content from those for which screening did not give a correct assessment. Salinity is unlikely to be the cause of the lower values obtained for screening since salinity does not vary greatly over the bay (Chrisitidis, 2010). A possible explaination for the lower screening results is the loss of Hg during the digestion. It was observed that during the digestion step, the cold finger 'popped' frequently during heating and it is possible that Hg was lost at this stage. It is also possible that the digestion procedure used in the screening method was not adequate to release all forms of Hg contained in these samples: while digestion in the screening method was performed at a relatively low temperature of 80 ℃ (using *aqua regia*), for quantitave determination microwave digestion was carried out at 175 ℃ (using concentrated HNO_3). The green colour obtained on digestion of 10 g of sample A8 colour was not a consequence of high Hg or OM content since higher Hg and OM content were determined at other locations. In later work carried out with the screening process, the green colour on the detecting papers was seen to occur frequently. Instability of the coating on the papers was considered the most probable cause (Burns, 2012).

In sediment from the Union Canal, initial screening of canal sediment (10 g sample size, standard solutions of concentration 10, 40 and 70 *μ*g/L, equivalent to 0.075, 0.300, 0.525 mg/kg for this mass of sample) resulted in a very intense colour on the detecting papers, darker than the colour obtained from screening the highest standard (Table 3.9). Screening was repeated using 1 g of sample in place of 10 g, and standard solutions of concentration 10, 40 and 70 *μ*g/L, equivalent to 0.75, 3.00 and 5.25 mg/kg for this mass of sample. However colour intensity was still greater than that of the highest standard with the exception of one replicate of sample 1 which indicated a concentration range of between 3 and 5.25 mg/kg (Table 3.9). On use of 0.1 g sample with standard solutions of concentration 10, 50 and 100 *μ*g/L, equivalent to 7.5, 37.5 and 75 mg/kg, (Figure 3.10, Table 3.9), sample 1 gave a screening result of < 7.5 mg/kg however sample 3 was still over range therefore standard solution concentrations were increased to the maximum possible for the

method of 200 μg/L (Section 2.5.1.3) equivalent to 150 mg/kg. A screening result in the range 75 - 150 mg/kg was obtained for sample 3: However, the concentration of Hg in samples 5 and 9 remained above the maximum screening level possible of 150 mg/kg (Figure 3.11, Table 3.9).

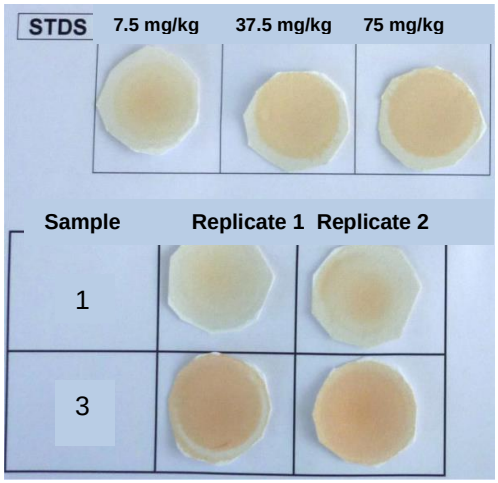


Figure 3.10 Screening results for Hg in Union Canal sediment using 0.1 g of sample and standard solutions of concentration 10, 50 and 100 μg/L, equivalent to 7.5, 37.5 and 75 mg/kg for this mass of sample.

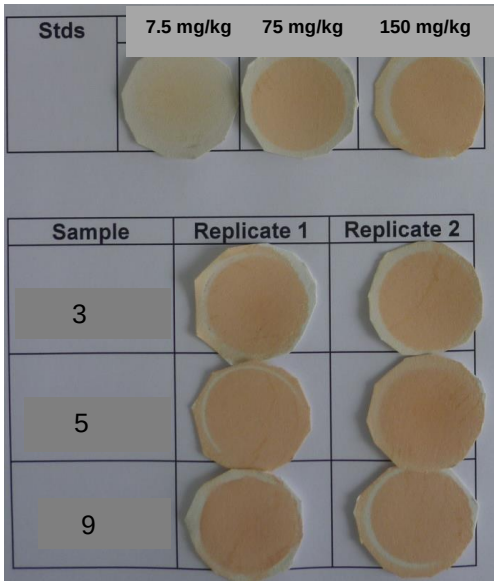


Figure 3.11 Screening results for Hg in Union Canal sediment using 0.1 g of sample and standard solutions of concentration 10, 100 and 200 μg/L, equivalent to 7.5, 75 and 150 mg/kg for this mass of sample.

Quantification of Hg in sediement from the Union Canal indicated concentrations of Hg between 4.56 and 452 mg/kg (Table 3.9). While these concentrations are

considerably lower than before the dredging of the canal they are still extremely high, well in excess of the PEL for Hg in freshwater sediment of 0.486 mg/kg (CCME, 1999) above which concentration adverse effects to aquatic organisms are frequently observed. Results obtained for all samples using both screening and quantification were in agreement, although for samples 5 and 9 these were expressed only as an upper limit.

3.5.3 Contamination factors (C_f) and Geoaccumulation Index (I_{geo})

It has already been noted that the selection of an appropriate background level in assessing sediment contamination can strongly influence the results (Section 3.2). Using both background values for Hg of 0.08 mg/kg found in continental crust (Taylor, 1964) and 0.4 mg/kg found in shale (Turekian and Wedepohl, 1961), C_f and I_{geo} and associated contamination levels for the four areas in this study were calculated (Table 3.10). As expected from the screening and quantitative results, for the West of Scotland, U.K., and Kifissos River, Greece, the use of both background levels resulted in classification at the lowest levels of contamination for C_f (low contamination) and I_{geo} (uncontaminated). Despite concentrations of Hg in the Union Canal being over 150 times greater than Hg concetrations in Elefsina Bay, based on the lower background level of 0.08 mg/kg, the C_f classification resulted in the highest classification of contamination for all sampling locations in both study areas. Using the higher background level of 0.4 mg/kg, four locations in Elefsina Bay were classified as having considerable contamination, whilst Elefsina positions A5 and A11, and all of the Union Canal samlpes were classified as having very high contamination levels. Calculation of I_{geo} gave more insight into relative contamination level, which is to be expected since there are more classification categories. A clear distiction was made in the contamination levels in Elefsina Bay. Locations A11 (ship yard) and A5 (discharge of industrial effluent) were classified as having a higher level of contamination than A2, A4 and A8, regardless of which background level was used, while the classification of A1 was dependent on the background value selected. Use of the higher background value in I_{geo} calcuations also allowed distinction to be made between the Union Canal samples, with position 1 having a lower classification than the other three positions.

Table 3.10 Contamination levels in sediment from four locations based on the calculation of contamination factors (C_f) and geoaccumulation index (I_{geo}) values using two different background values of Hg

Area	Locations	C_f (C_b = 0.08) and contamination level		C_f (C_b = 0.4) and contamination level		I_{geo} (B_n = 0.08) and contamination level		I_{geo} (B_n = 0.4) and contamination level	
West of Scotland, U.K.	All locations	0.838	low contaminatiom	0.168	low contaminatiom	-0.841	uncontaminated	-3.16	uncontaminated
Kifissos River, Greece	All locations	0.838	low contaminatiom	0.168	low contaminatiom	-0.841	uncontaminated	-3.16	uncontaminated
Elefsina Bay, Greece	A1	24.1	very high contamination	4.82	considerable contamination	4.01	strongly to extremely contaminated	1.76	moderately contaminated
	A2	23.5	very high contamination	4.70	considerable contamination	3.97	strongly contaminated	1.65	moderately contaminated
	A5	37	very high contamination	7.40	very high contamination	4.62	strongly to extremely contaminated	2.30	moderately to strongly contaminated
	A8	17.5	very high contamination	3.51	considerable contamination	3.55	strongly contaminated	1.22	moderately contaminated
	A4	18.8	very high contamination	3.75	considerable contamination	3.64	strongly contaminated	1.32	moderately contaminated
	A11	35.7	very high contamination	7.14	very high contamination	4.57	strongly to extremely contaminated	2.25	moderately to strongly contaminated
Union Canal, Scotland, U.K.	1	57	very high contamination	11.4	very high contamination	5.2	extremely contaminated	2.93	moderately to strongly contaminated
	3	1520	very high contamination	305	very high contamination	10.0	extremely contaminated	7.67	extremely contaminated
	5	5650	very high contamination	1130	very high contamination	11.9	extremely contaminated	9.56	extremely contaminated
	9	2010	very high contamination	403	very high contamination	10.4	extremely contaminated	8.07	extremely contaminated

3.6 Conclusions

In total 20 samples from four different locations were screened for Hg content and the results compared with quantitative Hg determination by CVAAS. The screening method successfully indicated the presence or absence of Hg in all cases. For ten samples (4 marine sediment and 6 freshwater sediment), in which OM ranged from 0.11 to 10.1%, no Hg was detected by either method. For six samples (2 marine and 4 freshwater) where Hg concentration ranged from 1.88 to 452 mg/kg and OM ranged from from 6.7 to 16.4%, the CVAAS results was in the range of the screening result. For the remaining four marine sediment samples where Hg ranged from 1.4 to 2.86 mg/kg and OM ranged from 8.09 to 11.8%, while the screening method identified the presence of Hg, the ranges obtained were lower than the CVAAS results. It is possible that at concentrations below the lowest Hg content identified by screening as contaminated but above the ISQG of 0.13 mg/kg, the method would not identify Hg contamination. It may be that the digestion process used in screening, that involved heating in a waterbath at 80 ℃, was not vigorous enough to release all Hg species from the samples, or that Hg was lost during digestion. The nature of the detecting paper itself can also be variable and may influence results (Yallouz, 2010). However, the screening method successfully identified Hg in all contaminated samples with Hg concentrations ranging from 1.4 to 452 mg/kg for a range of freshwater and marine samples of different sample matrix, indicating its suitablilty as a quick, inexpensive method of screening for Hg. Based on C_f and I_{geo} values, sediment from the West of Scotland, U.K., and Kifissos River, Greece, was uncontaminated. Locations in Elefsina Bay, Greece, in the vicinity of a shipyard and at the outfall of industrial effluent indicated a higher degree of contamination than other locations in the bay, while three locations in the Union Canal, Scotland, U.K., were extremely contamined regardless of assumed background values. Elefsina Bay, Greece, and the Union Canal, Scotland, U.K. are considered in more detail in Chapters 4, and Chapters 5 and 6 respectively.

CHAPTER 4 TOTAL MERCURY IN SEDIMENT AND FISH FROM ELEFSINA BAY, MERCURY MOBILITY AND SEASONAL VARIATION

4.1 History of Elefsina Bay

An area of major environmental concern (EEA, 2005), Elefsina Bay, located to the West of Athens (Section 3.4.1.4) forms part of the Thriassion Plain of Greece, with Mount Parnitha to the North and Mount Egaleo to the East. The surface area of the bay is 67 km^2 and its greatest depth is 33 m, although the depth of most of the bay is no more than 18 m. In winter the waters are mixed, but stratification occurs during the summer months. The water and sediment quality of Elefsina Bay have been badly affected by the discharge of effluent from many industries over the last few decades. There are four major sources of pollution into the bay:

1) discharge of sewage effluent from the Greater Attica area
2) industrial waste from distilleries, steel works, fertiliser plants, dye works, shipyards and ship disassembly units
3) waters from the Agios Georgios stream that receives effluent from industries including tanneries, paper works and cement works
4) leachate from the Ano Liosia landfill site situated to the northwest of Athens

Concentrations of several metals, excluding Hg, had been monitored in water and sediment over the last two decades by the Organisation for the Development of the Thriasion Plain (Section 3.4.1.4) before the organisation was closed in the year 2011. Samples were analysed twice yearly, once in the summer when the waters in the bay were stratified, and once in the winter, from nine locations in the bay, six nearshore and three from the centre of the bay. Metals determined were copper (Cu), cadmium (Cd), chromium (Cr), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn). Other water quality indicators monitored included temperature, pH, dissolved oxygen, transparency, biological oxygen demand (BOD) and chemical oxygen demand (COD). A decline in BOD and COD values was observed following the installation of sewage treatment facilities that started operation in 1994 with primary effluent treatment. Following transposition of the urban wastewater directive (EC, 1991) into Greek legislation (FEK192B, 1997) installation of secondary effluent treatment facilities in 2004 led to a further decline in BOD and COD values. The use of effluent treatment plants in some industries also lowered organic load (Christidis A., 2010). However there has been no decrease in metal concentrations (Pantazidou *et al.*,

2007). With regard to Hg contamination little information is available: in a study in the year 2000 total Hg concentrations in sediment were found to range from 0.31 to 6.40 mg/kg at nearshore locations while at central locations Hg concentrations were between 0.13 and 0.27 mg/kg (Stathopoulou *et al*., 2001). A study in 2004 determined Hg concentration in sediment in central locations in the bay to be in the range 0.1 - 0.5 mg/kg (Kanellopoulou *et al*., 2004). In the present study quantification of total Hg concentration in nearshore sediments indicated that Hg concentrations were above PELs of 0.7 mg/kg (CCME, 1999) and Hg enrichment factors indicated moderately to extremely contaminated sediment (Section 3.5.3). Screening suggested that Hg species may be in a tightly adsorbed form (Section 3.5.2) and therefore pose low risk of mobility and bioaccumulation, however there have been no studies considering the mobility of Hg species. Additionally the effect of seasonal variation on both Hg concentration and mobility has not been considered and could be significant: in surface sediment of St. Jorges Bay, Chile, where pollution sources were varied and ongoing, and included shipping and industrial wastes, a significant increase was reported in sediment Hg concentration in summer relative to winter from 0.404 to 0.820 mg/kg (Valdes *et al.*, 2010). The increase was attributed to the increase in biological activity in the bay in the summer and the transfer of the resultant increase in OM to the sediment from the water column. It was postulated that this also transferred Hg bound to OM from the water column to the sediment, thus increasing summertime Hg concentrations.

4.2 Aim

The aim of the work in Chapter 4 was to determine the mobility of Hg species in Elefsina Bay sediment using the procedure of Han *et al.* (2003) with a modified microwave assisted step 1 as recommended by Rahman and Kingston (2005). The relationship between OM content and total Hg concentration, the effect of seasonal variation on Hg concentration and mobility and OM content, and the bioaccumulation of Hg in fish, specifically flathead mullet, (*Mugil cephalus*), were also considered.

4.3 Materials and methods

4.3.1 Sediment sampling and preparation

Three sampling trips were carried out, one in July 2010 (summer sampling) at three central locations in the bay, K1, K3 and K5 (Figure 4.1), and two in March 2011 (winter sampling), at the same central locations and at the nearshore locations described in Section 3.4.1.4. The sampling procedure and sample preparation were carried out as described in Sections 3.4.1.4 and 3.4.2.4 respectively.

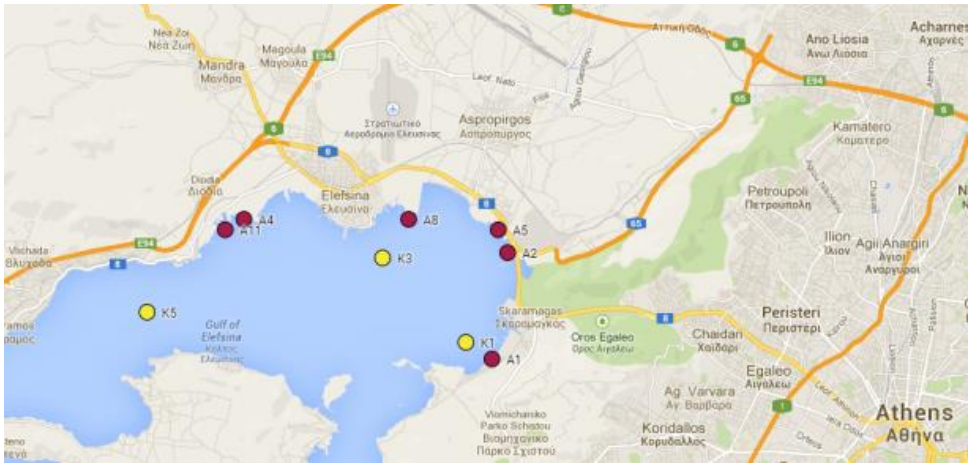


Figure 4.1 Nearshore and central sampling locations in Elefsina Bay, Greece.

4.3.2 Fish samples

Flathead mullet (nine) (Appendix E) from Elefsina Bay were caught on 24/2/2012 (Tselios P., 2012), stored on ice in a styrofoam box and delivered within 24 hours for analysis.

4.3.3 Procedures

4.3.3.1 General procedures as in Section 3.4.2.1

4.3.3.2 Solution preparation - in addition to those listed in Section 3.4.2.2

A 4 M HNO_3 solution for extraction of mobile species was prepared by addition of 255 mL HNO_3 to approximately 250 mL DI water in a 1000 mL volumetric flask and made up to 1000 mL with DI water.

A 5.33 M HNO_3 solution for extraction of semimobile species was prepared by combining 1 part HNO_3 with 2 parts DI water (1:2(v/v) HNO_3:H_2O).

4.3.3.3 Moisture content and OM content

Moisture content and OM content were determined as described in Sections 3.4.2.5 and 3.4.2.6.

4.3.3.4 Extraction procedure

4.3.3.4.1 Overall extraction procedure

The concentration of operationally-defined mobile Hg species, (such as inorganic $HgCl_2$, $Hg(OH)_2$, $Hg(NO_3)_2$, HgO and Hg-OM complexes soluble in weak acid, and organic MeHg and EtHg species), was initially determined (Rahman and Kingston, 2005). This was followed by the extraction of semimobile species such as Hg^0 and strongly complexed Hg-OM compounds (Han, 2003). Nonmobile species such as HgS and HgSe were determined from the difference between total Hg concentration and the sum of the concentrations of mobile and semimobile species.

4.3.3.4.2 Procedure for removal of mobile and toxic Hg species

Approximately 1 g of each sample was accurately weighed (4 d.p.) (analytical balance Kern 870, KERN & SOHN GmbH, Balinger, Germany) into a microwave digestion vessel (microwave digestion vessels with pressure release valves, Berghof Products + Instruments GmbH, Eningen, Germany) and 10 mL of 4 M HNO_3 added. Following microwave irradiation (100 ℃, 10 min.) (Berghoff Speedwave MWS-2 microwave system, Berghof Products + Instruments GmbH, Eningen, Germany), samples were allowed to cool and, after filtration of the supernatants, the solutions were made up to 50 mL with DI water, stored at 4 ℃ and analysed with 4 days. The solid residues were kept for extraction of semimobile species. Extractions were performed in duplicate.

4.3.3.4.3 Procedure for removal of semimobile Hg species

In order to remove chloride ions that can promote the release of Hg species from the nonmobile fraction, DI water (5 mL) was added to the residues of the mobile step following which samples were transferred to centrifuge tubes (VWR, Leuven, Germany). The samples were vortexed (1 min.) (Vortex Genie, K-550-GE, Scientific Industries Inc. N.Y., USA) and centrifuged (3200 rpm, 5 min.) (table top model CT6E, himac CT6E, Hitachi koko Co. Ltd., VWR International GmbH, Leuven, Germany), following which the supernatants were removed using a plastic pipette and tested for the presence of chloride ions by the addition of three drops 0.1 M silver nitrate solution (($AgNO_3$), Fluka, Sigma-Aldrich Company, Life Science Chemilab A.E.,

Athens, Greece) (indicated by turbidity in the supernatant). Steps from the addition of DI water to testing supernatant for the presence of chloride were repeated until no chloride was detected and were not needed more than twice in any sample. For removal of the semimobile species 5.33 M HNO_3 (5 mL) was added to the residues. The samples were vortexed (1 min.), heated to 95 ℃ in a water bath (PD Group, Medingen, Dresden, Germany) for 20 min. loosely capped, centrifuged (3200 rpm, 5 min.) and the supernatants collected. The steps from addition of the extraction solution to collection of supernatants were repeated. Following this, 5 mL DI water was added to the residues, and after vortex shaking (1 min.) and centrifuging (3200 rpm, 5 min.) the supernatant was combined with the other supernatants. The combined supernatants contained the semimobile Hg species. These were made up to 50 mL with DI water and stored at 4 ℃ until analysis and always with 4 days.

4.3.3.5 Digestion procedure for determination of total Hg concentration

Digestions were carried out as in Section 3.4.2.7

4.3.3.6 Preparation of fish for analysis

The length and weight of each fish was recorded (Appendix E), following which the fish were scaled, skinned, and muscle tissue removed using a stainless steel knife**.** Only muscle tissue was analysed since this is the portion mainly consumed.

4.3.3.7 Digestion of fish samples

Approximately 0.25 g of fresh muscle tissue from each fish was weighed (4 d.p.) into a microwave digestion vessel. After addition of HNO_3 (10 mL) the samples were digested using the microwave conditions in Table 4.1 (Berghoff, 2006). The efficiency of this digestion could not be verified since no reference material was available within the laboratory, however samples appeared digested and no filtration was necessary. Following digestion samples were made up to 100 mL with DI water. Digestions were carried out in duplicate.

Table 4.1 Microwave programme used for digestion of fish tissue

Step	1	2
T (℃)	160	205
Power (%)	80	90
Time (min.)	15	15

4.3.3.8 Determination of Hg concentration

Determination of Hg concentration was carried out as described in Section 3.4.2.9 after calibration with reagent-matched standards.

4.4 Results and discussion

4.4.1 Total Hg concentration and OM content in Elefsina Bay during summer and winter seasons

Total Hg concentrations in sediment sampled in summer ranged from 1.40 mg/kg to 2.96 mg/kg for the six nearshore locations (Table 3.9) and from 0.312 to 0.821 mg/kg for the three central locations (Figure 4.2). Generally lower concentrations in the centre of the bay were consistent with Hg entering the bay from land. The highest concentrations were observed at locations A5 and A11 probably due to discharge of industrial waste and the use of Hg-containing paints respectively (Section 3.5.2). In sediment sampled in winter, total Hg concentrations for the nearshore locations were between 0.220 and 2.07 mg/kg, and for the central locations ranged from 0.461 to 0.580 mg/kg (Figure 4.3). Highest concentrations were found at A2 (in the vicinity of an oil refinery), and position A5. The variation in Hg concentrations at the different sampling locations was attributed to the different industries operating in the area (Section 3.5.2).

The OM content ranged from 5.80 to 11.8% in the summer and from 2.26 to 10.1% in the winter (Figures 4.2 and 4.3), with highest OM found at position A4 possibly due to its proximity to an oil refinery and lowest OM found at central location K1 in both sampling periods. A similar trend was seen in the OM content between the sampling locations during summer and winter (Figures 4.2 and 4.3).

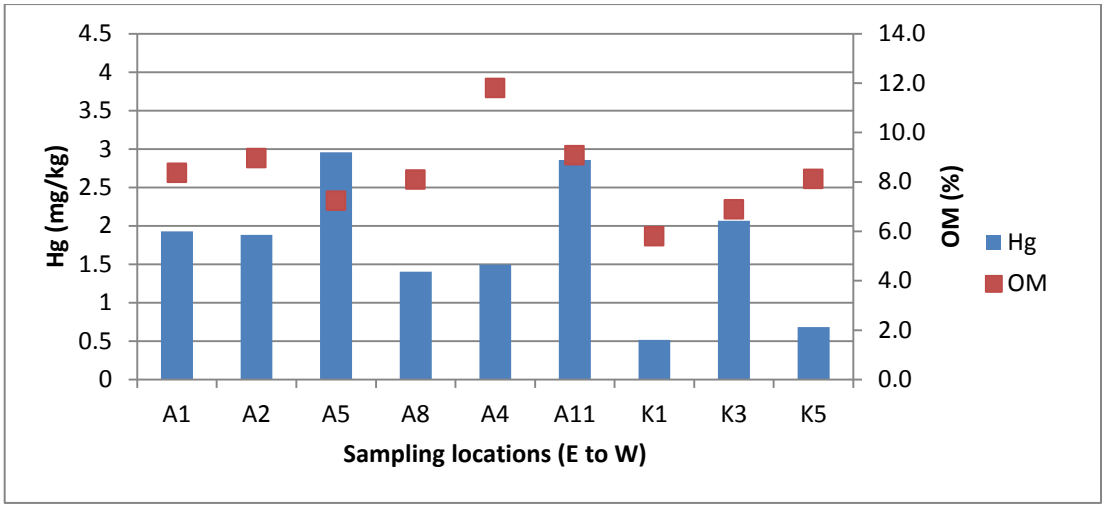


Figure 4.2 Total Hg concentration and organic matter (OM) content at sampling locations in Elefsina Bay, Greece, during summer. Locations with the prefix A are nearshore locations and locations with the prefix K are central locations (mean based on two values, Appendix F).

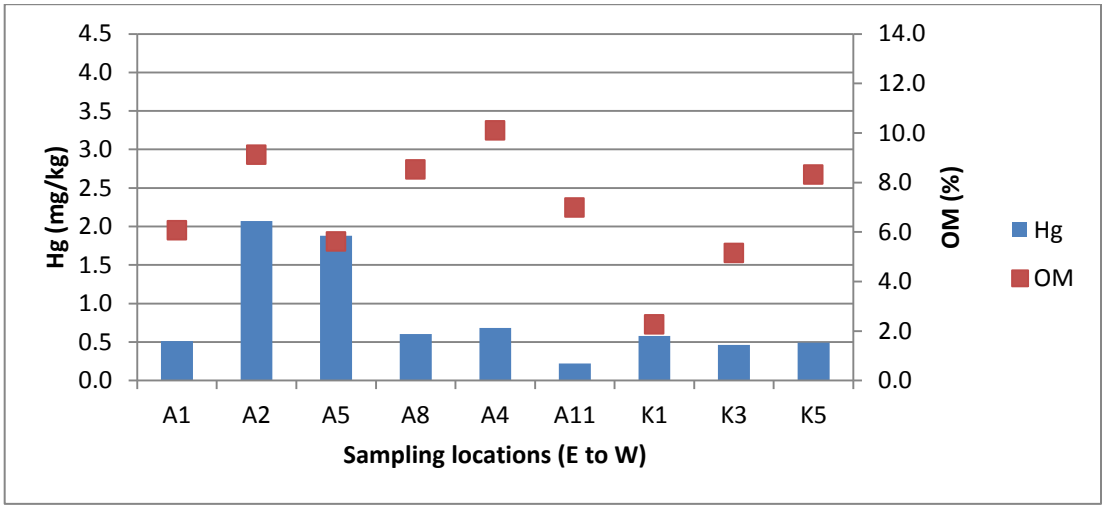


Figure 4.3 Total Hg concentration and organic matter (OM) content at sampling locations in Elefsina Bay, Greece, during winter. Locations with the prefix A are nearshore locations and locations with the prefix K are central locations (mean based on two values, Appendix F).

Despite the general observation of positive association between Hg and OM (Zhang *et al*., 2013), a weak correlation was found between the two parameters in the present study (r^2 = 0.0812). This is in agreement with Kwokal *et al.* (2012) who found a weak correlation between Hg concentration and OM in surface sediment from the Sundarban mangrove wetlands where total Hg concentration ranged from 7.3 to 93.3 *μ*g/kg. In studying the spatial variation and speciation of Hg in Guanabara Bay, Brazil, Covelli *et al.* (2012) observed that for locations where Hg concentration was > 1 mg/kg, concentration was mostly affected by nearby contamination sources, not organic content. Similarly in Elefsina Bay where Hg concentrations were > 1 mg/kg at

all nearshore locations with the exception of A1 (winter), increases in Hg concentration were probably a result of pollution source, rather than a result of increased Hg in association with increasing OM content.

4.4.2 Seasonal variation in total Hg concentration and OM content

A difference in Hg concentration and OM content between summer and winter was not supported by the data (Wilcoxon matched pairs T-test, Tcalc > Tcrit, n = 9, $p < 0.05$).

4.4.3 Mobility of Hg species

The mobility of Hg species at different locations in the bay in summer and winter varied as indicated in Figure 4.4 and Figure 4.5 respectively. A difference in Hg species mobility in summer and winter was not supported by the data (Wilcoxon matched pairs T-test, Tcalc > Tcrit, n = 9, $p < 0.05$). Concentrations of mobile Hg species in the summer ranged from < 0.067 (A11) to 1.66 mg/kg (56%, A5). Concentrations of semimobile species were low, ranging from < 0.067 to 0.107 mg/kg (3.6%, A5). Nonmobile species accounted for a maximum > 95% of total Hg concentration (A11).

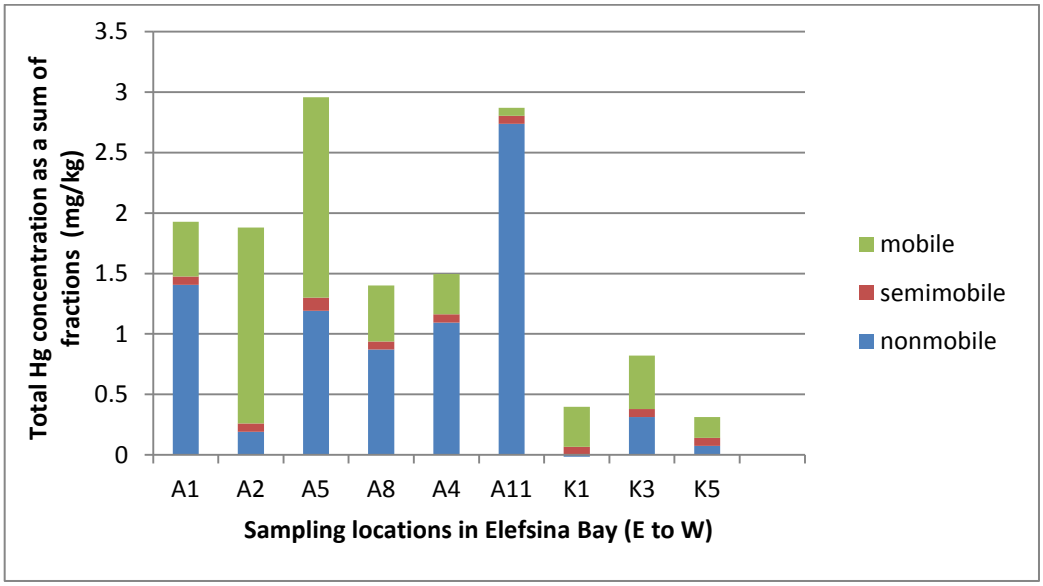


Figure 4.4 Total Hg concentration expressed as a sum of Hg concentration in fractions for sampling locations in Elefsina Bay, Greece, in summer (mean based on two values, Appendix F).

The high concentration of nonmobile species at locations A1, A8, A4 and A11 (73, 62%, 73% and 96% of the total Hg concentration respectively) could account for the

underreporting of Hg concentration obtained when sediment from these locations was screened (Section 3.5.2). In contrast, screening of sediment from positions A2 and A5, where nonmobile species accounted for a smaller percentage of the total (10% and 40% respectively), yielded results in the correct range for total Hg concentration (Section 3.5.2).

Concentrations of mobile species in the winter ranged from < 0.067 (A11) to 1.36 mg/kg (66%, A2) (Figure 4.5). Concentrations of semimobile species were < 0.067 mg/kg at all locations. Nonmobile species accounted for a maximum of 66% (1.24 mg/kg) of the total Hg concentration (A5).

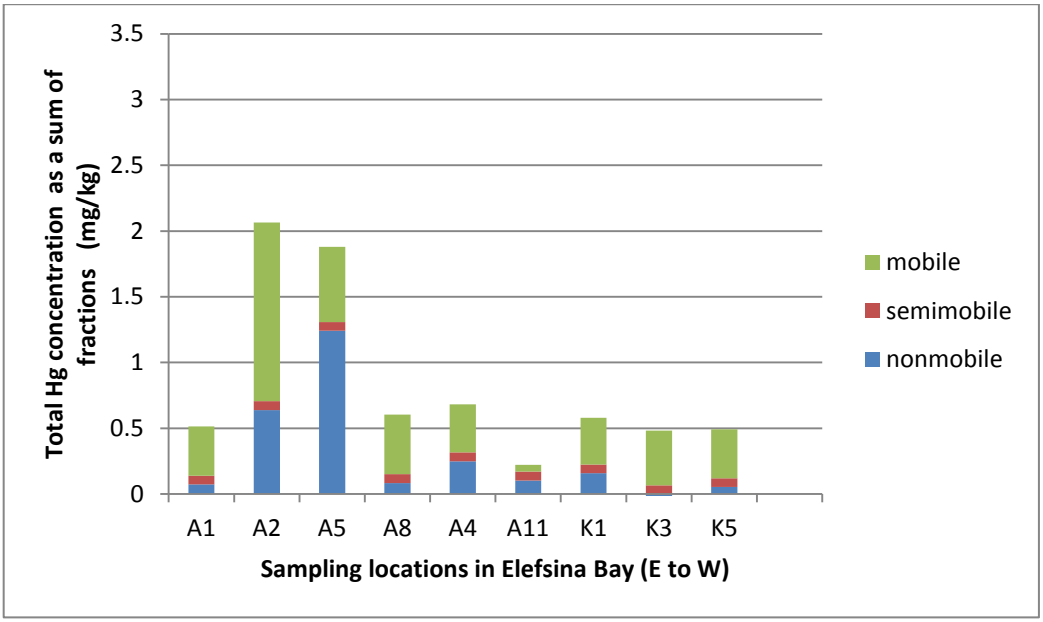


Figure 4.5 Total Hg concentration expressed as a sum of Hg concentration in fractions for sampling locations in Elefsina Bay, Greece, in winter (mean based on two values, Appendix F).

Total Hg concentrations were above the PEL of 0.7 mg/kg at all six nearshore locations in the summer and at locations A2 and A5 in winter. At concentrations above the PEL, aquatic organisms in contact with the sediment are likely to exhibit adverse effects (CCME, 1999), but, where Hg species are predominantly in nonmobile forms, the release of Hg will be limited, and as a consequence, so will methylation.

4.4.4 Hg in flathead mullet

The Hg content of flathead mullet was below the LOD of 0.267 mg/kg wet weight in all cases, below the maximum allowable concentration of 0.5 mg/kg (EC, 2006). In a previous study, mussels in the bay tested for Hg bioaccumulation were not found to contain Hg (Christidis, 2010). Flathead mullet is found around coastal areas in the Mediterranean and tropics and, since it moves within restricted ranges, could be exposed to Hg in contaminated sediment (Verdouw *et al*., 2011). Larger, older fish tend to accumulate higher concentrations of Hg. At maturity, the length this fish type is over 32 cm (Fish base, 2012). Six of the fish collected from Elefsina Bay were longer than this (Appendix E) and can be considered mature, but species of fish and trophic level are also important. Flathead mullet is a low trophic level omnivore, feeding mainly on zooplankton and dead plant matter (Akin and Winemiller, 2006), and generally has low levels of Hg in its flesh (Squadrone *et al*., 2013). Regularly fished from the bay for consumption, this fish type can be assumed to pose no human health risk with regard to Hg content. It is possible that fish at a higher trophic level may have higher Hg content; however it was not possible to obtain a suitable number of such fish.

4.5 Conclusions

Sediment Hg concentrations in Elefsina Bay ranged from 0.220 to 2.96 mg/kg. Lower concentrations were found at central locations in the bay, consistent with Hg entering from land. Higher Hg concentrations were observed at nearshore locations in the vicinity of shipping activities and at the outfall of the Agios Georgios stream, probably as a result of the use of Hg-containing paints in ships, and Hg-containing waste from industries including cement manufacture and metal extraction. The OM content of the sediment ranged from 2.26 to 11.8% with highest OM found at a nearshore location in the vicinity of an oil refinery. There was no significant difference between sediment Hg concentration, mobility and OM content between summer and winter. A poor correlation was found between Hg concentration and OM content, probably since Hg concentration was affected directly by on-going inputs to the bay and was not solely a result of Hg transportation and deposition after association with OM. While the PEL of 0.7 mg/kg (CCME, 1999) was exceeded at approximately half the locations, where species were mainly present in nonmobile forms release of Hg from the sediment would be limited. High concentrations of nonmobile species at four locations (62 to 96%) may account for the underreporting of total Hg concentrations at these locations obtained using screening (Section 3.5.2). The Hg concentration of flathead

mullet collected from the bay was < 0.267 mg/kg wet weight, below the maximum allowable concentration of 0.5 mg/kg (EC, 2006), indicating no adverse health effects to humans from Hg with consumption of this fish type.

CHAPTER 5 TOTAL MERCURY, MERCURY MOBILITY AND SPECIATION IN UNION CANAL SEDIMENT

5.1 Introduction

5.1.1 The Union Canal

The Edinburgh to Glasgow Union Canal, commonly referred to as the Union Canal, was the last large public barge canal to be built in Scotland. The impetus for its construction was the creation of a transport route for coal between Edinburgh in the East and the Lanarkshire coalfields in the West. The canal, which was built between 1818 and 1822 based on a design proposed by Hugh Baird, engineer of the Forth and Clyde Canal, was described as *'commencing from the Lothian Road near the city of Edinburgh to join the Forth and Clyde navigation, near Falkirk in the county of Stirling…….The length of the canal is thirty miles, the depth of water 5 feet, and is on one level from Edinburgh to its western extremity, where it falls 110 feet, in one series of locks, into the Forth and Clyde Canal'* (Priestly, 1831). It was supplied with water from Cobbinshaw Reservoir via the River Calder and a feeder channel. Three aqueducts with cast iron troughs were built to carry it over the River Almond (Almond Aqueduct), River Avon (Avon Aqueduct) and the Water of Leith (Slateford Aqueduct). Scotland's oldest tunnel, a 600 m tunnel through the solid rock of Prospect Hill, was dug for the passage of the canal as an alternative route to Callander Estate, whose owner refused permission for the canal to cross his property. The working lifetime of the canal however was short. The arrival of the railways, and particularly the Edinburgh to Glasgow Railway in 1842, led to the gradual decline of the canal. The locks connecting it to the Forth and Clyde Canal were in-filled in 1933 and it was officially closed by Act of Parliament in 1965, as the Forth and Clyde had been in 1962 (Smith and Lassiere, 2000).

The closure of the canals resulted in a deterioration of the canal environment and led to concerns about safety, particularly from the canalside community. In response to mounting pressure, the governmental navigation authority British Waterways, (BW), responsible for the canal network, launched plans for the reconnection of the Forth and Clyde and Union Canal in 1994, and secured the £78 million required in 1997 from local authorities, the Scottish Enterprise Network, the European Regional Development Fund and the Millennium Commission. Named the 'Millennium Link' project the plan involved reopening the two canals, making them navigable and

joining them through a rotating boat lift, the only one of its kind in the world, to be called the Falkirk Wheel. Work on the project started in 1999 and included the extension of the Union Canal by approximately 2 km westwards from its end at Greenbanks Road (West) to connect to the Forth and Clyde Canal at the Falkirk Wheel. The Royal Opening was in May 2002. Today the Union Canal is 52 km long, has a navigable depth of 1.07 m and a sediment depth of 1 m.

5.1.2 History of the Westquarter Detonator Factory

The Westquarter Detonator Factory was situated in the Reddingmuirhead area of Falkirk. The first industrial activity to take place on the land which was to become the factory was a tile works built in the 1860s situated on the north bank of the Union Canal. The tile works was taken over in the 1870s and redeveloped as the Westquarter Chemical Company. Sulfuric acid manufactured by the company was supplied to the British Dynamite Company in Ardeer on the Clyde Estuary on the West Coast of Scotland, a company set up by Alfred Nobel producing nitroglycerine for dynamite. In 1878, Nobel took over the Westquarter Chemical Company renaming it the Nobel Explosives Company. A small factory was later built to the South of the canal. Named the Westquarter Detonator Factor, or the Nobel Detonator Factory after its founder, it was used for the manufacture of mercury fulminate, $Hg(CNO)_2$, for munitions. The manufacturing process involved the dissolution of Hg in nitric acid to produce mercury nitrate, followed by the addition of ethanol. A grey crystalline powder, $Hg(CNO)_2$ exploded readily once dry on shock or with friction. It was therefore stored moist in canvas bags. Lead (Pb) based detonating agents were also manufactured. The factory was expanded during the 1890s and neighbouring land to the East of the factory, known as the Redding Bing, was used for stockpiling material. The factory was reported to be the largest of its kind in the world covering approximately six acres of land on the north and 21 acres of land on the south of the canal (Figure 5.1). During its operation over 73 million detonators were produced (Smith and Lassiere, 2000).

Figure 5.1 Scanned image of Luftwaffe vertical air photograph of the Westquarter Detonator Factory taken in 1940 (Scotland's Places, 2004).

In the 1950s, the south site was taken over by Imperial Chemical Industries (ICI). Shortly thereafter, production ended and ICI cleared the site by 1966. The factory buildings were decontaminated and demolished before the explosives licence was determined in 1968 by H.M. Explosives Inspectorate, a licence being determined only when all explosive material has been removed.

5.2 Hg contamination in and around the Union Canal

Contamination in the Union Canal was first identified in 1992 following a sediment sampling scheme by BW along their 3000 km canal network to determine levels of contaminants and allow appropriate sediment disposal. Results of this sampling exercise revealed that five locations along a 10 km stretch of the Union Canal between Falkirk and Polmont were contaminated with Hg (Figure 5.2) (BW, 1992).

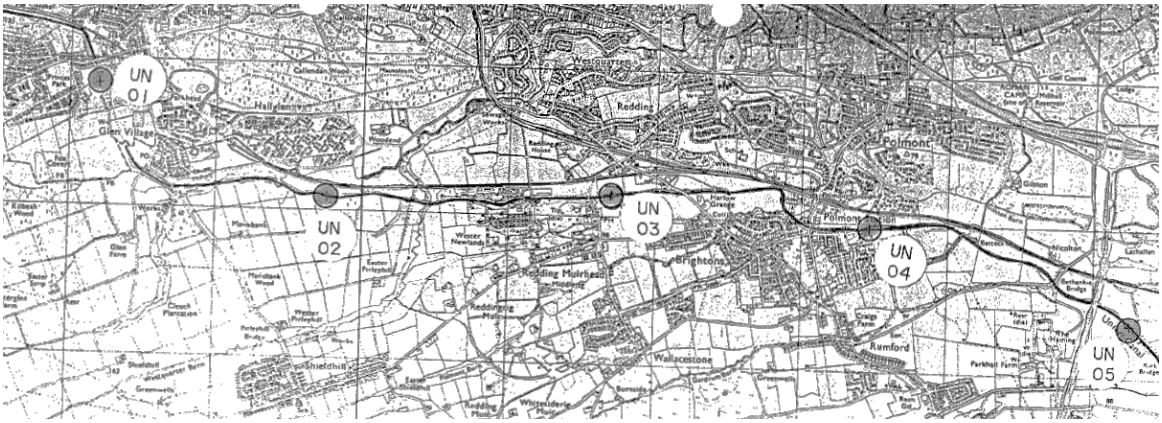


Figure 5.2 Sampling locations between Falkirk and Polmont in the Union Canal, Scotland, U.K. where Hg contamination was detected in the 1992 British Waterways sediment sampling scheme (after BW, 1992).

Concentrations of Hg were UN1: 1304 mg/kg, UN2: 1571 mg/kg, UN3: 1410 mg/kg, UN4: 80 mg/kg and UN5: 13.2 mg/kg, exceeding the trigger value of 20 mg/kg for amenity grass or public open spaces (ICRCL, 1987).These trigger values indicated levels above which the risk of hazard was considered significant (ICRCL, 1987). Located between UN2 and UN3, the probable pollution source was identified as being the Westquarter Detonator Factory. In the production of $Hg(CNO)_2$, acidic vapours containing ethanol and Hg were usually condensed and collected on site. Wastewater however, produced after filtering the reacting mixtures or from washings that also contained Hg waste, was historically disposed of to the land surrounding the production plants (Camps *et al.,* 2009). In the case of the Westquarter Detonator factory, it is possible this waste was discharged directly into the canal since no controls were in place at the time on effluent disposal.

Detailed investigations to determine the extent of Hg contamination were carried out by BW throughout 1992-1993. In 1992 a second canal sampling scheme was implemented: Sixteen canal sediment samples were taken from the canal end at Greenbanks Road, Falkirk, to Polmont at 500 m intervals. Total Hg concentrations were found to range from a minimum of 24.2 mg/kg at Polmont (RP16) to a maximum of 12,100 mg/kg in front of the factory (RP12), an order of magnitude larger than found in the first sampling scheme in 1992 (Figure 5.3) (BW, 1994).

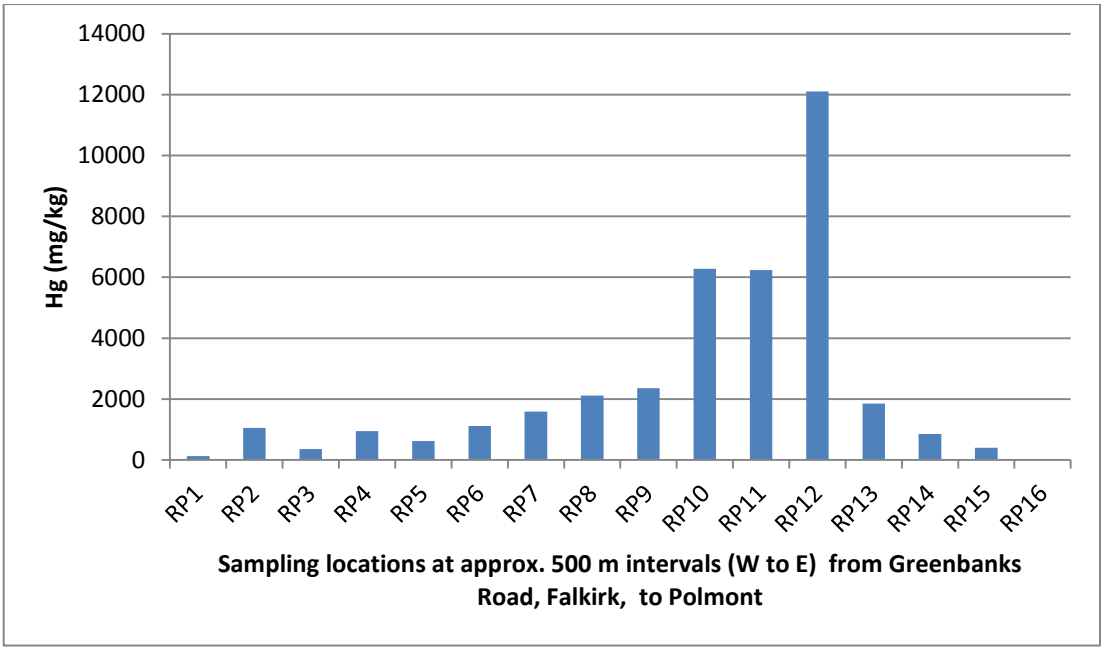


Figure 5.3 Total Hg concentrations found in sediment of the Union Canal, Scotland, U.K., between Falkirk and Polmont, in British Waterways investigations in 1992 (after BW, 1994).

At Greenbanks Road, (RP1), approximately 5 km from the factory, 123 mg/kg of Hg was found, clearly indicating the transfer of contaminated sediment westward, in the same direction as water flow. 'Organic' Hg was determined by extraction with 6 M hydrochloric acid (HCl). Although considered to possibly remove inorganic Hg in addition to organic (BW, 1994) with no correction being made for this, organic Hg concentration was between 0.026 and 2.27 mg/kg and was not proportional to total Hg content. In leaching tests, < 0.0001 mg/L to 0.071 mg/L Hg was leached from the sediments indicating Hg species in the sediment were not soluble in the leachant used. The leachant was not specified (BW, 1999). Since the canal sediments were likely to be fully anaerobic, as indicated by high sulfide content, and Hg species were found to be insoluble, it was assumed that Hg in the sediment would probably be in the form HgS (BW, 1999).

To determine the effect of Hg on the fish community, predatory fish, specifically pike and perch, were caught by electrofishing from the contaminated stretch of water and levels of Hg determined in fish muscle tissue. Concentrations ranged from 0.9 mg/kg to 1.5 mg/kg for perch and from 1.8 mg/kg to 2.5 mg/kg for pike (wet weight) (MAFF, 1993). The EQS (Section 1.6.) for Hg in fish tissue used to assess contamination was 0.3 mg/kg (wet weight) for fish caught locally in areas receiving significant inputs of Hg (EC, 1984) indicating that all fish analysed were contaminated. In assessing the public health hazard posed by the contaminated fish, Environmental Health

(Scotland) Unit suggested a background Hg content in canal fish be determined. In one fish sample composed of seven fish from an uncontaminated section, similar in size to those analysed from the contaminated area, Hg concentration in muscle tissue was found to be 0.07 mg/kg, indicating no background Hg contamination in fish (BW, 1994). Despite the elevated values of Hg, advice from Environmental Health (Scotland) Unit, was that in the absence of human consumption of fish caught in the contaminated area of the canal, there was no threat to public health (Environmental Health (Scotland) Unit, 1993).

Soil sampling was also carried on the factory grounds north and south of the canal. On the north site, total, leachable and organic Hg were determined in 30 soil samples (top 25 cm). Total Hg concentration ranged from 1.55 to 84,000 mg/kg, and exceeded the 20 mg/kg trigger value (ICRCL, 1987) at 20 locations. Organic Hg concentration ranged from 0.0002 mg/kg to 135 mg/kg and leachable Hg concentration ranged from < 0.0001 mg/L to 3.88 mg/L (BW, 1994). On the land south of the canal Hg concentrations were determined from 29 trial pits at different depth ranges, 0 - 50 cm, 50 - 100 cm and 100 - 500 cm in a study carried out by the University of Glasgow (Halliday Fraser Munro, 1998). No Hg was detected at nine of the pits. A maximum of 19,000 mg/kg was found at one pit, Hg concentration was > 1000 mg/kg at two pits and ranged from 1 mg/kg to 360 mg/kg at the remaining pits. Concentration remained constant where more than one depth was sampled indicating Hg was not confined to the top 25 cm of soil, but rather extended down to a depth of between 100 and 500 cm and could have been deeper.

5.3 Remediation of the Union Canal and the Reddingmuirhead site

Despite the Hg contamination identified in the Union Canal, on the land to the north and south of the former detonator factory and at locations on the canal towpath previously used for disposal of canal dredgings, it is possible that remediation work would not have been carried out due to the immense scale of the contamination and the costs that would be incurred. However a requirement of the Millennium Link project was the deepening of the canal and the removal of contaminated sediment. With an estimated cost of £3 million, this would have been a fruitless exercise if contamination from the land re-entered the canal. Therefore in addition to dredging, the remediation of the contaminated land to the north and south was also deemed necessary. Falkirk Council reached an agreement with the Redding Park Development Company (RPDC) whereby the council-owned land would be sold to

the RPDC, who would remediate the site and develop the land. It was proposed that land remediation and dredging be completed together to avoid contaminated material entering the canal after dredging. In December 1999 two specialists were contracted to carry out the canal clean-up: Land and Water Services Limited were contracted for dredging and Shanks Waste Services for disposal. It was estimated that the process, which was scheduled to commence in March 2000 and take 60 weeks, would remove 80,000 tonnes of contaminated sediment from the canal (Smith and Lassiere, 2000). The high contamination levels necessitated special disposal. A processing plant was established opposite Her Majesty's Young Offenders Institute (HMYOI), Polmont, to the East of the former factory where the dredged, wet sediment was solidified by addition of lime, after which it was transported to a monocell landfill at Avondale, Falkirk, and disposed of as stabilised non-reactive hazardous waste. Removal of contaminated sediment initially involved using an excavator on a floating platform to load the sediment onto floating pans that were then pushed by tugs to the plant (Figure 5.4). Bridges and narrow stretches of the canal where there was little room to maneuver caused difficulty, as did the 600 m tunnel at Glen Village. Pushing the pans to the plant became increasing difficult as distance increased. Finally this procedure was abandoned in favour of drying the canal and digging out the sediment (Coyle, 2000). Dredging was completed in 2001.

Figure 5.4 A tug pushing pans filled with dredged sediment along the Union Canal, Scotland, U.K. to the processing plant located in Polmont (after MMLink Resources, 2000).

Following dredging Hg levels in sediment were determined three times in 2002, twice in 2003 and once in 2007 by BW at the Redding location only (Figure 5.5). This location corresponds approximately to RP12 in the 1992 sampling (Figure 5.3). The first analysis in 2002 indicated that post dredging Hg concentration was 2640 mg/kg, which dropped to 390 mg/kg in 2007. Based on these results it can be said that

contamination was reduced at Redding after dredging, but not cleared. Values still exceeded the 20 mg/kg trigger value (ICRCL, 1987). How appropriate the use of this guideline, which referred to soil, was in the assessment of sediment contamination was questionable, since, for example, for freshwater sediment the Canadian SQG for Hg was (and still is) 0.17 mg/kg (CCME, 1999) (Section 1.6) and based on the Dutch sediment pollution classification system, sediment containing > 10 mg/kg Hg is considered very polluted sediment (Kelderman *et al.,* 2000). Additionally the drop in Hg concentration observed post-dredging between 2002 and 2007 at the Redding location (Figure 5.5) could have been suggestive that Hg was being transported west, although no other sediment sampling was carried out to assess this.

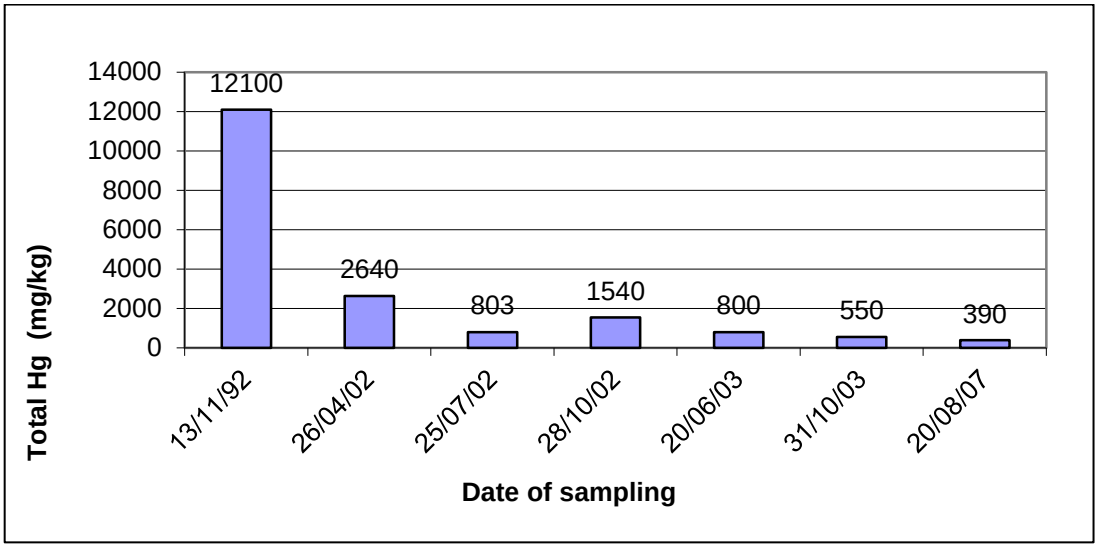


Figure 5.5 Total Hg concentration in sediment from the Union Canal at Redding pre and post canal dredging that was carried out in 2000 (after Scottish Canals, 2013).

Despite initial aims that canal dredging and land remediation be carried out within the same time frame, remediation of the contaminated land had not commenced by completion of canal dredging. Environmental consultants Wren and Bell were contracted by RPDC to carry out a Site Investigation and Assessment (1999) and a Pre-remediation Environmental and Mineral Investigation (2000/1) that included soil sampling and chemical analyses on both sites before remediation work began. The north site was to remain as a woodland/ public access area and the south site was to be redeveloped into housing, retail and leisure area including a pub/restaurant and a heritage centre. As expected, chemical analyses indicated Hg and Pb contamination,

Hg concentrations reaching a maximum of 129,000 mg/kg (Wren and Bell, 2001). Areas of higher contamination were linked to locations of detonator manufacture and storage, with no other obvious pattern to Hg contamination (Wren and Bell, 2001). Civil engineering company I&H Brown began the 67 week, £5.3 million, remediation work in March 2005 (Angus, B., 2014; I&Hbrown, 2014). In June 2006 earth works and the building of infrastructure for housing development commenced that lasted 54 weeks and cost £3.1 million. Remediation had two main aspects. The first was to remove any pathway for human contact with Hg and Pb at levels considered harmful and the second was to prevent contaminated run-off from the site entering the canal. Remediation work included the removal of trees and vegetation followed by the replacement of contaminated top soil and lower level weathered clay. Structural platforms on which housing would be built were then constructed using suitable unweathered boulder clay on top of which a 20 cm protection layer of weathered clay was placed (Figure 5.6).

Figure 5.6 Reddingmuirhead site pre (upper) and post (lower) remediation (after I&HBrown, 2014).

Contaminated soil was either disposed of at Avondale Landfill, Polmont, as stabilised non-reactive hazardous soil waste or at Port Clarence Landfill, Teeside, in the case of hazardous soil waste (Wren and Bell, 2006). Top soil, to be added by the house builder after remediation, was recommended to be at least 30 cm deep to allow rooting (Wren and Bell, 2006). Following remediation, soil samples from residential gardens (garden plots), open space areas (that included the canal bank, access roads and slopes between housing platforms), the central public open space area and the commercial area were validated to ensure soil guideline values (SGVs), which replaced ICRCL trigger values in 2002 (DEFRA, 2002), were met. Based on SGVs, (DEFRA-EA, 2002b), Hg values for residential garden soil with plant uptake

should not exceed 8 mg/kg. The SGV of 15 mg/kg for gardens without plant uptake was used for open space areas and the SGV of 480 mg/kg for commercial/industrial land was applied to soil in the area to be used for a pub/restaurant and hertitage complex. For the area to be developed as public open space where no SGV was specified Risk Based Corrective Action (RBCA) methodology was used to derive a value of 88 mg/kg (Wren and Bell, 2006). Similarly SGVs were used to assess remediation of soil with respect to Pb and copper (Cu). Values referred to surface soil concentrations (top 50 cm) since deeper soil was not considered a significant exposure pathway. For verification of successful remediation, soil was sampled at two depths, at 10 cm and at 35 cm, thus assessing both the protection material and the unweathered boulder clay. If the SGVs were exceeded in garden plots, the soil was removed and replaced from the plot in question and from plots on either side of it, after which it was resampled and retested. Soil removal and replacement had to be repeated more than once in some instances until levels were acceptable. For the open spaces, public open space and the commercial areas an identical procedure of soil removal, replacement and retesting was carried out as many times as necessary for SGVs or RBCA value to be met. In order to prevent contaminated run-off entering the canal, three sets of temporary lagoons were constructed after consultation with BW, the Scottish Environment Protection Agency (SEPA) and Historic Scotland to retain run-off and allow suspended solids to be reduced before water discharge into the canal (Figure 5.6). Levels of Hg, Pb and Cu were regularly monitored to ensure compliance with SEPA requirements (1, 50 and 20 *μ*g/L respectively) before discharge. Validation reports for the north and south site were provided by the Environmental Consultants (on behalf of the RPDC) in 2006.

The Falkirk Council Contaminated Land Team in relation to the Reddingmuirhead Development and the validation reports stated '*This information was reviewed by SEPA, Falkirk Council and Specialist Environmental Consultants URS Corporation (who it is understood were working on behalf of the Developer's Banking Investors). The information submitted for the site would appear to have met with the current legislative requirements and statutory guidance……the Contaminated Land condition placed upon the Planning Application for the site has not yet been purified and the temporary lagoons have not yet been decommissioned. The Falkirk Council Contaminated Land Team are awaiting a Validation Report for the decommissioning of the temporary* lagoons' (Laird, 2014). Today the site therefore is still classified as a contaminated site until validation reports are provided by RPDC, that meet legislative requirements. Property developers Kier Homes started building in 2007 which is

estimated to finish in 2017. Over half of the approximately 300 houses have been completed and sold (Kier Homes, 2014).

5.4 Aim

Despite dredging and remediation work carried out between 2000 and 2006, screening of sediment samples in 2012 from four locations in the canal indicated a high level of Hg contamination which was confirmed by quantitative analysis (Section 3.5.2). To determine the overall efficiency of the decontamination with respect to Hg along the Union Canal between Falkirk and Polmont where dredging took place, further examination of sediments was undertaken. More detailed sampling was carried out and sediment samples were screened for total Hg concentration using pXRF. Quantitative determination of total Hg concentration was carried out by CVAAS and by TDAAS. Mobility of the Hg species was examined using sequential extraction and TDAAS was used for species identification.

5.5 Materials and methods

5.5.1 General procedures

General procedures are described in Sections 3.4.2.1.

5.5.2 Solution preparation

Solution preparation is described in Section 4.3.3.2.

5.5.3 Sediment sampling and preparation

Sampling and sample preparation are described in Sections 3.4.1.5 and 3.4.2.4.

5.5.4 Determination of moisture content and OM

Moisture content and OM were determined as described in Sections 3.4.2.5 and 3.4.2.6. Determinations were performed in triplicate.

5.5.5 Procedures used for determination of total Hg concentration

5.5.5.1 Determination of total Hg concentration by pXRF

Total Hg concentration was determined using a Niton, XL3t portable XRF calibrated against a soil matrix (Aspray, 2013). Determinations were performed in triplicate.

5.5.5.2 Determination of total Hg concentration by TDAAS

Determination by TDAAS was carried out using a direct mercury analyser (LECO, model AMA-254) with gold amalgamation and AAS detection system using a silicon diode detector, at 253.6 nm (Reis, 2013). Determinations were performed in triplicate.

5.5.5.3 Determination of total Hg concentration by CVAAS

Determination by CVAAS was carried out as descirbed in Sections 3.4.2.7-9. Determinations were performed in triplicate.

5.5.6 Mobility of Hg species in Union Canal sediments

5.5.6.1 Sequential extraction of Hg species in Union Canal sediments

The mobility of Hg species was determined by sequential extraction as described in Sections 4.3.3.4.1-3. Determinations were performed in triplicate.

5.5.6.2 TD technique for Hg speciation

Species identification using TDAAS was carried out using a direct mercury analyser with gold amalgamation (LECO, model AMA-254) (Reis, 2013). Thermodesorption curves for samples were compared to desorption curves of $HgCl_2$, Hg associated with iron(III)oxide, (HgFe), a Hg-humic acid complex, (HgHA), and HgS.

5.5.7 Sampling locations

Eight sampling locations were selected from Tamfourhill (1) in the west to Polmont in the east (10) (Table 5.1) (Figure 5.7). Location 1 was part of the canal extension added in 2000-2001 as part of the Millennium Link project. At this location, sediment had not previously been tested for Hg.

Table 5.1 Location and coordinate of sediment sampling points in the Union Canal, Scotland, U.K. (W to E)

Location	Location Number	Grid Reference	X	Y	Latitude	Longitude
Tamfourhill	1	NS 85948 79597	285948	679597	55.995723	-3.8301385
Summerford	2	NS 86681 79415	286681	679415	55.994265	-3.818323
W exit of tunnel	4	NS 88107 79075	288107	679075	55.991541	-3.7953204
Glen Village	5	NS 88581 78186	288581	678186	55.983667	-3.7873703
Hall Glen	6	NS 89402 78170	289402	678170	55.983715	-3.7742007
1 km west of HMYOI	7	NS 91148 78099	291148	678099	55.983475	-3.7461984
HMYOI	8	NS 91846 78154	291846	678154	55.984135	-3.7350404
Polmont	10	NS 93076 77952	293076	677952	55.982592	-3.7152578

Figure 5.7 Map showing sediment sampling locations along the Union Canal, Scotland, U.K., and the location of the former Nobel Westquarter detonator factory (DF) (location 7).

5.6 Results and discussion

5.6.1 Total Hg concentration by CVAAS, TDAAS and pXRF

Total Hg concentrations 12 years after dredging ranged from a minimum of 22.0 ± 0.61 mg/kg at location 1, Tamourhill, to a maximum of 565 ± 48 mg/kg at location 5, Glen Village, as determined by CVAAS (Figure 5.8) (Appendix G) (Equivalent locations sampled in 1992 by BW are given in brackets).

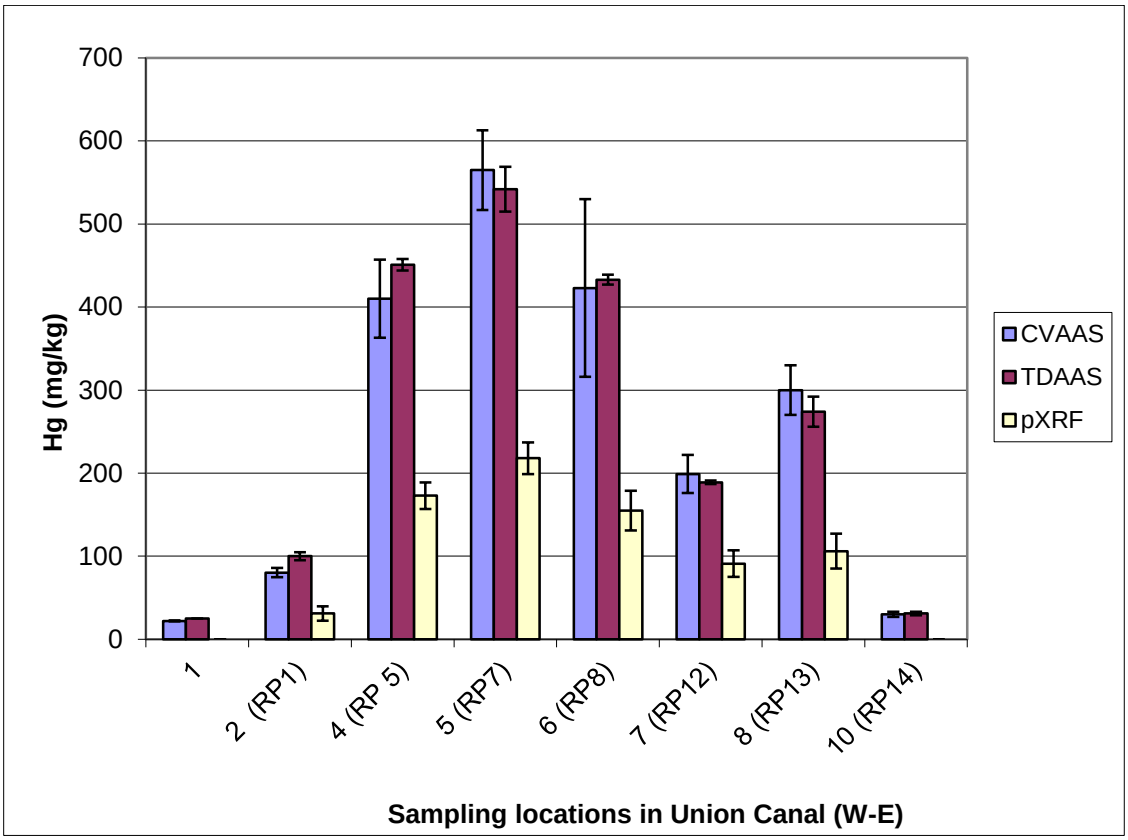


Figure 5.8 Total Hg concentrations as determined by CVAAS, TDAAS and pXRF in sediments of the Union Canal, Scotland, U.K. (mean ± SD, n=3).

No statistical difference was found between the results obtained by the CVAAS and TDAAS methods (Wilcoxon matched pairs T-test, Tcalc >Tcrit, n=8, $p < 0.05$). Total Hg concentration as determined by pXRF was statistically different to both CVAAS and TDAAS results (Wilcoxon matched pairs T-test, Tcalc. = Tcrit n = 6, $p < 0.05$), and ranged from 36 to 43% of CVAAS results at six locations where comparisons could be made (Appendix G). Locations 1 (22.0 ± 0.6 mg/kg by CVAAS) and 10 (30.3 ± 3.1mg/kg by CVAAS) were below the LOD of the pXRF (10 mg/kg for Hg) (Hurley, 2013). The correlation between results obtained by CVAAS and pXRF was strong (r^2 = 0.985) (Figure 5.9), indicating that pXRF can be useful in semi-quanititative

screening and with a suitable correction factor could perhaps be used for quantification.

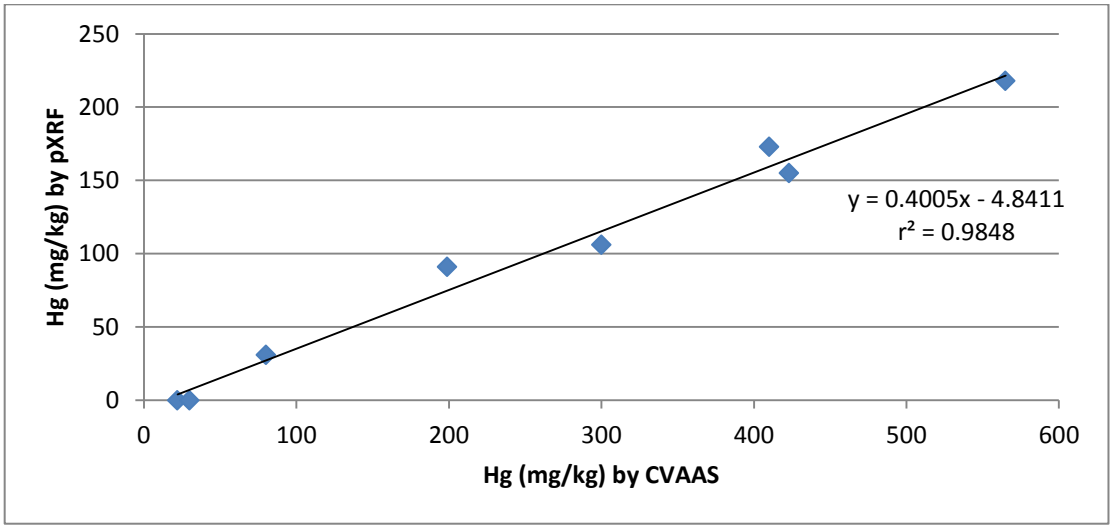


Figure 5.9 Correlation between CVVAS and pXRF total Hg determinations in sediments of the Union Canal, Scotland, U.K..

Concentrations of Hg in the current study were statistically different from concentrations in 1992 (Wilcoxon matched pairs T-test, Tcalc = Tcrit, n = 7, $p < 0.05$) indicating the Hg concentrations have decreased in the contaminated stretch of the canal. Dredging the canal therefore reduced Hg levels markedly; however, Hg contamination was still present along the canal. Location 7 in this study corresponded approximately to SP12 in the BW study (BW, 1994) (Section 5.2, Figure 5.3) and was the position in front of the former factory. Here a marked decrease from 12,100 mg/kg in 1992 to 199 ± 23 mg/kg in the present study was observed. In 1992 this location had the highest Hg concentration (BW, 1994). However in the present study, levels of Hg to both west and east were found to be higher than at this location, with a maximum of 565 ± 48 mg/kg determined at location 5 *ca.* 4 km west of this location in Glen Village. The BW sediment analysis carried out at RP12 (location 7) post dredging demonstrated that the canal was not successfully decontaminated at this location (Figure 5.5). Whether the remainder of the stretch was successfully decontaminated and was then recontaminated from RP12 cannot be said with certainty, although it seems unlikely. Possibly, the tunnel at Glen Village may have limited sediment removal at location 5, resulting in the higher Hg concentration determined at this location in the present study. Not only was the long-term decontamination of the canal not entirely achieved, but the new section of the canal at Tamfourhill has also become contaminated (location 1, 22.0 mg/kg) indicating the movement of contamination westward. With concentrations of Hg > 10

mg/kg, all locations were classified as having heavily polluted sediment (Kelderman *et al.*, 2000). Also interesting is the spread of contamination via the Falkirk Wheel as indicated by analysis of Forth and Clyde Canal sediment immediately upstream and downstream of the Falkirk Wheel junction where Hg concentrations of 7.54 mg/kg and 10.2 mg/kg respectively were found (Cortis, R., 2013). Soil run-off may have increased contamination in the canal. The canal tow path was found to be contaminated in 1992 investigations (BW, 1994), however, land remediation covered only the factory grounds. The towpath could therefore be a source for re-introduction of Hg to the canal. Additionally, land remediation commenced four years after dredging, during which contaminated soil could have entered the canal.

The concentration of Hg at all locations sampled was well above the PEL of 0.486 mg/kg, the level above which the incidence of adverse biological effects in aquatic life is frequent (CCME, 1999) (Section 1.6). Thus the Hg concentrations still present are potentially significant in relation to the aquatic community. The possibility of human exposure should also be considered. Pike and perch, found to be contaminated in 1993 investigations carried out by BW (Section 5.2), are not commonly eaten by the Scottish population, however, a Scottish Government report (Scottish Government, 2010) recommended their inclusion in investigations into the levels of contaminants in Scottish freshwater fish since they are commonly eaten by recent immigrant communities particularly from Poland. The consumption of fish caught in the canal is not permitted but is known to take place among immigrant communities (Lamont, 2013). As sediment is contaminated the concentration of Hg in fish should be determined since there is potential for human exposure through fish consumption: In sediments from Florida estuaries, a positive relationship was found ($r = 0.52$, $p < 0.05$) between total Hg concentration in sediment and Hg concentration in a variety of fish including catfish and perch, where Hg concentrations ranged from 1 to 219 *μ*g/kg and Hg in fish muscle ranged from 0.03 to 2.22 ng/kg wet weight (Kannan *et al.,* 1998).

5.6.2 Total Hg concentration and OM content

A weak correlation was found between Hg concentration and OM content, which ranged from 6.12 ± 1.4 to 18.1 ± 1.1% (r^2 = 0.376; Figure 5.10, 5.11).

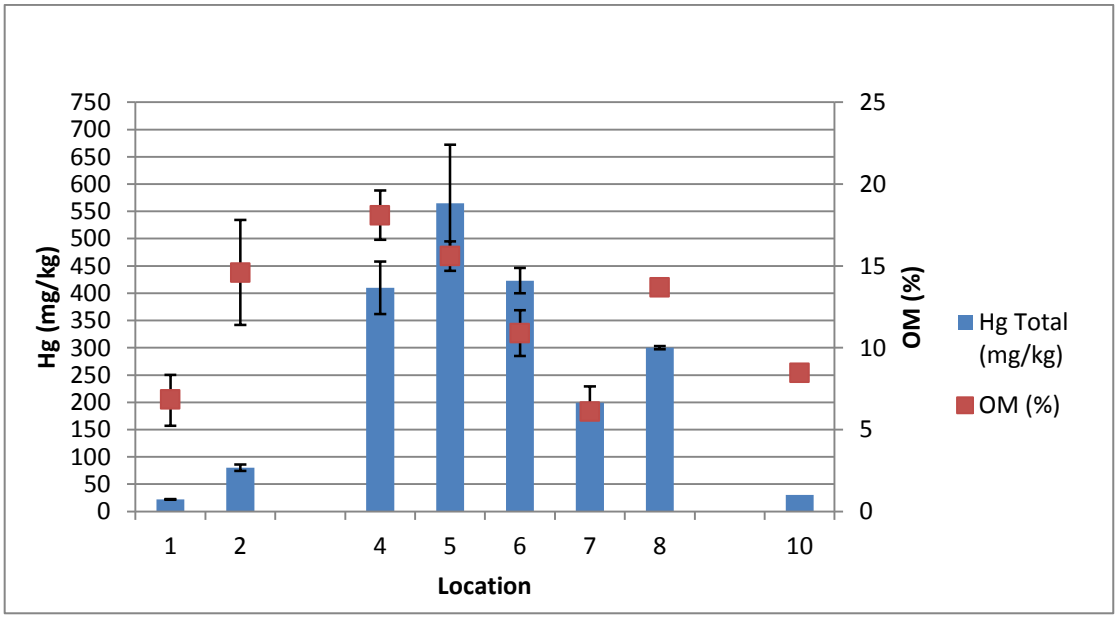


Figure 5.10 Total Hg concentration and OM content in sediments of the Union Canal, Scotland, U.K. (mean ± SD, n=3).

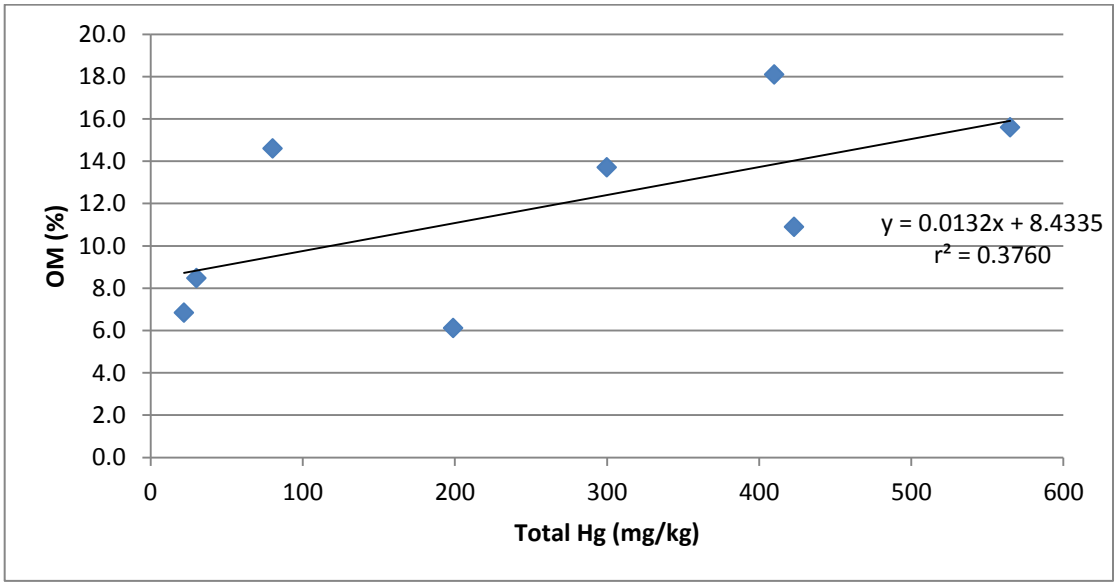


Figure 5.11 Relationship between Hg concentration and OM content in sediments of the Union Canal, Scotland, U.K..

Many authors have observed a positive correlation between concentrations of Hg and OM. For example Zhang *et al.* (2013) found a strong positive correlation ($r = 0.912$, $p < 0.01$) in sediments of the Fujian and eastern Guangdong Provinces where total Hg concentration ranged from 1.1 to 87.4 *μ*g/kg. The OM levels were not

specified in the study. In sediment of the Scheldt Estuary, Belgium, where total Hg concentrations ranged from 144 to 1192 *μ*g/kg, an increase in total Hg concentration was observed with increasing organic matter (1-11%), ($r = 0.84$, $p < 0.01$) (Muhaya *et al*., 1997). Likewise in sediment of the South Florida estuaries where total Hg concentration ranged from 1 to 219 *μ*g/kg and OM content was < 8%, a positive relationship was observed between the two parameters ($r = 0.58$, $p < 0.05$) (Kannan *et al*., 1998). The concentrations of Hg in those cases were up to five orders of magnitude lower than those of the Union Canal, where the higher Hg concentrations may be significant in Hg associations.

5.6.3 Mobility of Hg species by sequential extraction

Of the total Hg content, between 75.9% (429 mg/kg, location 5) and 94.0% (187 mg/kg, location 7) was extracted in the first step (4 M HNO_3) which represented inorganic Hg species such as water soluble $HgCl_2$, weak acid soluble species such as HgO, other weakly bound Hg^{II} compounds such as a portion of Hg bound to OM, and extractable organic Hg species such as CH_3HgCl and CH_3CH_2HgCl (Section 1.7.3.2.2) (Figure 5.12). Between 1.08% (3.25 mg/kg, location 8) and 12.0%, (2.63 mg/kg, location 1) was in a semimobile form (such as strongly complexed Hg^{II} including HgOM, and Hg^0) and between 1.22% (0.268 mg/kg, location 1) and 22.9% (129 mg/kg, location 5) was in a nonmobile, nonbioavailable form (such as HgS) (Figure 5.12) (Appendix H).

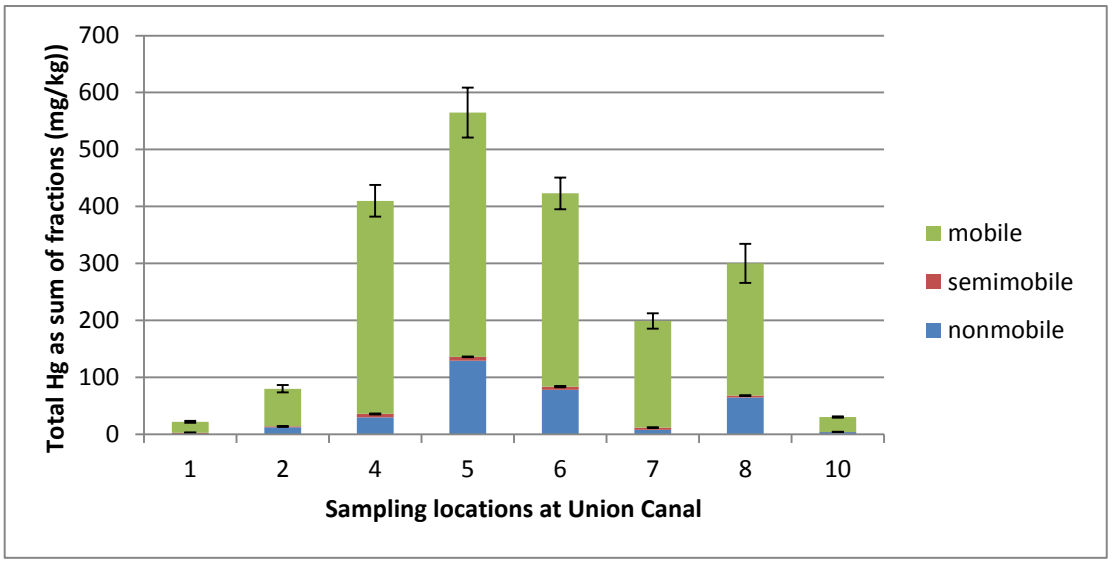


Figure 5.12 Total Hg concentration expressed as a sum of Hg in fractions in sediments of the Union Canal, Scotland, U.K. (mean ± SD, n=3).

5.6.4 Hg species by TDAAS

Thermodesorption curves were obtained for the standard materials $HgCl_2$, HgFe, HgHA and HgS (Figure 5.13). The curves for HgFe and $HgCl_2$ showed peaks at 75 - 285 ℃ (maximum at 170 ℃) and 95 - 285 ℃ (maximum at 170 ℃) respectively. Temperatures up to 250 ℃ are generally assigned to the release of Hg species weakly associated with the mineral matrix (Biester *et al.*, 1999). Desorption of HgS occurred over the temperature interval 240 - 370 ℃, (maximum at 306 ℃). The curve obtained for HgHA spanned the temperature range 125 - 655 ℃ (maximum at 240 ℃). The curves for standards $HgCl_2$ and HgFe completely overlapped preventing identification of these species individually from thermodesorption curves alone. While HgS was well separated from $HgCl_2$ and HgFe, there was overlap with desorption from HgHA.

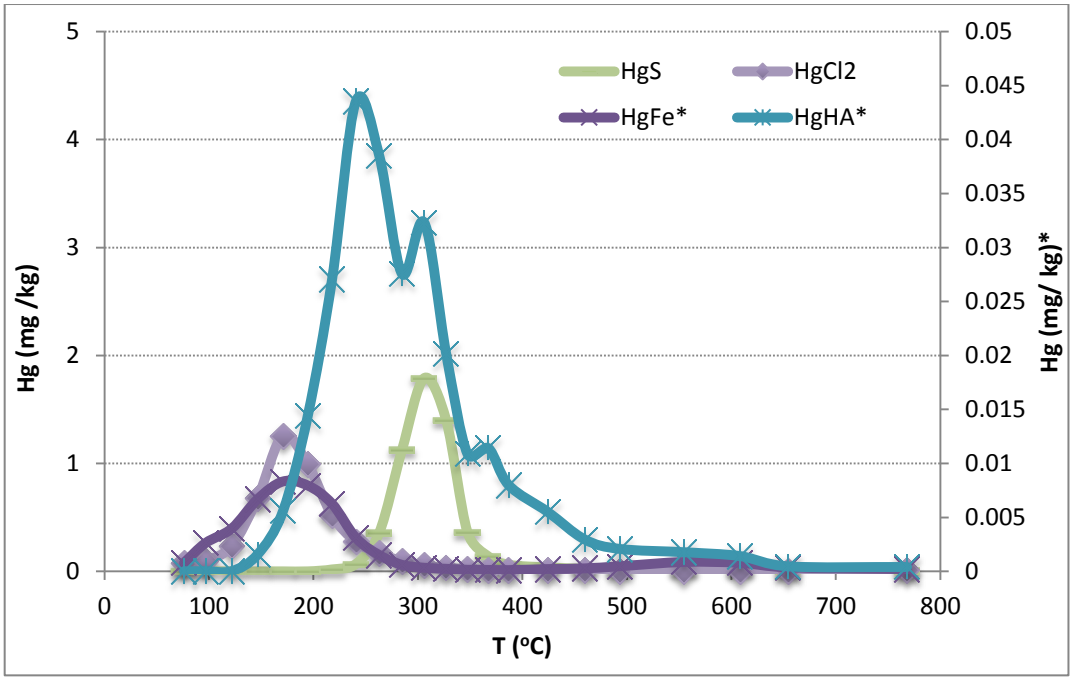


Figure 5.13 Thermal desorption curves for standard Hg materials (Reis, 2013).

Results for samples as obtained (Reis, 2013) were recalculated to dry weight content to allow comparisons to be made with the findings of the sequential extraction method (Appendix I). Desorption of > 67% of Hg species in all samples occurred in the temperature range 100 - 250 ℃ consistent with $HgCl_2$ and/or HgFe. Specifically, samples 2, 5, 6 and 7 showed one peak consistent with $HgCl_2$ and/or HgFe corresponding to ≥ 94% of the total Hg present (Figure 5.14).

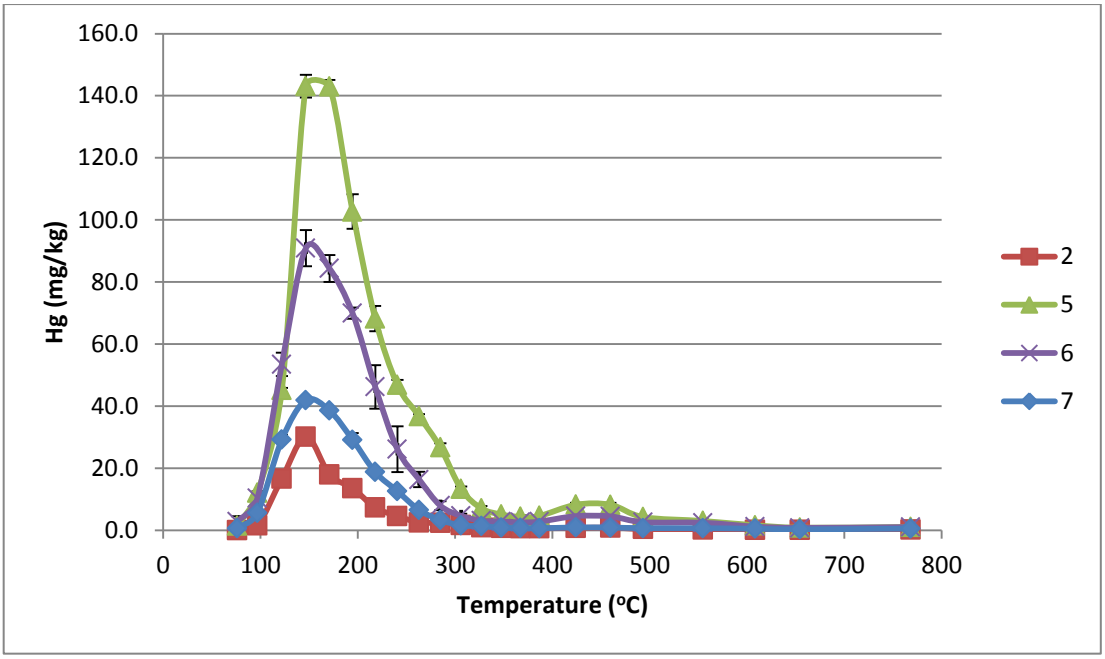


Figure 5.14 Averaged thermodesorption curves for Hg in sediment samples 2, 5, 6 and 7 from the Union Canal, Scotland, U.K. (mean ± SD, n=3).

In samples 1, 4, 8 and 10, 73% (19.7 mg/kg), 68% (297 mg/kg), 90% (248 mg/kg) and 81% (28.6 mg/kg) of the total Hg concentration was also desorbed as $HgCl_2$ and/or HgFe (Figures 5.15; 5.16). A second desorption peak was observed in samples 1, 4 and 10 consistent with HgHA or HgS representing 23% (6.18 mg/kg), 31% (136 mg/kg) and 14% (4.95 mg/kg) of the total respectively (Figures 5.15; 5.16).

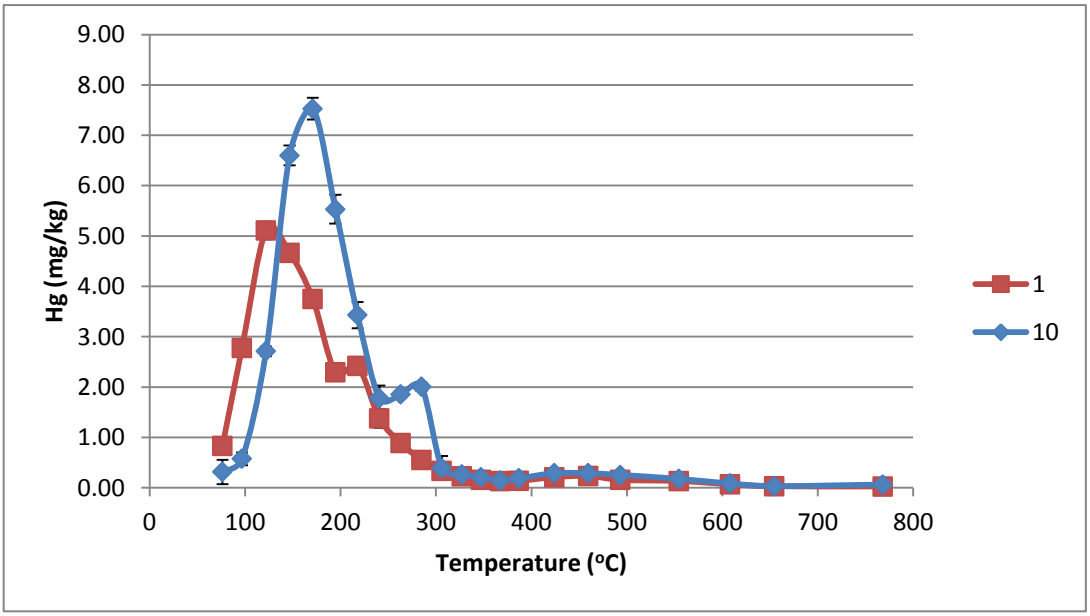


Figure 5.15 Averaged thermodesorption curves for Hg in sediment samples 1 and 10 from the Union Canal, Scotland, U.K. (mean ± SD, n=3).

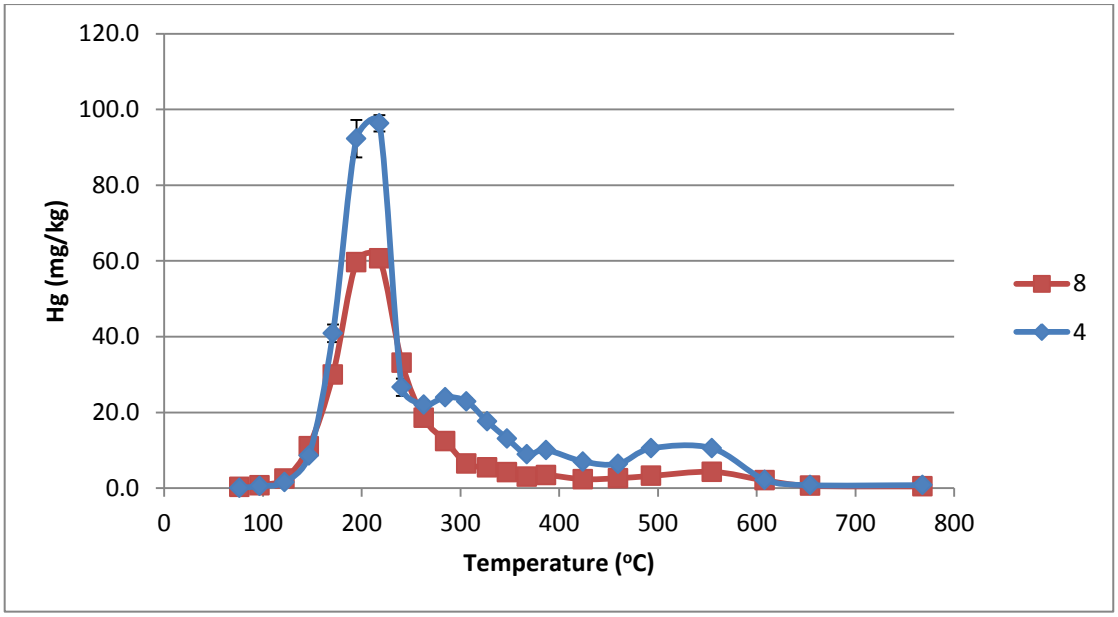


Figure 5.16 Averaged thermodesorption curves for Union Canal sediment samples 4 and 8 indicating desorption at 550 ℃ (mean ± SD, n = 3).

A peak observed at maximum T = 550 ℃ in samples 4 and 8 (Figure 5.16) could be assigned to HgO using the literature value for thermodesorption of this species found in Biester *et al.* (1999). Coufalik *et al*. (2014) however found the desorption of HgO to occur over the temperature range 110 - 400 °C, with a maximum at 240 ℃.

5.6.5 Identity of Hg species

While the exact nature of the Hg species in the canal sediment was not determined in this study, information obtained from both sequential extraction and TD allowed conclusions to be drawn about the nature of Hg species. Both the results from sequential extraction and TD indicated that the contribution of nonmobile species to the total Hg concentration was little: results from sequential extraction indicated that nonmobile Hg content ranged from 1.2% (0.27 mg/kg) to 23% (129 mg/kg) and results from TD indicated the possibility of HgS in only three samples, corresponding to between 14% (4.95 mg/kg) and 31% (136 mg/kg) of the total Hg concentration.

In contrast, the BW Union Canal Contamination Review (BW, 1999) concluded that the main form of Hg in the sediment was probably HgS (Section 5.2). The conclusion of BW review (BW, 1999) was based on the low Hg concentration observed in solution following leaching tests, indicating Hg species were not soluble in the leachant used, and the high sulfide content of the sediment. If however the leaching test was carried out with DI water as leachant, only water soluble species would be released. This does not imply the remaining species were HgS. Also, the high sulfide

concentrations found in the canal sediment may in fact encourage the formation of more soluble polysulfides (Paquette and Helz, 1997).

Both sequential extraction and TD results indicated that Hg species were mobile. Sequential extraction indicated that > 75% of Hg was extracted by 4 M HNO_3 in forms considered mobile. While this could have included weak HgOM complexes, TD indicated that > 67% of the Hg species in all samples were desorbed below 250 ℃, temperatures which are consistent with desorption from the mineral matrix and not from association with OM from which desoprtion occurs at higher temperature (Biester *et al*., 1999). The absence of significant HgS and strong HgOM associations and the indication that Hg species were mobile (Carter, A. and Briscoe, M., 2013; Han *et al*., 2003) could explain the movement of Hg in the canal.

Whether the main Hg species was $HgCl_2$, a possibility suggested by both methods, Hg associated with Fe, or another extractable, mineral-matrix bound Hg species which desorbed at the same temperature could not be concluded. Due to its high solubility and stability in solution, (Kim *et al.*, 2004b), $HgCl_2$ is not usually adsorbed to sediment except in areas with high $HgCl_2$ contamination for example as a results of its use as a preservative in wood treatment (Bollen *et al*., 2008). The presence of HgO as the main Hg species, a possibility suggested by the extraction, was rejected on the basis of TD. Additionally, HgO does not occur naturally and is generally associated with mine tailings as a result of the roasting process (Biester *et al.*, 1999).

Association of Hg species directly with the mineral matrix without OM contribution is unlikely. Although EXAFS laboratory studies have confirmed adsoption of Hg to Fe and Al - (hydr)oxide surfaces in the absence of OM (Kim *et al*., 2004a, 2004b) this is not to be expected in the presence of OM (Section 1.3.4.2). However indirect adsorption to the mineral matrix may occur through an ‘organic bridge’ in which the carboxyl and/or amino group of the organic molecule (L), interact directly with the mineral surface group (M) and the thiol group of the organic molecule interacts with Hg^{II} to form a ternary surface complex, >M–L–Hg^{II}, as shown in an adsorption - desoprtion study where cysteine was used to represent OM (Senevirathna *et al*., 2011). The desorption of Hg in this case was found to be inhibited over the pH range 3 - 7, and the temperature range 15 - 35 ℃ compared to the desorption of direct mineral matrix-bound Hg. There are no TD studies that consider Hg desorption temperature for > M-L-Hg^{II} association, probably due to lack of reference materials, although the results are likely to be dependent on the nature of the OM.

In the absence of significant HgS and HgOM association, the reaction of Hg with iron(II) sulfides could also be considered. In adsorption - desorption experiments, Hg has been shown to from surface bonds with both pyrite (FeS_2) (Behra *et al.,* 2001) and mackinawite (FeS_m) (Jeong *et al*., 2007) both of which are abundant in sediment (Rickard and Luther, 2007). Again however the role of OM must be considered since in the one study which considered Hg-FeS_m-OM interactions, Hg was found to dissolve FeS_m in the presence of OM, with the formation of HgS and HgOM complexes, albeit at very high %OM (> 95%) (Skyllberg and Drott, 2010).

5.7 Conclusions

Following canal dredging carried out in 2000, Hg concentrations along the contaminated 10 km stretch of canal between Falkirk and Polmont were significantly lower than levels determined in 1992. Concentrations of Hg ranged from 22 ± 0.6 to 565 ± 48 mg/kg as determined by CVAAS. No statistical difference was found between CVAAS results and results obtained by TDAAS, while results obtained using pXRF ranged from 36 to 43% of the CVAAS results indicating that pXRF was a useful screening method was Hg. Despite the reduction in Hg concentration, sediment was still classified as being heavily polluted (Kelderman *et al*., 2000) and levels were still well above the PEL of 0. 486 mg/kg for freshwater sediment (CCME, 1999) indicating adverse effects on aquatic organisms were likely. A weak correlation was found between Hg concentration and OM content, and assessment of species mobility using sequential extraction and TD indicated that Hg species were primarily present in forms considered mobile. The analytical techniques used in this chapter provided no information on the degree of Hg methylation in the sediment. If present, MeHg species would have been removed with the extractable species in the extraction scheme implemented (Section 4.3.3.4.1). Determination of MeHg is important because this is the form that is more readily taken up by aquatic organisms (Section 1.4). Methylation in the sediment of the Union Canal is considered in Chapter 6.

CHAPTER 6 MERCURY METHYLATION IN UNION CANAL SEDIMENTS

6.1 Introduction

Contamination in sediment from the Falkirk to Polmont section of the Union Canal generated media interest following presentation of levels of Hg up to 565 mg/kg found in this study (Section 5.6.1) at the 13th International Conference on Mercury as a Global Pollutant (ICMGP) (Cavoura *et al*., 2013). In response to an article in the national press regarding the findings (Scotsman, 2013), a press statement was issued by Falkirk Council that stated *'Following an academic paper which was recently presented at a conference in Edinburgh, Scottish Canals, SEPA, Falkirk Council and The University of Strathclyde met to discuss the presence of mercury in the sediment of the Union Canal, close to the site of the former Nobel munitions factory in Reddingmuirhead near Falkirk. Although regular monitoring over the past 10 years has shown that mercury levels in the canal are on a downward trend, it was agreed that an action plan would be developed to give us a better insight into the current situation at Reddingmuirhead'* (Laird, 2014). Better understanding into the situation required speciation analysis to determine levels of MeHg in the canal sediments since this is the form that is bioaccumulated and biomagnified (Section 1.4). The relationships between MeHg, total Hg and sediment parameters OM and pH are considered in this chapter.

6.2 Factors affecting Hg methylation in sediment

To what extent Hg will be methylated in the aquatic environment is influenced by microbial populations and sediment characteristics. An overview of methylation in the aquatic environment is given in Figure 6.1. Sediments adsorb and store inorganic Hg^{II} and MeHg. Adsorption may not be permanent however and, through, for example, the formation of soluble complexes either with sulfide or OM (Merrit and Amirbahman, 2007; Faganeli *et al.,* 2003), or the reduction of Fe^{III} and Mn^{IV} (oxy)hydroxide surfaces on which Hg species are adsorbed, Hg may be released, as indicated by higher dissolved Hg concentrations found in water immediately overlying sediment when compared to water nearer the surface. The amount of Hg released from sediment varies, with inorganic Hg being released less readily than MeHg as a result of its stronger sorption. Covelli *et al.* (1999) estimated that up to 25% of total sediment Hg may be released annually in sediments from the Gulf of Trieste, Italy, of which up to 23% could be MeHg.

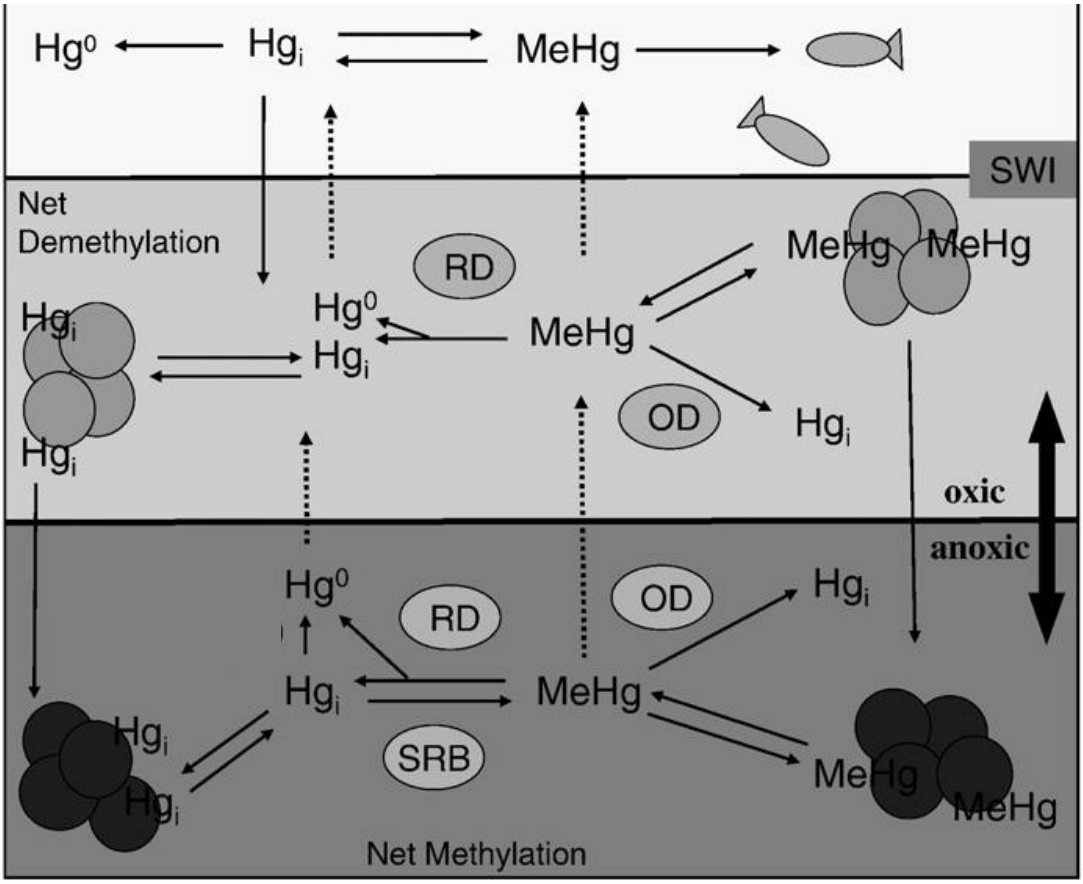


Figure 6.1 Methylation dynamics of Hg in the aquatic environment (adapted from Merrit and Amirbahman, 2009).

Hgi = Inorganic Hg, SRB = sulfate reducing bacteria; RD = Reductive demethylation; OD = oxidative demethylation; SWI = sediment-water interface, circles represent adsorption to particulate matter, solid downward arrows represent sedimentation or deposition, dotted upward arrows represent diffusion processes.

Methylation of inorganic Hg^{II} may occur after release from sediment since only dissolved Hg^{II} species are methylated (Benoit *et al*., 1999; Farrell *et al*., 1998). Although predominantly a biotic process, abiotic methylation does occur to a limited degree (Section 1.3.4.4). Biotic methylation was first demonstrated in 1969, when it was shown that $HgCl_2$ was methylated in freshwater bottom sediments, although no methylation was observed in sterilised sediment (Jensen and Jernelov, 1969). Since then it has been shown that methylation is carried out by certain strains of sulfate and iron reducing bacteria (Section 1.3.4.4). Being an intracellular process, dissolved inorganic Hg must be in a form able to cross the cell membrane. Transportation of Hg species across the cell membrane occurs by the passive diffusion of small, uncharged or non polar Hg complexes such as $HgCl_2$ (Mason *et al.*, 1996), $HgS^0_{(aq.)}$ and $Hg(SH)_2$ (Benoit *et al.*, 2001; Drott *et al.*, 2007). More recently the uptake of polar

Hg-cysteine complexes has also been demonstrated (Schaefer and Morel, 2009), indicating an active transport mechanism. Bacteria then either accumulate the MeHg internally or excrete it into the water column where it is adsorbed to sediment or plankton (USGS, 2000). MeHg attached to sediment may be directly taken up and assimilated in aquatic organisms (Gagnon and Fisher, 1997), whereas plankton serves as the food source for higher trophic levels.

Sediment parameters such as pH, sulfide, temperature and OM also influence methylation since they can affect the microbial community and the available inorganic Hg^{II} (Mauro *et al.,* 1999). For example, OM can stimulate microbial activity, and, can also affect Hg^{II} speciation and solubility (Drott *et al.*, 2007). The effect of chloride and sulfide on methylation is also significant. In marine and estuarine environments, methylation rates decrease with increasing salinity due to the formation of negatively charged chloride complexes that cannot be taken up by bacteria (Boszke *et al*., 2003; Ullrich *et al.,* 2001). Sulfide also affects Hg methylation through controlling speciation. Mildly sulfidic conditions (dissolved sulfide < 10 μM), are favourable for the formation of the neutral soluble Hg complexes such as $HgCl_2$ and HgS^0 that can be taken up and methylated by bacteria (Benoit *et al.*, 2001). At higher sulfide concentrations, methylation is inhibited due to the formation of insoluble $HgS_{(s)}$ or the formation of soluble charged sulfide complexes such as HgS_2^{2-} and $HgHS_2^-$ for which passive diffusion is not possible (Benoit *et al*., 1999). At sulfide concentrations > 1 mM, the presence of S^0 (as polysulfide complexes), formed through the reaction of sulfide and elemental sulfur, promotes the dissolution of cinnabar (Paquette and Helz, 1997) and results in the formation of soluble Hg –polysulfide complexes (Jay *et al.*, 2000). Such complexes though are not bioavailable due to their charge and size (Jay *et al*., 2002).

The overall degree of methylation is dependent not only on the rate of methylation but rather on the balance between rates of methylation and demethylation. Photodegradation is known to degrade MeHg (Sellers *et al*., 1996), as are two biotic processes: reductive demethylation and oxidative demethylation (Schaefer *et al*., 2004). Reductive demethylation is mediated by Hg-resistant bacteria as part of their mercury resistance (*mer*) system in both aerobic and anaerobic environments (Merritt and Amirbahman, 2009; Barkey *et al.,* 2003). In the presence of Hg, these bacteria express *mer* genes that encode for enzymes to degrade MeHg to Hg^0. The *merB* gene produces the organomercury lyase enzyme, MerB, which cleaves the Me-Hg bond producing methane and Hg^{II}, whereas the *merA* gene produces the mercuric

ion reductase enzyme MerA that can then further reduce Hg^{II} to Hg^{0} which may in turn be lost to the atmosphere (Barkey *et al*., 2003). Oxidative demethylation, (also carried out by aerobic and anaerobic bacteria) results in the oxidation of the methyl group to carbon dioxide, with methane also being formed (Marvin-Dipasquale and Oremland, 1998; Barkey *et al.,* 2003). It is not thought to a detoxification process since the Hg^{II} formed is still available to bacteria (Hintelmann, 2010).

6.3 Speciation procedures for determination of MeHg in sediment

For the determination of MeHg in sediment, samples are usually analysed fresh since an increase in MeHg concentration has been observed in air dried samples (Leermakers *et al.,* 2005). Following sampling, MeHg species must be removed without destroying the C-Hg bonds and without the generation of MeHg. Distillation or acid or alkaline extraction are commonly used for the extraction of MeHg, each procedure with its own benefits and drawbacks. Distillation, where the more volatile MeHg is first removed using acid and then distilled in an air stream at 150 ℃, was first proposed for use in river sediments by Nagase *et al*. (1980). Initially a popular method due to a high MeHg removal efficiency of nearly 100%, artificial methylation was found to occur in the presence of humic acids in both sediment and freshwater. Similarly artificial methylation was encountered in alkaline extraction of sediment, limiting this extraction to biological samples (Leermakers *et al.*, 2005; Tseng *et al*., 1997). Acid extraction using HCl was first proposed by Westöö (Westöö, 1966) for the removal of MeHg from foodstuffs. Since then many modifications have been proposed for the acid extraction of MeHg from sediment with less artifacts formation than alkaline extraction or distillation (Bloom *et al.,*1997). Following extraction MeHg is typically removed into an organic solvent such as dichloromethane or isooctane and derivitised either with Grignard reagents or tetraalkylborates ($NaB(Alk)_4$) to allow GC separation. Due to the time consuming and laborious nature of Grignard derivitisation, which requires strictly non aqueous media, the use of alkylation with $NaB(Alk)_4$, which can be performed in aqueous media, has gained popularity. The derivitisation reaction for MeHg with $NaB(Alk)_4$ is described in De Smaele *et al*., (1998) and Tutschku *et al.* (2002) as shown in Equation 6.1:

$$MeHg^{+} + NaB(Alk)_4 \longrightarrow MeHgAlk + B(Alk)_3 + Na^{+} \qquad \textbf{Equation 6.1}$$

The derivitisation reaction for inorganic Hg with $NaB(Alk)_4$ is described as shown in Equation 6.2:

$$Hg^{2+} + 2\ NaB(Alk)_4 \rightarrow AlkHgAlk + 2B(Alk)_3 + 2Na^+ \qquad \textbf{Equation 6.2}$$

Where Alk = ethyl, propyl or phenyl group (Tutschku *et al.*, 2002; Rapsomanikis and Craig, 1991).

If the derivitising agent is $NaB(Et)_4$ distinctions cannot be made between inorganic Hg and EtHg, as shown in Equations 6.3 and 6.4.

$$Hg^{2+} + 2NaB(Et)_4 \rightarrow EtHgEt + 2\ B(Et)_3 + 2Na^+ \qquad \textbf{Equation 6.3}$$

$$EtHg^{+} + NaB(Et)_4 \rightarrow EtHgEt + B(Et)_3 + Na^+ \qquad \textbf{Equation 6.4}$$

There are very few reports of EtHg in soil and sediment. Procedures that allow the distinction between inorganic Hg and EtHg to be made such as butylation (Cai *et al.,* 1997) and phenylation (Mao *et al.,* 2010) have resulted in the detection EtHg in sediments from the Florida Everglades, U.S.A, (Cai *et al.*, 1997) and from four Canadian wetlands (Holmes and Lean, 2006). For the determination of MeHg in Union Canal sediment, acid extraction using 4% w/w HCl was used (Bermejo-Barrera *et al.*, 1999) on freshly sampled, wet, canal sediment. Derivitisation was carried out using sodium tetra-n-propylborate ($NaBPr_4$).

6.4 Materials and methods

6.4.1 General procedures

General procedures were carried out as noted in Section 3.4.2.1.

6.4.2 Solution preparation

10 mg/kg MeHg in methanol

A stock solution containing 10 mg/kg MeHg in methanol MeOH (AnalaR NORMAPUR BDH Prolabo – VWR International, Lutterworth, U.K.) was prepared from methylmercury(II)chloride powder, (Pestanal analytical standard, Sigma-Aldrich

Company Ltd. Dorset, U.K.). Appropriate amounts were used to prepare 0.5, 1.0 and 5 *μ*g/kg standards in isooctane (≥ 99% ACS Reagent, Sigma-Aldrich Company Ltd. Dorset, U.K.).

Calcium chloride solution, 0.01M

A 0.01 M $CaCl_2.2H_2O$ solution (pH = 5.45) was prepared by dissolving 1.47 g of $CaCl_2.2H_2O$ (≥ 99%, ACS reagent, Sigma-Aldrich Company, Life Science Chemilab A.E., Athens, Greece) in distilled water and making up to 1 L.

Derivitising agent

Derivitising agent 1% $NaBPr_4$ (w/w) was prepared by addition of 1 g $NaBPr_4$ (Chemos GmbH, Regenstauf, Germany) to DI water to produce 100 g aqueous solution and was stored at -20 ℃ until use.

6.4.3 Sediment sampling and sample preparation

Sampling was carried out as described in Section 3.4.1.5 at locations 1, 5, 6, 7, 8 and 10 (Table 5.1). For total Hg determination sediment was dried as described in Section 3.4.2.4.

6.4.4 Determination of MeHg

6.4.4.1 Extraction

To approximately 1 g of well mixed and accurately weighed (weighing balance AB 204-S/FACT, Mettler Toledo, Leicester, U.K.) (4 d.p.) wet sediment, 3 mL of 4% (w/w) HCl (prepared from 30% HCl for trace analysis, Sigma-Aldrich Company Ltd. Dorset, U.K.) was added. The samples were shaken mechanically for 2 min. and then centrifuged (3000 rpm, 10 min.) (CENTRIFUGE ALC 4218, ALC International SRL, Milano, Italy). The supernatant was transferred to a clean glass vial. A further 2 mL of 4% (w/w) HCl was added to the residue, shaken mechanically for 2 min. and centrifuged (3000 rpm, 10 min.). The supernatant was again removed and combined with the first supernatant to give a 5 mL combined extract. Vials were weighed after each removal of HCl in order to obtain the exact weight of HCl added. Extractions were carried out in triplicate. Certified reference material ERM CRM CC580

(estuarine sediment containing 132 ± 3 mg/kg total Hg and 75 ± 4 μg/kg MeHg, Geel, Belgium,) was treated in the same manner using a 0.1 g test portion.

6.4.4.2 Derivitisation

Following extraction, 1 mL of the HCl extract was transferred into a glass vial and 5 mL of 0.1 M acetate buffer solution (University of Aberdeen) added. The pH was adjusted to 3.9 ± 0.1 (pH meter HI 8521, Hanna Instruments, Leighton Buzzard, U.K.) using tetramethyl ammonium hydroxide (TMAH) (25% w/w aqueous solution, Alfa, Aesar, Heysham, U.K.) and acetic acid (AA) (AnalaR NORMAPUR BDH Prolabo – VWR International, Lutterworth, U.K.). To this, 1 mL of isooctane was added followed by 1 mL of 1% (w/w) $NaBPr_4$. The samples were then allowed to stand for 30 min. in order to ensure complete derivitisation, after which they were mechanically shaken for 5 min. to extract derivitised species into the organic layer. In order to facilitate separation of the organic and aqueous layers, the samples were then centrifuged (3000 rpm, 5 min.). The organic layer was removed into an amber GC vial and stored at -20 ℃ until analysis. Calibration standards were derivitised in the same manner.

6.4.4.3 Operating conditions

Following extraction and derivitisation, GC-ICP-MS (GC Hewlett Packard HP 6850, Agilent Technologies U.K. Ltd., Wokingham, U.K.; ICP-MS 7500c Series, Agilent Technologies UK Ltd., Wokingham, U.K.) was used for Hg species separation and detection. The GC was fitted with a capillary column Rtx-1 (Crossbond 100% polydimethylsiloxane (PDMS) Silcosteel, 30 m, 0.59 mm internal diameter, 1 μm film diameter). Instruments were connected via a heated Silcosteel transfer line (1 m length, in thermally insulated sheath (in-house)). Operating conditions are listed in Table 6.1. An isooctane blank was also analysed in the same manner. Tuning parameters for the ICP-MS are given in Appendix J.

Table 6.1 Operating conditions for GC-ICP-MS

GC temperature programme	
Initial temperature	50 ℃
Hold	1 min.
Temperature ramp	50 ℃/min.
Final temperature	250 ℃
Hold	7 min
Injection	1 μL volume, splitless injection
Injector temperature	240 ℃
Carrier gas	Helium, 10 mL/min. flow rate
Transfer line	220 ℃
ICP nebuliser rate	1 L/min. argon
Plasma flow rate	16 L/min. argon
MS operation	Full scan 100 - 300 m/z

6.4.4.4 Procedure for MeHg determination by GC-ICP-MS

Using manual injection, duplicate analyses were performed for each derivitised sample in a random order. The syringe was washed by rinsing five times with acetone between injections. To compensate for fluctuations in plasma sensitivity, a Tl internal standard (25 μg/L Tl in 1% HNO_3, University of Aberdeen) was continuously aspirated and mixed with the sample emerging from the transfer line. MeHg counts were then averaged with Tl counts over the same time period. Determination of MeHg was carried out at the Department of Chemistry at the University of Aberdeen, Scotland.

6.4.4.5 Data analysis

OriginlabData analysis and technical graphics software was used for chromatogram generation and peak integration. The response for the most abundant Hg isotope, ^{202}Hg (RSC, 2014), was used for data analysis.

6.4.5 Determination of total Hg concentration, moisture content and OM content

Total Hg concentration was determined after samples were dried and 2 mm sieved as described in Sections 3.4.2.4, 3.4.2.7-9. Determination of moisture content and OM content were carried out as described in Sections 3.4.2.5 and 3.4.2.6.

6.4.6 Determination of pH

The determination of pH was carried out as specified in European Standard TC WI: 2003 (EN, 2003). Approximately 5 g (2 d.p.) of each dried sediment sample was weighed into a 50 mL polyethylene centrifuge tube (ALC International, Milano, Italy)

and 25 mL of 0.01 M $CaCl_2.H_2O$ added. The tubes were mechanically shaken (roller Mixer Stuart SRT9, Life Science Chemilab A.E., Athens, Greece) for 60 min. and allowed to stand for 1 hr.. Immediately before pH measurement was taken (pH meter pH mobile, Metrohm 826, Alfa Analytical Instruments, Athens, Greece), the samples were shaken to provide a suspension of the particles.

6.5 Results and Discussion

6.5.1 Retention times, calibration and LOD

Species retention times are given in Table 6.2. Calibration was carried out using standard solutions of concentration 0, 0.5, 1 and 5 μg/L MeHg (Appendix K). The LOD for $Me^{202}Hg$ was 0.231 μg/L (Gajdosechova, Z., 2103) corresponding to a MeHg concentration of 1.16 μg/kg in sediment for the procedure used.

Table 6.2 Retention times of mercury species and Tl internal standard

Species	Retention time (s)
Hg^0	30 - 60 s
Tl internal standard	90 - 120 s
MeHgPr	130 - 175 s
EtHgPr	180 - 200 s
$HgPr_2$	200 - 220 s

6.5.2 CRM and the detection of EtHg

The recovery for MeHg from CRM 580 was 87.5% indicating that the extraction, derivitisation and detection procedure used was adequate. Analysis of the CRM indicated the presence of EtHg (Figure 6.2).

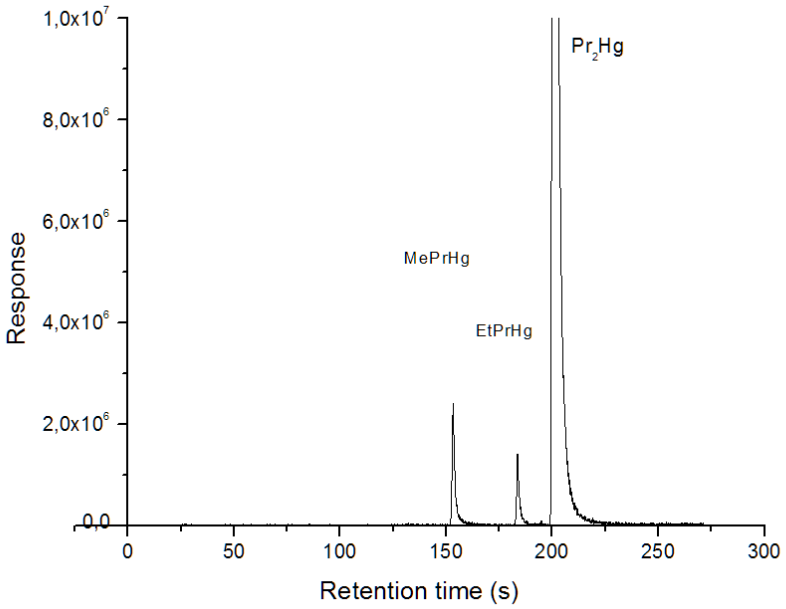


Figure 6.2 GC-ICP-MS chromatogram obtained for CRM 580 indicating the presence of EtHg.

Although not reported in the CRM certification (IRMM, 2004) the detection of EtHg in CRM 580 was recently reported (1.42 ± 0.07 *µ*g/kg in a 0.2 g sample) (Kodamatani H. and Tomiyasu T., 2013). In the determination of MeHg in Union Canal sediment, calibration was not carried out for EtHg specifically, however EtHg concentration in the CRM was estimated to be 40 *µ*g/kg using the MeHg response from the standard solutions. This was possible since the Hg response obtained by the MS was the response to Hg ions while the different species were identified by their retention times. However, the use of $NaBPr_4$ for derivitisation has been found to produce EtHg artifacts proportional to the concentration of inorganic Hg up to a maximum of 1 *µ*g Hg in the derivitisation solution (Huang, 2005). In the absence of standards to confirm the extraction/ derivitisation procedure for EtHg, the concentration obtained was an estimate. The certification of a CRM for EtHg would allow the efficiency of extraction procedures for this species to be determined.

6.5.3 Total Hg, MeHg and EtHg concentrations in Union Canal sediments

Total Hg and MeHg concentrations were determined as was MeHg as a percentage of total Hg concentration (%MeHg) (Table 6.3). In all but two samples, which contained the lowest concentrations of Hg, EtHg was detected and an estimate for EtHg concentration and %EtHg was made based on the MeHg response (Table 6.3)

(Appendix L). At location five, where the highest Hg concentration was determined, Hg^0 was also detected (Figure 6.3). Total Hg concentration ranged from a minimum of 35.3 ± 7.3 mg/kg at location one to a maximum of 1200 ± 180 mg/kg at location five. Concentrations at all locations were higher than those determined on the previous sampling trip in 2012 (Section 5.6.1) and well above the PEL value (Section 1.6) of 0.486 mg/kg for freshwater sediment (CCME, 1999). Excluding the possibility of a new external pollution source, this indicated either that contaminated soil entered the water from the banks of the canal, or that more contaminated sediment deeper in the sediment profile had been disturbed possibly by activities on the canal such as the use of barges, canoeing and fishing.

Table 6.3 Concentrations of total Hg, MeHg and EtHg, %MeHg and %EtHg in Union Canal sediment (mean ± SD, n = 3)

Location	Total Hg (mg/kg)	MeHg (*μ*g/kg)	%MeHg	EtHg (*μ*g/kg)	%EtHg
1	35.3 ± 7.3	8.17 ± 2.1	0.023	< 1.16	NC
5	1200 ± 180	10.8 ± 2.9	0.001	6.12 ± 1.0	0.0005
6	571 ± 70	6.11 ± 2.1	0.001	2.49 ± 1.1	0.0004
7	742 ± 94	18.6 ± 4.2	0.003	4.11 ± 1.0	0.0006
8	787 ± 220	9.93 ± 1.2	0.001	3.73 ± 0.6	0.0005
10	71.7 ± 8.2	6.02 ± 2.0	0.008	< 1.16	NC

NC = not calculated since EtHg concentration < LOD

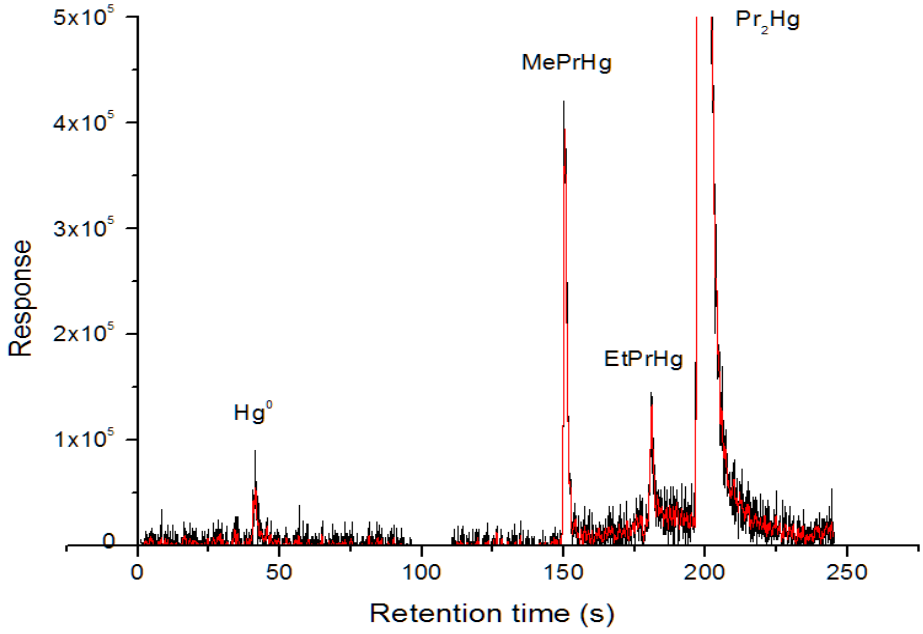


Figure 6.3 GC-ICP-MS chromatogram showing mercury species detected at location five in the Union Canal, Scotland, U.K..

Despite the large difference in total Hg concentrations MeHg concentrations did not vary greatly. Concentrations of MeHg ranged from 6.02 ± 2.0 *µ*g/kg to 18.6 ± 4.2 *µ*g/kg, below the Dutch (VROM) target value of 0.3 mg/kg for MeHg concentration in sediment (GESAMP, 2014). The SQG value for total Hg concentration in freshwater sediment (0.17 mg/kg) is lower than the Dutch target value for MeHg highlighting the non-uniformity of guideline values (Section 1.6). A weak correlation was found between total Hg and MeHg concentrations (Figure 6.4).

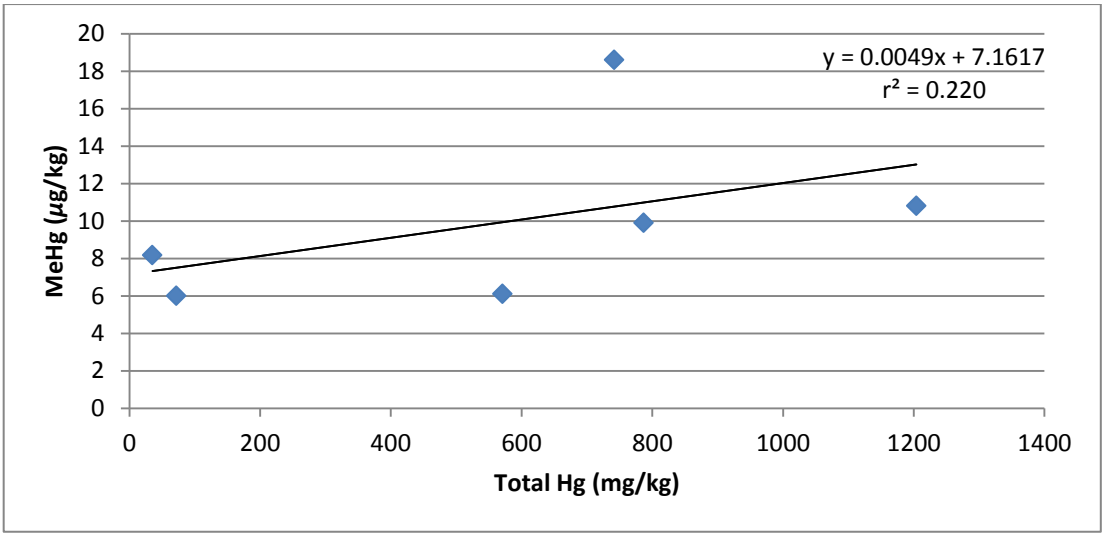


Figure 6.4 Relationship between total Hg and MeHg concentrations in sediments of the Union Canal, Scotland, U. K..

Net methylation has been found to increase with increasing total Hg concentration particularly at low (background) concentrations. For example by using an isotopically enriched ^{202}Hg spike, Orihel *et al.* (2006) found a positive correlation between MeHg production and $^{202}Hg^{II}$ where background Hg sediment concentrations were 0.004 - 0.007 mg/kg. Positive correlation between total Hg and MeHg concentrations has also been found in the field. For example in the Scheldt estuary, Belgium, total Hg and MeHg concentrations were highly correlated ($r = 0.82$, $p < 0.01$), in sediment where total Hg concentration ranged from 0.144 to 1.19 mg/kg and MeHg concentration ranged from 0.8 to 6 µg/kg (Muhaya *et al.,* 1997). Likewise in mangrove sediment from the Jiulong River Estuary, China, where total Hg concentration ranged from 0.170 to 0.620 mg/kg and MeHg concentration ranged from 0.23 to 0.87 µg/kg, a positive correlation was found between the two parameters ($r = 0.558$, $p < 0.05$) (Wu *et al.,* 2011b). Positive correlation has also been found in coastal sediments. For example in sediments along the Fujian coast, total Hg concentration, which ranged from 1.1 to 87.4 µg/kg, was found to be highly correlated to MeHg concentration, which ranged from 11 to 290 ng/kg ($r^2 = 0.84$, $p < 0.01$) (Zhang *et al.,* 2013). On examining the distribution of mercury and MeHg in sediments of the Vigo Ria in the Iberia Peninsula, Canario *et al.* (2007) concluded that for concentrations of Hg between 0.75 - 2.5 nmol/g (0.15 - 0.5 mg/kg), MeHg concentrations were independent of Hg concentration and were in fact constant, although at Hg concentrations between 2.5 and 10 nmol/g (0.5 - 2 mg/kg) MeHg concentration was significantly positively correlated with Hg ($r = 0.91$, $p < 0.05$). As background values of Hg increase however, a different relationship between Hg and

MeHg is often observed. In considering the relationship between total Hg and MeHg concentrations in sediments of a contaminated coastal lagoon in Italy, Trombini *et al.* (2003) determined higher MeHg concentration in sediments with lower total Hg concentration, where total Hg concentration ranged from 0.2 to 250 mg/kg and MeHg concentration ranged from 0.13 to 45 *μ*g/kg. No correlation was found between the two species in polluted sediments of the Lenga Estuary, Chile, where Hg concentration ranged from 0.5 to 129 mg/kg, and MeHg concentration ranged from 11 to 53 *μ*g/kg (Yanez *et al.,* 2013). The variation in results indicates that methylation is influenced by site-specific parameters.

As in the case of MeHg, concentrations of EtHg in the canal sediment did not vary greatly and ranged from < 1.16 *μ*g/kg to 6.12 ± 1.0 *μ*g/kg. Besides MeHg, EtHg is the only other monoalkyl Hg compound to be found in the environment (Hintelmann, 2010). Unlike MeHg, it does not appear to bioaccumulate (Zhao *et al.*, 2012; Batsita *et al.*, 2011) and is not persistant in the environment (Hintelmann, 2010). Despite this, its occurrence is important with regard to the cycling of Hg in the environment. With few reports of EtHg in sediment, information on its behaviour and relationship with parameters such as total Hg concentration and OM content is very limited. In addition to the detection of EtHg in the studies of Cai *et al.,* (1997) and Holmes and Lean (2006), (Section 6.3) EtHg has also been identified in industrially-contaminated sediments from the Kosseine River, Germany (Hintelmann *et al*., 1995). In that particular case, the occurrence of EtHg was attributed to the wastewaters from a fungicide plant producing EtHg (Hintelmann *et al*., 1995). However its presence in other locations was hard to attribute to a particular source. In the Florida Everglades for example, where Hg species occur from natural sources such as natural mineral and peat deposits and from distant pollution sources, and MeHg occurs mainly through biotic methylation, EtHg was found to be widespread. Total Hg concentrations ranged from 26.6 *μ*g/kg to 433 *μ*g/kg and EtHg concentrations ranged from < 0.01 *μ*g/kg to 4.91 *μ*g/kg (SDs were not specified). Biotic ethylation is not known to occur (Hintelmann, 2010) and, with no point sources releasing EtHg, no explanation could be given for its source (Cai *et al.*, 1997). One suggestion offered by the authors was that EtHg was produced by abiotic (chemical) alkylation, similar to the alkylation reported when high-octane gasoline containing tetraethyl lead ($PbEt_4$) was mixed with $HgCl_2$, producing ethylmercury chloride (EtHgCl).

A strong correlation (r^2 = 0.957) was found between total Hg and EtHg concentration in Union Canal sediments (Figure 6.5).

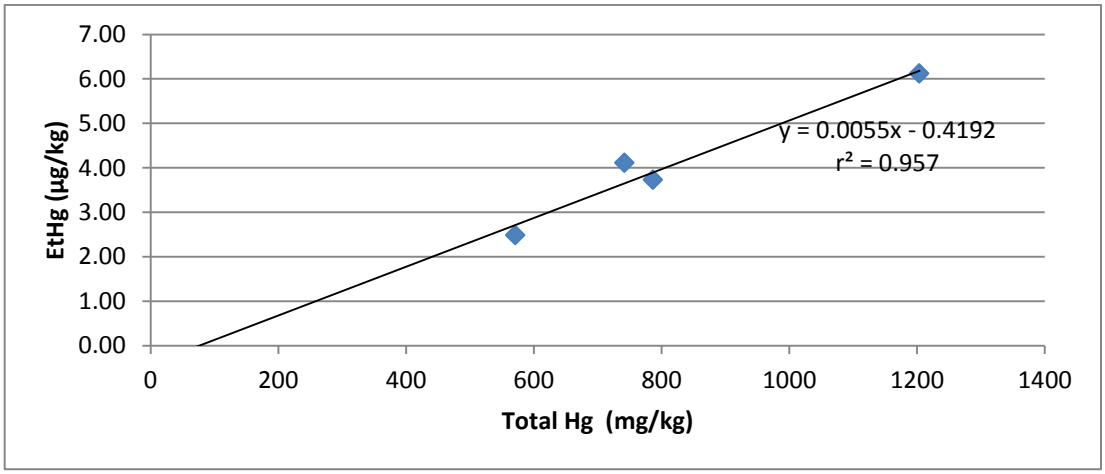


Figure 6.5 Relationship between total Hg and EtHg concentrations in sediment of the Union Canal, Scotland, U. K..

Processing of the data produced from the study of Cai *et al*. (1997) yielded a positive relationship between total Hg and EtHg concentrations (r^2 = 0.534), although this was not as strong as the relationship found in the Union Canal sediment (Figure 6.6).

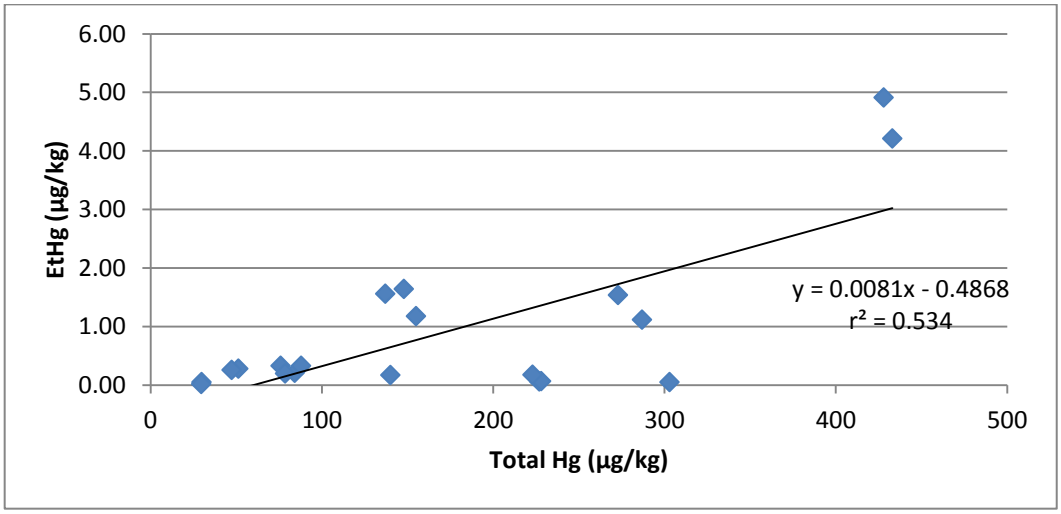


Figure 6.6 Relationship between total Hg and EtHg concentrations in sediment of the Florida Everglades, U.S.A. Based on results for total Hg and EtHg concentration reported in Cai *et al*. (1997).

Processing data from the study of Holmes and Lean (2006), where the Hg concentrations found in Canadian wetland sediments ranged from 66.1 ± 22.5 *µ*g/kg to 319 ± 57.6 *µ*g/kg and EtHg concentrations ranged from 0.3 ± 0.3 *µ*g/kg to 3.7 ± 0.5 *µ*g/kg, indicated a very weak relationship between total Hg concentration and EtHg

concentration (r^2 = 0.039). It should be noted that there were only four data pairs and SDs were high, for example the minimum EtHg concentration detected was 0.3 *µ*g/kg with a SD of 0.3 *µ*g/kg.

6.5.4 Total Hg concentration, %MeHg and %EtHg

The percentage of total Hg content that was methylated, reflecting the proportion of methylated species in relation to the total Hg concentration rather than the concentration of MeHg, ranged from 0.001% (locations 5, 6 and 8) to 0.023% (location 1), theThis was a particularly small percentage of the total Hg content, since typically around 0.5% of total Hg content in sediment is present as MeHg (Hines *et al*., 2000; Zelewski *et al.,* 2001). An inverse relationship was found between total Hg concentration and %MeHg (Figure 6.7).

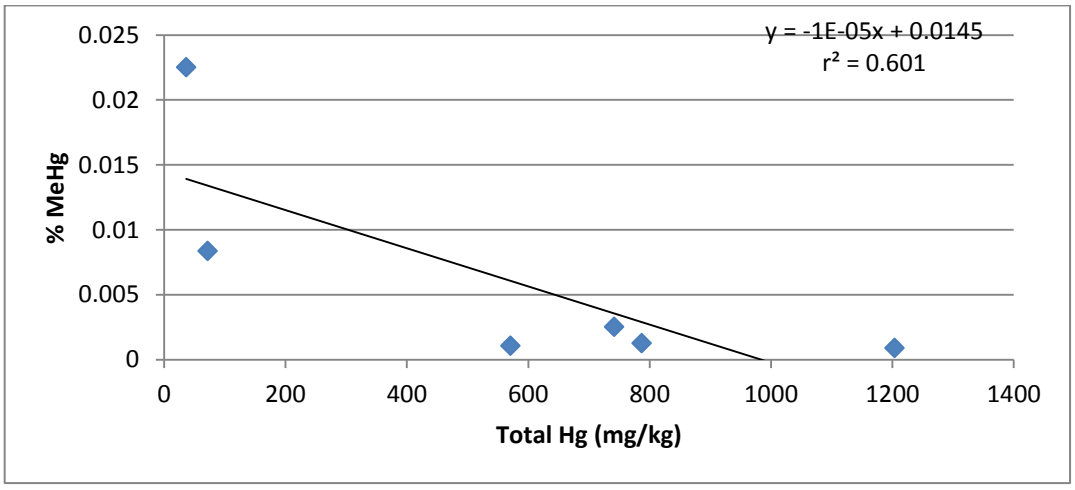


Figure 6.7 Relationship between total Hg concentration and %MeHg in sediments of the Union Canal, Scotland, U.K., in 2013.

Processing of data from the 1992 BW investigations (BW, 1994) indicated that the pre-dredging organic Hg content as a percentage of the total Hg concentration in the contaminated stretch of the canal was also low, ranging from 0.005% (where total Hg concentration was 2360 mg/kg) to 0.146% (where total Hg concentration was 24.4 mg/kg). Their data (BW, 1994) yielded a weak negative relationship between total Hg concentration and % organic Hg (Figure 6.8).

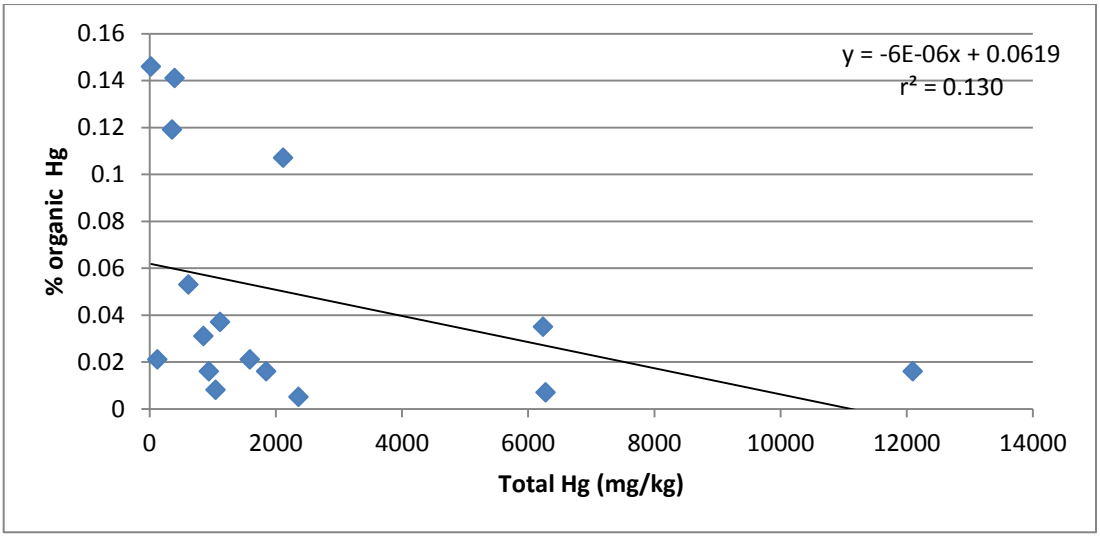


Figure 6.8 Relationship between total Hg concentration and % organic Hg in sediments of the Union Canal, Scotland, U.K. in 1992. Calculated from BW results for total Hg and organic Hg concentrations (BW, 1994).

The low net methylation observed in the Union Canal sediments has been observed in other contaminated environments. In Tokuyama Bay sediments, Japan, where Hg concentrations ranged from 10.9 to 22.2 mg/kg, no MeHg species were found (Nakanishi *et al*., 1989). Boszke *et al.* (2003) attributed this to the high Hg concentrations that inhibited the growth of methylating bacteria. Inhibition of methylating bacteria was also offered as an explaination by Chen *et al*. (1996) for the low percentage of MeHg detected in river sediments of the Carson River when spiked with $HgCl_2$, at ≥ 15.3 mg/kg. In freshwater environments, it has been observed that %MeHg decreased as total Hg concentration increased. For example Schaefer *et al*. (2004) in comparing freshwater Hg and MeHg concentrations from two sites, one affected by industrial inputs (where total Hg concentration ranged from 113 to 4200 ng/L and MeHg concentration ranged from 0.08 to 1.6 ng/L) and one considered pristine (where total Hg concentration ranged from 0.3 to 5.4 ng/L and MeHg concentration ranged from 0.03 to 0.34 ng/L) found an inverse relationship between total Hg concentration and %MeHg ($r^2 = 0.804$, $p < 0.001$). The presence of *merA* genes and a high rate of reductive demethylation ($K_{deg} = 0.19\ day^{-1}$) in the microbial community from the contaminated waters compared to the absence of *merA* genes and a low rate of oxidative demethylation ($K_{deg} = 0.01\ day^{-1}$) in the microbial community from the uncontaminated waters provided evidence that MeHg degradation was directly related to Hg^{II} concentration. It was proposed that in highly contaminated waters mercury-resistance (*mer*) genes are expressed which regulate

reductive demethylation and that at lower levels of Hg these genes cannot be expressed.

The relationship between total Hg concentration and %MeHg in sediment has not been considered in many studies since most frequently concentrations of total Hg and MeHg are compared. A previous report of such a relationship has been described in river sediments in Kazakhstan (Ullrich *et al*., 2007) where total Hg concentration ranged from 9.95 to 306 mg/kg and MeHg accounted for < 0.1% on average. Based on a strong inverse relationship between total Hg concentration and %MeHg ($r = 0.761$, $p < 0.001$) it was proposed that, although MeHg production is controlled by background total Hg concentrations where these are low, in contaminated sediment, net methylation is limited as a result of more efficient bacterial demethylation rather than the inhibition of methylation. This is supported by microbial assays of freshwater sediment using radiolabeled MeHg (as $^{14}CH_3HgI$) at levels between 15 and 2400 *μ*g/kg that indicated demethylation rate increased with increasing Hg concentration (Marvin-DiPasquale *et al.*, 2000). In Union Canal sediments, the presence of *merA* genes has been confirmed (Rodriguez-Gil *et al*., 2013). It is proposed that the extremely high Hg contamination in the sediment 'limits' net methylation. The small percentage of MeHg and the inverse relationship found between total Hg concentration and %MeHg in sediment of the Union Canal, supports the proposal that net methylation depends on the contamination level of the sediment through the effect on demethylating bacteria. An examination of the demethylation rate in these extremely contaminated sediments would provide useful information on the microbial activity.

A weak relationship was found between total Hg concentration and %EtHg in Union Canal sediments ($r^2 = 0.155$) (Figure 6.9). Similarly, no meaningful relationship was observed between the two parameters ($r^2 = 0.091$) after processing data from the study on EtHg in the Florida Everglades (Cai *et al*., 1997).

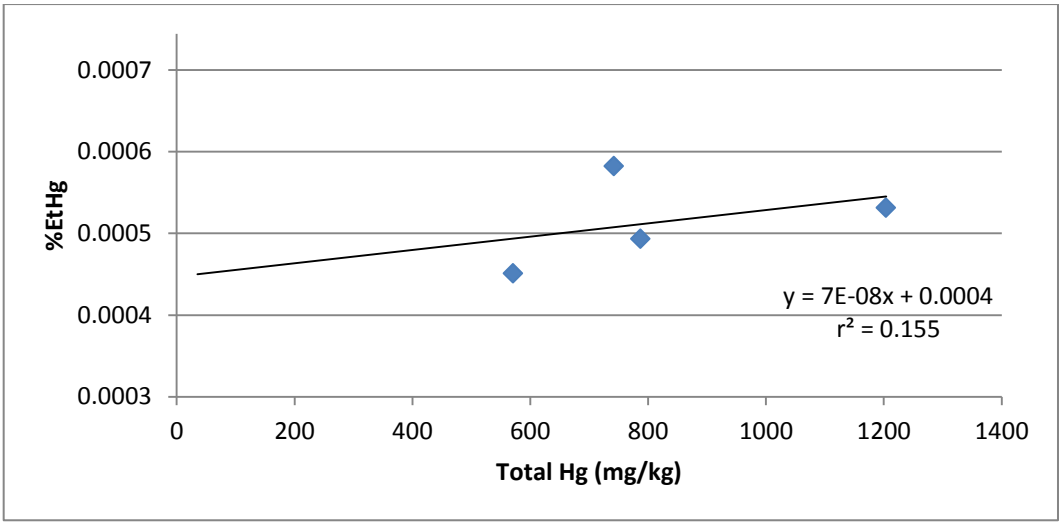


Figure 6.9 Relationship between total Hg concentration and %EtHg in sediments of the Union Canal, Scotland, U.K..

While the results are limited to two studies, it seems that increasing Hg concentration does not affect the overall %EtHg in sediment. While %MeHg in sediment appears to be negatively related total Hg concentration, probably a result of increased microbial demethylation rate with increasing Hg concentration, since ethylation is not microbially mediated, or at least there is no evidence of this to date, (Hintelmann, 2010) such an effect is not seen in %EtHg as Hg concentration increases.

6.5.5 Relationship between MeHg and EtHg concentrations and OM content

OM content in canal sediment ranged from 5.10 ± 2.0 to 13.9 ± 2.5% (Appendix L). A weak positive relationship was found between MeHg concentration and OM content (Figure 6.10).

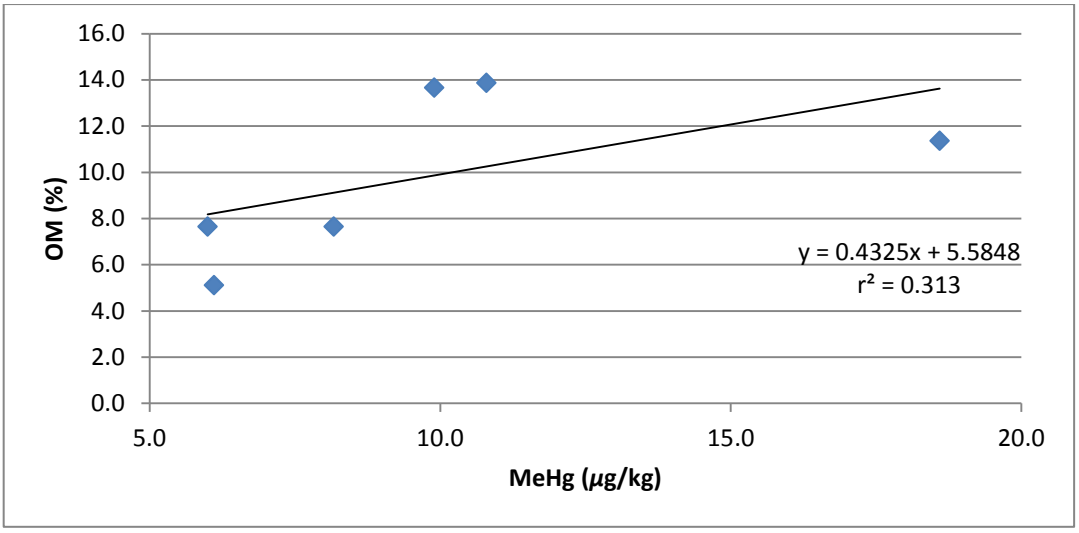


Figure 6.10 Relationship between MeHg concentration and OM content in sediments of the Union Canal, Scotland, U. K..

Many studies have found a positive relationship between MeHg concentration and OM content in sediment (Hammerschmidt *et al.,* 2008; Muhaya *et al*., 1997; Choi and Bartha, 1994). This can be explained by the stimulation of methylating bacteria from nutrients released during breakdown of OM. A positive relationship between MeHg concentration and OM content is not always found however. In estuarine sediment from the Fugong Mangrove Area, Fujian, China, Liang *et al*. (2013) found a weak negative relationship between MeHg concentration and OM content (r^2 = 0.284, $p < 0.01$) in the dry season where MeHg concentration was between 0.15 and 1.8 *µ*g/kg and OM content was between 2.4 and 5.8% and a weak positive correlation in the rainy season (r^2 = 0.170, $p < 0.05$) when MeHg concentration ranged from 0.081 to 0.58 *µ*g/kg and OM content from 1.6 to 3.2%. Combination of the results for both seasons yielded a conic relationship (y = -0.1638x2 + 1.033x – 1.0632) where maximum methylation occurred at an OM content of 3%, above and below which methylation was reduced. These results may be explained by the contrasting roles OM has in methylation. While it is true that OM can stimulate methylating microbes, organic compounds formed from the break down may complex Hg^{II} and reduce bioavailability (Barkey *et al.,* 1997). On the other hand, it has been demonstrated that depending on the Hg complex formed and the nature of the bacteria, methylation may be increased; in laboratory experiments with the bacterium *G. sulfurreducens*, methylation increased on addition of cysteine, due to the uptake of the Hg-cysteine complex formed (Schaefer and Morel, 2009). The role of OM in methylation remains unclear but must certainly vary depending on the overall environment; in extremely

contaminated environments like the Union Canal the role of OM in the stimulation of methylating bacteria and demethylating bacteria, should be further examined.

A stronger relationship was found between EtHg concentration and OM content (r^2 = 0.598, Figure 6.11) than between MeHg concentration and OM content (r^2 = 0.313, Figure 6.10).

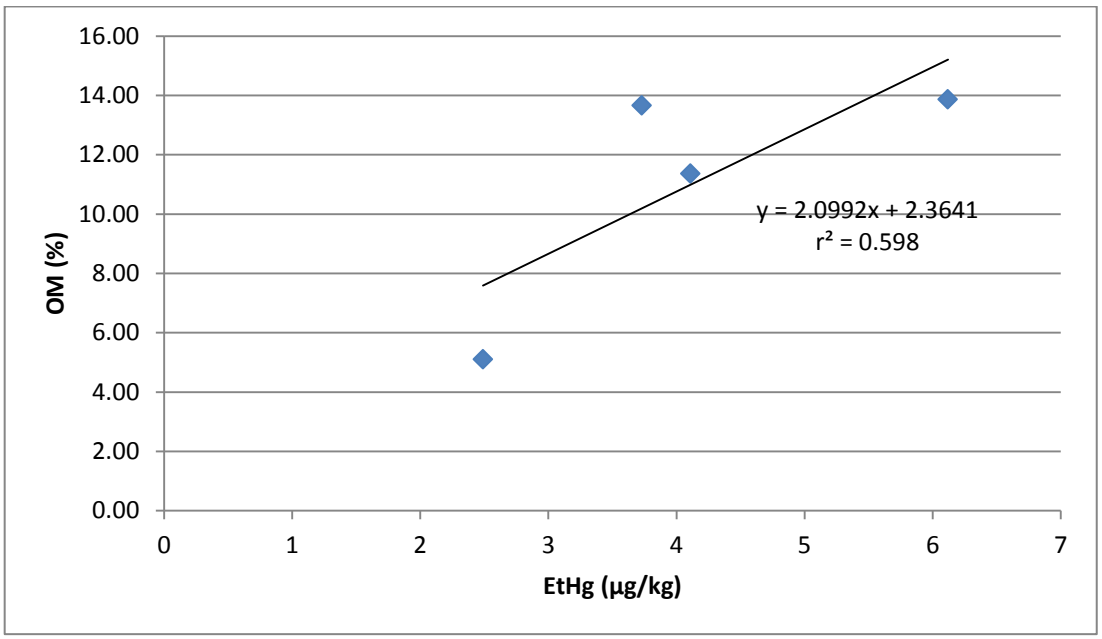


Figure 6.11 Relationship between EtHg concentration and OM content in sediments of the Union Canal, Scotland, U.K..

A similar relationship between EtHg concentration and OM content was found by processing data from a study examining MeHg flux in four Canadian wetlands by Holmes and Lean (2006): EtHg concentration ranged from 0.3 ± 0.3 *µ*g/kg to 3.7 *µ*g/kg, OM content ranged from 9.3 ± 2.1 to 90 ± 3.5% and a linear correlation with r^2 = 0.560 existed between the two parameters. Processing data from the study on EtHg in the Florida Everglades, where EtHg ranged from < 0.01 to 4.91 *µ*g/kg (Cai *et al*., 1997) and OM content ranged from 9.1 to 94.3%, yielded a weak relationship between EtHg concentration and OM (r^2 = 0.183). These limited results did not allow any conclusions to be drawn on the effects of OM content on EtHg concentration.

6.5.6 MeHg, EtHg and pH

The sediment pH ranged from 5.71 to 6.94 (Appendix L), within the range where Hg^{II} adsoption is favoured (pH 4 - 10) (Lui *et al.,* 2012). There was no observed relationship between pH and MeHg concentration (r^2 = 0.003) in sediment of the

Union Canal however a decrease in the %MeHg was observed with decreasing pH (Figure 6.12).

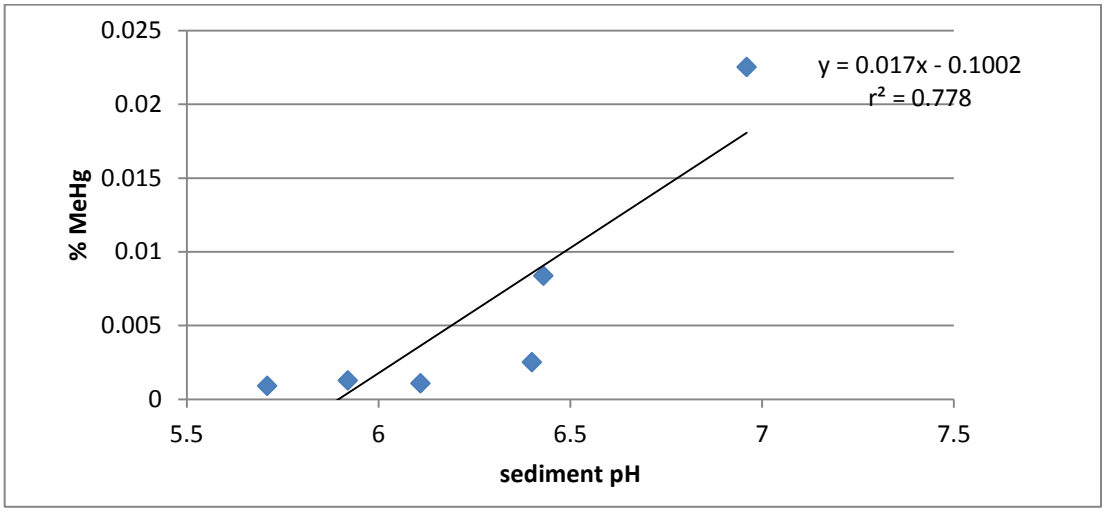


Figure 6.12 Relationship between pH and %MeHg in sediments of the Union Canal, Scotland, U. K..

Although in acidic waters MeHg concentrations are generally elevated relative to waters at higher pH, methylation is in general found to decrease with decreasing pH in anaerobic sediment. In laboratory experiments for example, a decrease in methylation by over 65% was observed in lake sediment spiked with isotopic Hg (1-2 μg $^{203}Hg^{II}$ per 15 mL sediment sample) and acidified from an initial pH of 6.1 to pH 4.5 (Steffan *et al*., 1988). Although microbial activity overall has been found to be little affected over the pH range 5 - 7 (Miskimmin *et al*., 1992), the activity of sulfate reducing bacteria may be affected at lower pH, at least in soil (Connell and Patrick, 1968). Ullrich *et al*. (2001) suggested that the decrease in methylation with decreasing sediment pH was a result of increased demethylation, which could explain the decrease in %MeHg with lower pH, however demethylation rates have been found to be unaffected over the pH range 8.0 - 4.4 (Steffan *et al.*, 1988). Changes in pH may directly affect methylation or indirectly influence it, by affecting partitioning. By spiking and incubating samples of Ottawa river sediment-water with 1 mg/kg $^{203}HgCl_2$, Miller and Akagi (1979) found that total MeHg production was unaffected over the pH range 7 - 5, however partitioning between the sediment and water was altered, with double the amount of MeHg in the water at pH 5. At low pH, MeHg is more soluble. Partitioning of MeHg into the water column may explain the decrease in %MeHg with lower sediment pH.

The relationship between pH and both EtHg concentration and %EtHg was weak as indicated in Figures 6.13 and 6.14.

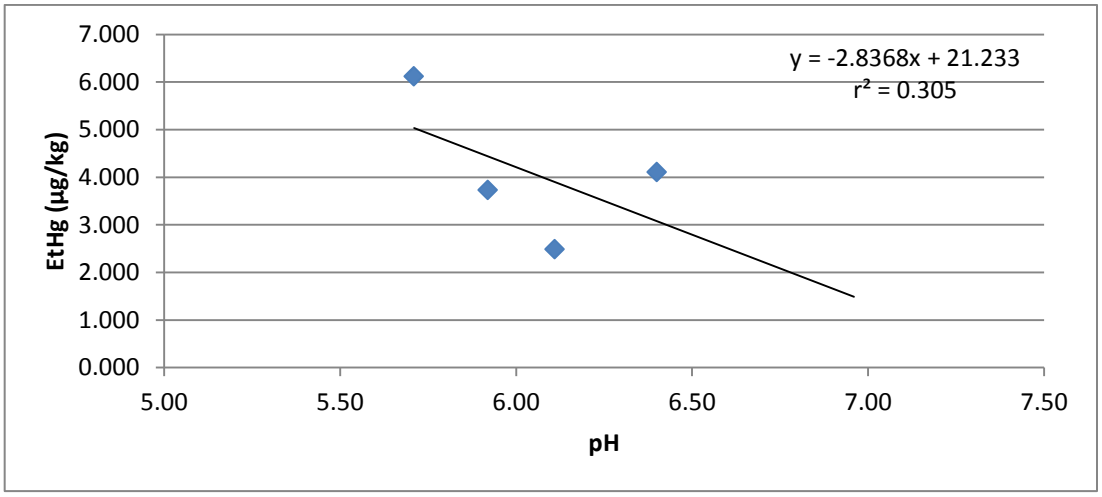


Figure 6.13 Relationship between pH and EtHg concentration in sediments of the Union Canal, Scotland, U. K..

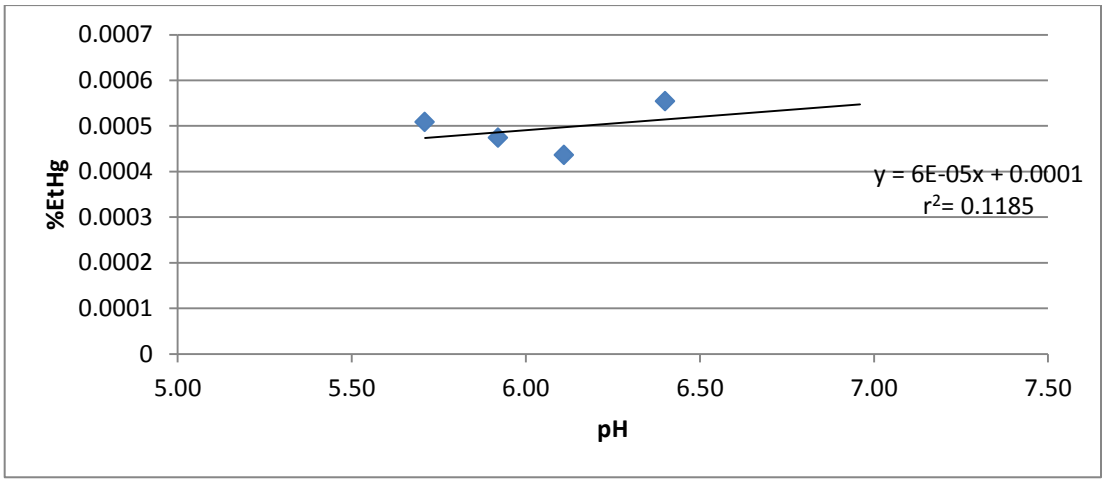


Figure 6.14 Relationship between pH and %EtHg concentration in sediments of the Union Canal, Scotland, U. K..

Since ethylation is not microbially mediated, any effect of pH changes on %EtHg content would possibly be a result of EtHg partitioning. While there are no field studies specifically addressing EtHg partitioning in sediment, in the study of Holmes and Lean (2006), where sediment, porewater and water column were analysed for EtHg and MeHg content, EtHg was detected only in sediment, while MeHg was detected in all three media, highlighting the difference in behaviour of these two species.

6.6 Conclusions

Net methylation in sediments of the Union Canal was low, with %MeHg < 0.023% of total Hg content. The concentration of MeHg ranged from 6.02 ± 2.0 *μ*g/kg to 18.6 ± 4.2 *μ*g/kg, below the Dutch EQS of 0.3 mg/kg for MeHg in sediment. The high total Hg concentrations (between 35.3 ± 7.3 mg/kg and 1200 ± 180 mg/kg) may enhance demethylation as indicated by the negative relationship between %MeHg and total Hg concentration (r^2 = 0.601). Lower %MeHg was also found in more acidic sediment (r^2 = 0.778) possibly due to the partitioning of MeHg into the water column, and the influence of OM was small with a slight increase in methylation with increasing OM (r^2 = 0.313). The detection of EtHg in sediment of the Union Canal was an interesting result with regard to the cycling of Hg in the environment. Since no point sources exist, its occurrence may be a result of abiotic alkylation. While biotic ethylation has not been observed, the possibility should not be completely overlooked. Concentrations of EtHg may be positively associated with total Hg concentration although data supporting this is limited.

CHAPTER 7 CONCLUSIONS AND FUTURE WORK

The behaviour of Hg in the environment remains an area where many questions remain unanswered. While much has been learned about the cycling of Hg, this information is often site-specific. This study optimises and evaluates analytical methods for Hg determination and provides insight into the environmental behaviour of Hg species, particularly in contaminated environments.

A critical issue in reducing human exposure to Hg, which primarily occurs through the consumption of contaminated fish, is the identification of contaminated marine environments. To this end a colorimetric method for Hg screening in freshwater sediment (Yallouz *et al*., 2008) was more fully characterised and its applicability to marine samples determined. The method involved the digestion of solid samples with *aqua regia* at 80 ℃ using a water bath and reduction of Hg^{II} species to Hg^0 following which Hg^0 was trapped on a detecting paper coated with Cu_2I_2 to produce a coloured Hg complex the intensity of which was proportional to the Hg concentration in the sample. The screening method was successfully implemented using a pump flow rate of 1 L/min over a range of standard concentrations from 5 to 200 *μ*g/L. Its performance was not found to be influenced by a soil OM content of 10%, above the typical soil OM content of 5% (CTAHR, 2014). If samples contained higher OM content, method testing should be extended to cover the required range.

Comparison of the screening results with quantitative Hg determination carried out by CVAAS following microwave digestion with concentrated HNO_3 at 175 ℃, indicated that the screening method successfully identified the presence or absence of Hg in 20 sediment samples from four locations that varied in salinity, Hg and OM content. Marine sediment from the West of Scotland, U.K., was found to be uncontaminted, as was freshwater sediment from Kifissos River, Greece. For the remaining two sites - Elefsina Bay, Greece, and the Falkirk to Polmont area of the Union Canal, Scotland, U.K. - screening indicated Hg contamination, a finding that was confirmed by CVAAS. In certain sediment samples from Elefsina Bay quantitative Hg determination by CVAAS indicated a higher level of Hg contamintion than the screening method. This could have been a result of 1) less mobile species being present in these samples that required more vigous digestion conditions than those provided in the screening method for the release of Hg, 2) the loss of Hg during the digestion process or 3) the variable nature of the detecting paper. For three canal

sediment samples, Hg concentrations exceeded the working range of the screening method of 200 *µ*g/L. However the colorimetric method successfully identified Hg contamination in all freshwater and marine sediment samples regardless of variation in sample matrix and could therefore be widely implemented for the screening of sediment, which to date is not possible without analytical instrumentation.

The behaviour of Hg was studied further in two locations where screening had indicated Hg contamination of sediment, Elefsina Bay and the Union Canal. The nature of the sites varied as did the behaviour of Hg. Elefsina Bay is a marine environment, where Hg contamination is a consequence of ongoing and varied pollution sources primarily from industrial discharges and shipping activities. Further sampling both at nearshore and central locations indicated sediment concentrations of Hg ranged from 0.220 ± 0.04 to 2.96 ± 1.1 mg/kg, with higher concentrations found at nearshore, relative to central, locations, indicating that Hg enters the bay from the land. The OM content ranged from 2.26 ± 0.1 to 11.8 ± 1.1%. In contrast to previous work (Valdes *et al*., 2010), no seasonal variation was observed in Hg concentration or OM content. The PEL level of 0.7 mg/kg (CCME, 1999) was exceeded at approximately half of the sampling locations indicating sediment Hg concentrations posed a threat to organisms in contact with it. In this environment a poor correlation was found between Hg concentration and OM content (r^2 = 0.081), probably because Hg concentration was affected directly by on-going inputs. The mobility of the Hg species in Elefsina Bay sediment was explored for the first time in this study and was found to vary between sites. For example the concentration of nonmobile Hg species ranged from < 0.067 mg/kg to 2.74 mg/kg (representing 96% of the total Hg species) again indicating the importance of pollution source and site-specific environments. Mobility of species was not affected by seasonal variation. Generally for background concentrations of Hg it is accepted that Hg is mainly found associated with S ligands either as HgS or with S ligands in OM, forms less mobile that are not methylated. Nonmobile Hg species in sediments of Elefsina Bay may have been present as HgS and thus not mobile, however, mobile species were a significant contribution to the total Hg content at certain locations associated with different pollution sources, even at total Hg concentrations below 0.5 mg/kg. For example over 80% of Hg was mobile at location K1 (summer) where total Hg concentration was 0.33 mg/kg. No Hg was detected in flathead mullet collected from the bay possibly due to the low bioavailability of Hg species in the sediment and the low trophic level of the fish type.

Therefore consumption of this fish, which is common among residents in the area (Tselios, 2012), has no associated risk of Hg exposure.

The second contaminated site considered in this study was the Falkirk to Polmont area of the Union Canal, where contamination was most probably a consequence of the manufacture of detonators between 1876 and 1968 in a factory on the banks of the canal in this area (Smith and Lassiere, 2000). In the year 2000, following the determination of Hg in canal sediment at levels exceeding 1570 mg/kg (BW, 1992), the canal was dredged in this area. In the present study, concentrations of Hg were found to vary between 22 ± 0.6 and 565 ± 48 mg/kg (sampling trip 1 in March 2012) and between 35.3 ± 7.3 and 1200 ± 180 mg/kg (sampling trip 2 in September 2013). The higher concentrations measured in the second set of samples may have been a result of perturbation of deeper sediment by barges or canoes bringing more contaminated sediment to the surface. Total Hg concentration in sediment samples determined by three analytical methods - CVAAS, TDAAS and pXRF - was compared. No statistical difference was found in Hg concentrations as determined by CVAAS and TDAAS. Results obtained using pXRF ranged from 36 to 43% of the CVAAS results and there was a strong relationship between the results from the two methods (r^2 = 0.985) indicating that pXRF can be a useful screening method. Despite the reduction in Hg concentration following dredging, sediment was still heavily polluted (Kelderman *et al*., 2000). Therefore, either dredging did not efficiently remove all contaminated material or contaminated material has re-entered the canal from the surrounding areas, for example through run-off of soil from the canal banks if they are also contaminated (BW, 1994). Levels were still well above the PEL of 0.486 mg/kg for freshwater sediment (CCME, 1999) at all locations, the concentration above which adverse effects on aquatic organisms are likely. Similar to Elefsina Bay, a weak correlation was found between Hg concentration and OM content (r^2 = 0.376). Unlike Elefsina Bay, Hg contamination in the canal is not considered to be an ongoing process of variable origin and consequently the general adsorption associations with S were expected. However, assessment of species mobility, using both sequential extraction and TD techniques, suggested that Hg was not mainly present as HgS or species strongly adsorbed to OM. In determinations using sequential extraction, between 76 and 94% of Hg species were found to be mobile species, while using thermodesorption between 67 and 97% of Hg species were desorbed at temperatures consistent with $HgCl_2$ or Hg associated with Fe (Reis, 2013). This suggests that in heavily contaminated environments typical adsorption phases may not be found and species mobility is largely influenced by Hg

concentration, or by other site-specific parameters. The mobility of the species may also explain the movement of Hg in the canal, through the Falkirk Wheel and into the Forth and Clyde Canal. The increased mobility of Hg, which may also be encountered at other contaminated sites, may have a wider impact with regard to the spread of contamination, biomagnification potential and ultimately human exposure.

Despite high total Hg concentrations, net methylation in sediments of the Union Canal was low with %MeHg < 0.023% of total Hg content. Concentrations of MeHg in the Union Canal ranged from 6.02 ± 2.0 to 18.6 ± 4.2 *μ*g/kg, below the Dutch target value of 0.3 mg/kg for MeHg in sediment (Crommentuijn *et al*., 2000). A negative relationship between %MeHg and total Hg concentration was found (r^2 = 0.601), again considered to reflect the particular nature of heavily contaminated environments where bacterial demethylation is suggested to be more efficient than in less contaminated environments (Ullrich *et al*., 2007).

Lower %MeHg was also found in more acidic sediment (r^2 = 0.778) possibly due to the partitioning of MeHg into the water column, and the influence of OM was small with a slight increase in methylation with increasing OM (r^2 = 0.313). The detection of EtHg in sediment of the Union Canal was an interesting result with regard to the cycling of Hg in the environment and may occur in other contaminated environments. Since no point sources exist, its unusual occurrence may be a result of abiotic alkylation. While biotic ethylation has not been observed, the possibility should not be completely overlooked.

FUTURE WORK

There are several future research needs arising from this study. The successful application the colorimetric method to sea water and marine sediments potentially provides a means for the large scale screening of Hg in marine environments. This would support the Minamata Convention (UN, 2013) aimed at protecting human health from exposure to Hg through the identification of Hg-contaminated sites. The fact that only very simple analytical equipment is needed would enable its application in developing countries where access to instrumentation and analysts may be limited. Further research is necessary to assess the potential for development of the method into a viable commercial system. A first aspect of this should address the ‘standardisation’ of the detecting papers, that is, the production of detecting papers

without variability. The lifetime (shelf-life) of the papers should be determined. The effects of storage conditions on the papers should also be considered since they should be suitable for use worldwide in different climates: both temperature and humidity may affect the stability of the coating. The fading of the colour intensity of the coloured complex on the exposed detecting paper should also be addressed. While colour has been noticed to fade, both in this study and by Yallouz *et al*. (2008), why, or how quickly, this fading occurs has not been identified.

The transposition of the method from one requiring basic laboratory facilities, such as a water bath or hotplate and a power supply, to a field-based method should also be explored. Sample digestion in the field would have to be addressed, perhaps through the use of a portable battery. If this were achieved the need for even a basic laboratory would be removed, saving time in sample transportation and reducing costs. Incorporation of a hand-held spectrometer for more accurate assessment of colour intensity, or a digital camera to save images, could also be considered.

There are knowledge gaps with regard to Hg pollution in Elefsina Bay. Identification of specific industries contributing to Hg pollution is necessary both in order to allow strategies for reduction of Hg releases to be implemented and also to ensure regulations are complied with. Further monitoring of the bay to determine Hg speciation is needed. The concentration of MeHg in both water and sediment would provide insight into the net methylation in the bay. The extent to which MeHg biomagnification occurs should be determined by analysis of fish at higher trophic levels.

More research on the heavily contaminated sediment in the Falkirk to Polmont area of the Union Canal is merited. Further characterisation of the sediment is required. For example the determination of the Hg bonding environment using X-ray absorption spectroscopy would provide an insight into bonding at high sediment Hg concentrations. Also determination of S speciation should be performed, since at high sufide concentrations, S^0 has been shown to encourage dissolution of HgS with the formation of soluble polysulfides (Paquette and Helz, 1997) and may explain its absence in the sediment. In addition to further study on the associations of Hg in the sediment, it seems prudent to test Hg content in canal fish. The Hg concentration of bank soil should also be quantified to identify potential sources of Hg resupply to the canal. These steps would allow informed decisions to be taken with regard to canal management. For example should fish be contaminated, an advisory notice against

fish consumption because of associated health risks should be put in place and, in the case of contaminated soil on the banks of the canal, further remediation may be required.

While dredging of the canal sediment to remove contaminated sediment would have very high associated disposal costs, since the canal sediment was found to contain MeHg, EtHg and at one location Hg^0, rather than disposal, the potential use of the sediment as a CRM for speciation could also be considered, if the species were found to be stable. At present there is no CRM for EtHg and Hg^0. The detection of EtHg in CRM 580 in the present study, which has been reported on one previous occasion (Kodamatani and Tomiyasu, 2013) also merits further investigation. The quantification of the EtHg concentration and a study of its stability in the reference material could enable its use for speciation studies.

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Appendix A Theory of applied analytical techniques and statistical tests

The analytical techniques used for the determination of Hg and MeHg in this study were cold vapour atomic absorption spectrometry (CVAAS) and gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS) respectively. Method validation and data analysis were performed using a variety of statistical tests. The theory of both the analytical methods used and the statistical tests employed are discussed below.

Atomic absorption spectrometry

Theory of atomic absorption spectrometry

One of the main techniques employed for the determination of Hg is AAS. Atomic spectrometric techniques are commonly used for the quantification of metals. The techniques are based on the interaction of electromagnetic radiation (wavelength approx 180 - 800 nm) with the valence electrons of an atom to produce atomic spectra that can be utilized to quantify metal concentration. Each atom has a discrete set of energy levels of specific energy values E, between which electrons can move (Figure 1).

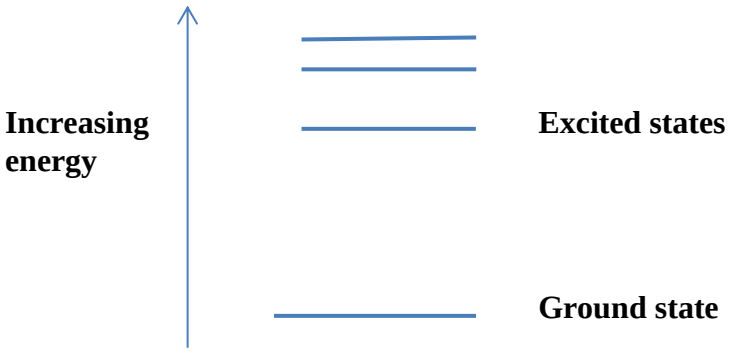


Figure 1 Atomic energy levels of increasing energy.

The difference in energy, ΔE, between these levels is given by Planck's Equation (Equation 1)

$$\Delta E = h\nu = hc/\lambda \qquad \textbf{Equation 1}$$

where h = Planck's Constant

ν = frequency

c = velocity of light

λ = wavelength

Electrons in an atom are usually in the ground state, the lowest available energy level. For a transition to occur to a higher energy level, or excited state, electrons must absorb energy at a specific wavelength, λ. This is achieved through the use of an external light source of the specific wavelength, λ. Valence electrons will absorb some of the energy from the light source as excitation occurs, resulting in a decrease in the light intensity. By measuring the difference in intensity, the absorbance (A) at a given concentration is determined as defined by the Beer-Lambert Law (Equation 2)

$$A = \log_{10}(I_o/I) \qquad \textbf{Equation 2}$$

where I_o is the measured intensity before absorption

I is the transmitted intensity after absorption ($I < I_o$)

Since absorption is proportional to the concentration, quantification can be carried out by comparing the absorbance of the sample with the absorbance of standard solutions of known concentration (Kenkel, 2003).

Cold vapour atomic absorption spectrometry

An AAS system requires three components: a light source to provide the required wavelength for analyte atoms to absorb, an atom cell to produce gaseous atoms of the analyte and a detecting system to measure the light absorbed. The light source typically used is a hollow cathode lamp (HCL) (Figure 2). The HCL consists of a sealed glass cylinder filled with an inert gas such as neon (Ne) or argon (Ar) inside which the anode and cathode are housed. The cathode is shaped like a hollow cylinder and is composed of, or contains a large proportion of, the metal to be determined. The process by which light is emitted from a HCL is shown in Figure 2.

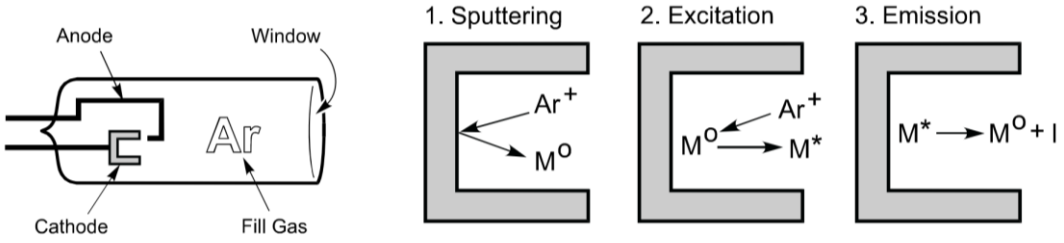


Figure 2 Hollow cathode lamp and emission of light through sputtering and electron excitation (after PE, 2006).

On application of a voltage between the anode and the cathode the electrical discharge causes the fill gas atoms to be ionised (Ar^+). The positively charged gas ions are accelerated towards the cathode where the kinetic energy of the ions is sufficient to eject metal atoms (M^o) from the cathode in a process called 'sputtering'. The sputtered metal atoms are further excited (M*) by electron collisions in the discharge and as these de-excite they emit a narrow line spectrum. Since the metal is of the same element as the analyte to be determined, the wavelength emitted is the correct wavelength needed for absorption by the analyte atoms. Atoms are directed into the path of the emitted light where absorption will occur. For the determination of Hg, an Hg HCL is used.

The atom cell is the part of the AA instrumentation where atoms of the element to be determined are formed. For most metals, the atom cell used is flame and/or electrically-heated furnace. However, because Hg can be easily reduced to the elemental form and vapourised, heat is not required. For Hg, atomisation is commonly performed using the cold vapour (CV) technique with sodium borohydride ($NaBH_4^-$) reductant (Kenkel, 2003). The CV accessory consists of an analyser assembly and a quartz cell assembly. The analyser is free standing and is placed adjacent to the sample compartment of the AA spectrometer (Figure 3).

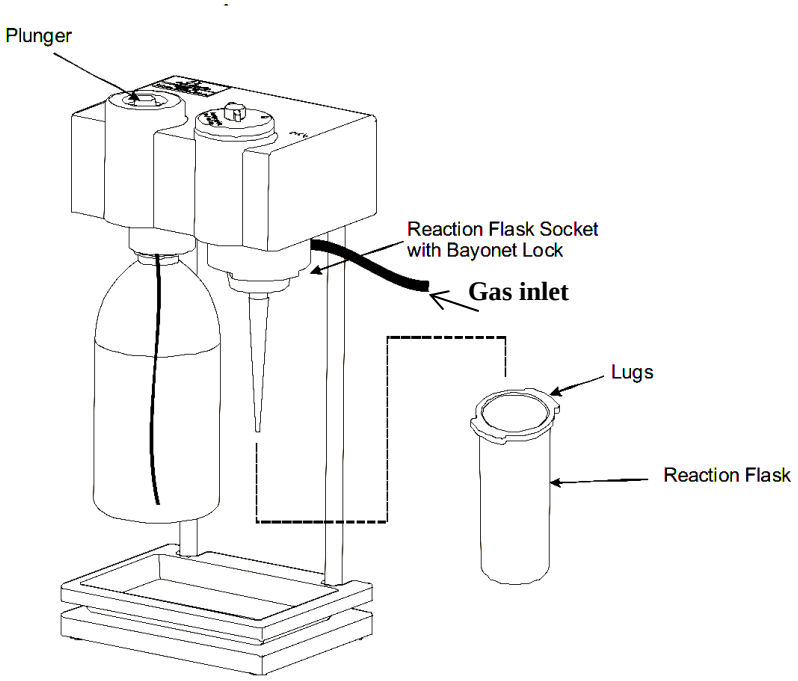


Figure 3 Cold vapour analyser used for the generation of mercury (Hg) atoms in the determination of Hg. Adapted from PE (2006).

The assembly includes a reaction flask, a reductant and the pneumatic component for control of the carried gas and transport of Hg^0 vapours. The analyser requires a supply of inert gas. After addition of the sample, the reaction flask is connected to the apparatus and tightly sealed through the lugs on top of the vessel. The inert gas stream flows through the vessel purging the system of air. To perform a determination the plunger is depressed and held. Reductant is forced into the sample solution where Hg^{2+} is reduced (Equation 3) following which Hg^0 vapour is transported to the quartz cell.

$$Hg^{2+} + 2BH_4^- \rightarrow Hg^0_{(g)} + H_2 + B_2H_6 \qquad \textbf{Equation 3}$$

The quartz cell assembly consists of a quartz cell and a holder (Figure 4). The cell is an open quartz cylinder with graphite cooling rings at both ends. It is connected to the analyser assembly by a silicone rubber transfer and is positioned in the path of the HCL by positioning it on the burner head of a standard flame AAS system.

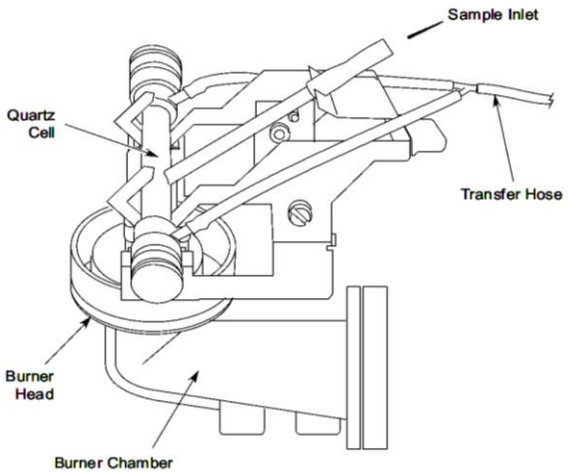


Figure 4 Positioning of quartz cell on atomic absorption spectrometer (after PE, 2006).

Detection is carried out using a photomultiplier (PM) tube based on the photoelectric effect, that is, the emission of electrons following exposure to light. The tube consists of a photocathode, a focusing electrode, a series of electron multipliers (dynodes) and an anode in a vacuum (Figure 5).

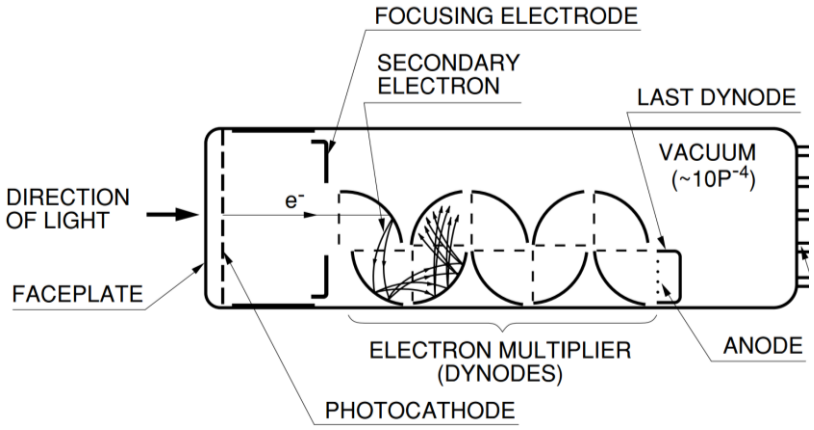


Figure 5 Photomultiplier tube (after Hamamatsu, 2006).

When the cathode is struck by a light beam it releases electrons proportionally to the intensity of the beam. The electrons are focused by the focussing electrode towards the first dynode. A dynode consists of a base material, for example glass, coated with a material that readily emits secondary electrons. Thus further electrons are emitted whenever an electron hits a dynode in a process called secondary emission. The signal is thus amplified when measured at the anode, but still proportional to the light intensity (Hamamatsu, 1998).

Gas chromatography-inductively coupled plasma-mass spectrometry

Coupled techniques

While identification of MeHg can be carried out by extraction from the sediment matrix and quantification using CVAAS, the coupling of GC with ICP-MS offers advantages: The GC system provides separation of organo-Hg species which are then transferred directly into the ICP, where they are atomised and ionised before selection and quantification of the Hg ion by MS. The GC system is coupled to the ICP by the use of a heated transfer line and a system of cones is used for pressure reduction at the plasma-MS interface (Figure 6).

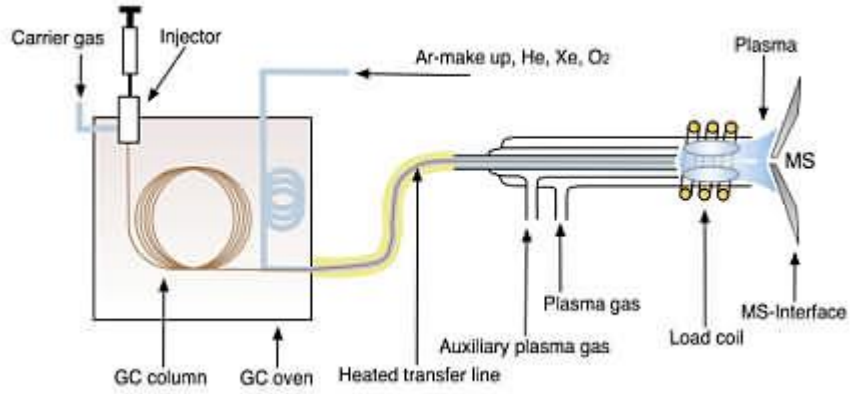


Figure 6 Coupling of analytical techniques: a gas chromatography (GC) system, and inductively coupled plasma-mass spectrometer (ICP-MS) (after Evisa, 2007).

Component separation by gas chromatography

For the separation of thermally stable, volatile compounds in a sample, GC techniques are commonly used. In gas-liquid chromatography, components are separated as a result of partitioning between a mobile gas phase and a stationary liquid phase adsorbed on a solid.

The main components of GC system are a carrier gas supply, the sample injection port, the column, the oven and the detector. The injection port is held at a high temperature to allow sample vaporisation. A small amount of sample (typically 1 μL) is injected through a rubber septum into a heated glass liner inside the GC oven (Figure 7). Injections can be made manually using a gastight syringe or can be automated using an autosampler. A split injection port can be used to separate off a portion of the sample before it reaches the column. The sample is carried through the column by means of a carrier gas such as helium (He) or nitrogen (N_2).

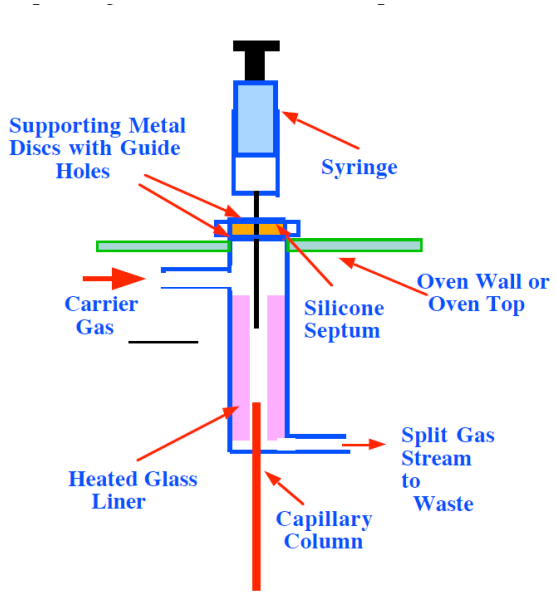


Figure 7 Example of gas chromatography (GC) injector system (after Scott, 2003).

Inside the oven individual components in the sample are separated based on their boiling points and their interactions with the stationary phase. Components with lower boiling points generally exit the column faster since they partition more favourably in the mobile (gas) phase. Components with high boiling points are retained by the liquid phase (stationary) to a greater degree and exit the column later. The oven can either be held at a constant temperature during the analysis (isothermal heating) or temperature can be increased during the analysis, a procedure called temperature programming, to speed up analysis time.

The properties of the column also affect component separation. Wound around a cage and positioned inside the GC oven (Figure 6), columns vary in length, internal diameter and stationary phase composition. The inert solid support of the column is made of fused silica and the stationary phase coating is typically polysiloxane. Varying the functional groups on the polysiloxane influences the column polarity. A compound with polarity similar to the column polarity will react with and be retained by the column, while compounds of different polarity will pass though faster. On exiting the column components can be identified by comparing their retention times, that is the time from analyte injection to component detection, to retention times of standards. Capillary columns are the most common column type.

Inductively coupled plasma-mass spectrometry

A plasma is a body of gas in which a large proportion of the species present are ionised. Characteristics include high temperature and a high electron density. Consequently there is energy available for ionisation of analyte atoms. The ICP torch where the plasma is created consists of three concentric quartz tubes through which Ar gas flows to carry the sample (inner tube), support the plasma (intermediate tube) and cool the torch (external tube). Water cooled copper coil is encircled around the outside cylinder, connected to a radio frequency generator (Figure 8).

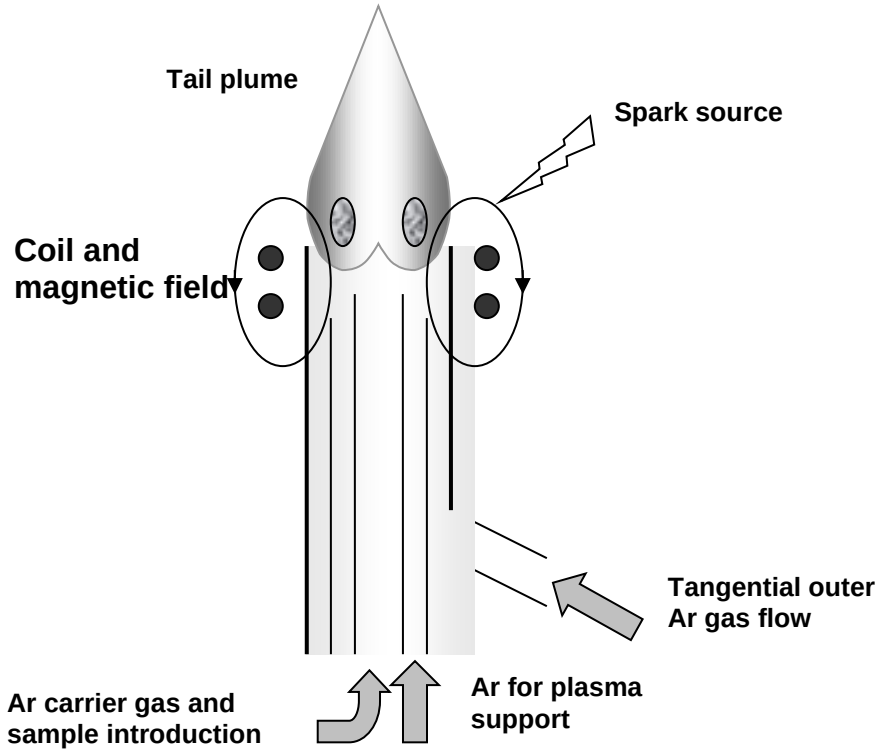


Figure 8 Inductively coupled plasma torch. Adapted from Davidson (2013).

For plasma formation, seed electrons are initially generated in the Ar stream using a spark from a Tesla coil. The radio frequency energy in the coil creates a magnetic field in which electrons are accelerated into circular paths. The electrons collide with the Ar atoms generating more electrons in a self-sustaining plasma (Equation 4).

$$e^- + Ar \rightarrow 2e^- + Ar^+ \qquad \textbf{Equation 4}$$

Some Ar^+ and e^- recombine in the plasma, giving off excess energy as heat and light. With temperatures in the plasma reaching 10000K, a separate flow of Ar is used to

cool the inside of the torch. The high temperature causes molecules entering the plasma to dissociate, atomise and ionise. Aligned horizontally to the MS, the ICP provides the ion source required for mass analysis (Figure 9). The ICP and MS are interfaced using two cones. The sampling cone is a water cooled cone with a small aperture in the tip to allow ions to pass into a low vaccum chamber. The second cone, called the skimmer cone further reduces or 'skims' the sample allowing a small proportion (approximately 1%) to enter the high vacuum chamber (Figure 9). (Kosler and Sylvester, 2003) where the beam is shaped by electrostatic lenses and accelerated into the mass analyser (quadrupole).

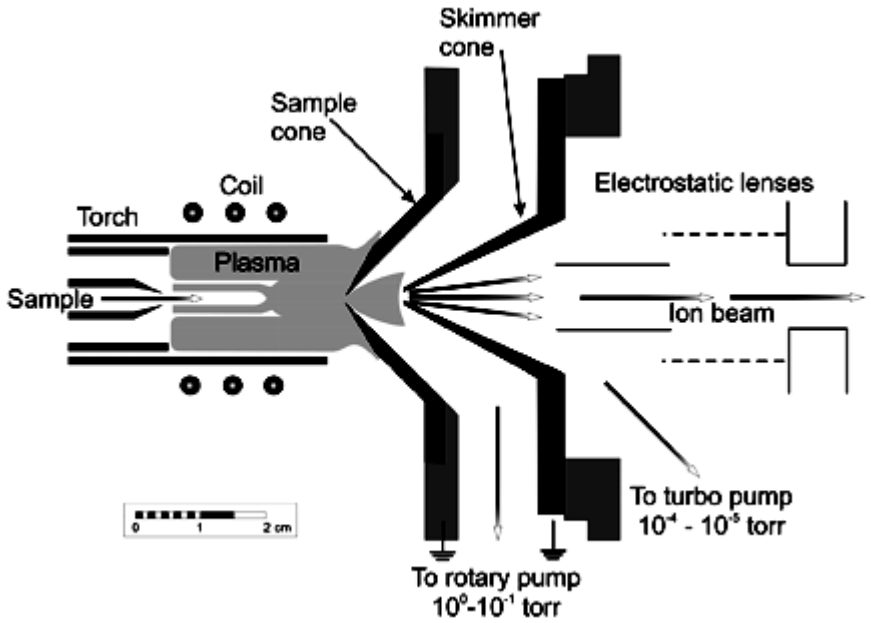


Figure 9 Interface of plasma and mass spectrometer (after Houk, 1986).

In the mass analyser ions are separated based on their mass (m) to charge (z) ratio m/z. In a quadrupole mass analyser ions are separated under the combined influence of direct (dc) and alternating (ac) fields. The quadrupole consists of four parallel metal rods (Figure 10). Each opposing rod pair is connected together. One pair of rods is held at + U volts (dc) and the other pair at –U volts (dc). An ac voltage is applied causing ions to undergo motion perpendicular to their line of flight. The resultant spiral ion paths cause most ions to collide with the rods and be neutralised (non resonant ions). Only ions of a certain m/z said to be 'in resonance' will reach the detector for a given ratio of voltages. This permits selection of an ion with a particular m/z (selected ion monitoring). Varying the ratio of dc to ac voltage allows specific ions to be determined (selective ion monitoring), or the full mass range can be swept (mass scanning).

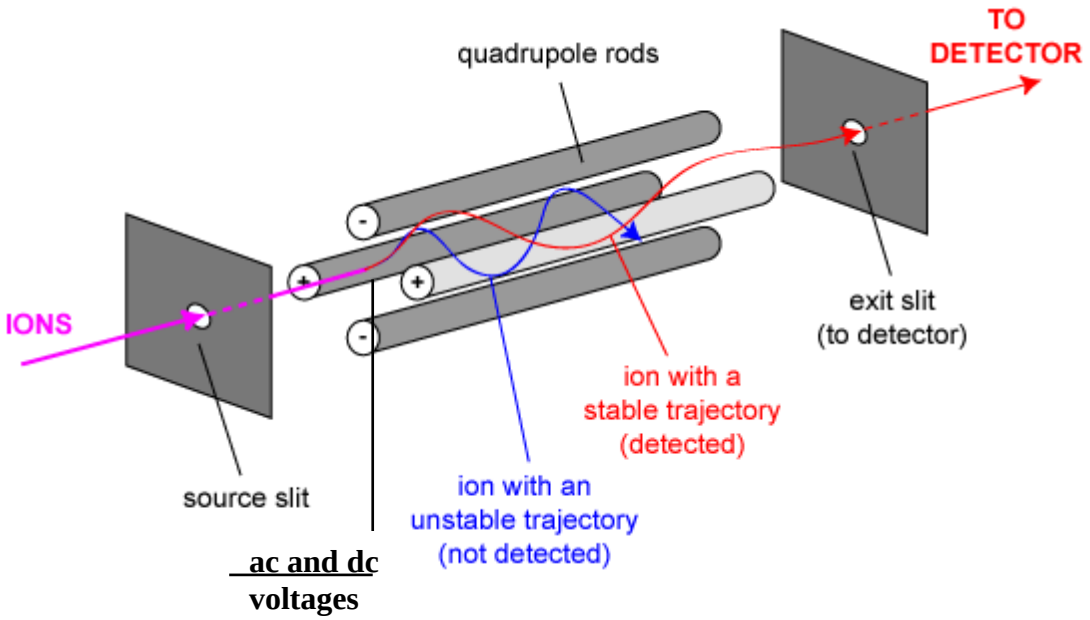


Figure 10 Ion paths through a quadrupole mass. (Adapted from NERC LSMSF, 2008).

On exiting the quadrupole mass analyser the relative abundance of the ions being monitored is determined by the detector. This is commonly achieved using a discrete dynode electron multiplier that converts low ion beam currents into amplified electron current (Restek, 2008). Discrete dynode electron multipliers consist of between 12 and 24 dynodes, with increasing electrical potential (voltage) for successive dynodes (Restek, 2008). When an ion emerges from the quadrupole, it strikes a conversion plate causing it to emit an electron. Similar to the PM tube, on striking successive dynodes, more electrons are released and the signal is amplified (Figure 11). The magnitude of the signal is directly proportional to the number of ions of the m/z being detected.

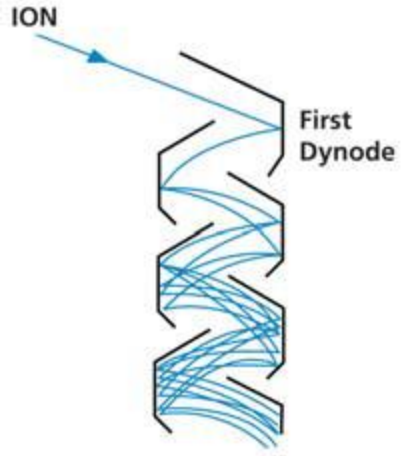


Figure 11 Discrete dynode electron multiplier showing electron multiplication at each successive dynode (after Restek, 2008).

The GC-ICP-MS chromatogram indicates the retention time of each component and the counts generated. A threshold value is set to discriminate between pulses obtained from components of interest and spurious emissions (Thomson, 2002). The identity of each peak is determined by its m/z ratio. The counts for the peaks of interest are normalised against the counts obtained from an internal standard passed through the ICP-MS to allow drifts in the plasma to be accounted for.

Statistical analysis

Software packages

Microsoft Excel 2007 software was used for statistical analysis.

Arithmetic mean concentration

The arithmetic mean, $\overline{x}$, or mean as it is more commonly called, represents the average of the measurements from which it is derived and was calculated using Equation 5

$$\overline{x} = \frac{\sum_{i=1}^{n} x_i}{n} \qquad \textbf{Equation 5}$$

where n is the number of observations, and x_i is the concentration for observation i.

Standard deviation and relative standard deviation

The spread of the values is expressed using the standard deviation, SD. The SD is calculated by taking into account the amount by which each value deviates from the mean as indicated in Equation 6.

$$\mathrm{SD} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \qquad \textbf{Equation 6}$$

The relative standard deviation, RSD, is used to express SD as a percentage of the mean, as indicated in Equation 7.

$$\mathrm{RSD}\ (\%) = \frac{SD}{\bar{x}} \times 100 \qquad \textbf{Equation 7}$$

Limit of Detection

The limit of detection (LOD) is derived from the smallest concentration that can be detected with reasonable certainty for a given analytical procedure (IUPAC, 2014). When using CVAAS this was calculated using Equation 8

$$\mathrm{LOD} = \frac{3 \times SD}{Gradient\ of\ the\ calibration\ slope} \qquad \textbf{Equation 8}$$

where SD was the standard deviation of 10 replicate absorbance values of the lowest concentration standards.

Correlation coefficient and coefficient of determination

The strength of a linear relationship between two variables x and y can be estimated by use of the Pearson product moment correlation, commonly called the correlation coefficient, denoted by r. The value of r is calculated as shown in Equation 9 and expresses the degree of association between the variables (Haynes, 1996)

$$\mathrm{r} = \frac{\Sigma\,(\mathrm{x}-\bar{\mathrm{x}})(\mathrm{y}-\bar{\mathrm{y}})}{\sqrt{\Sigma\,(\mathrm{x}-\bar{\mathrm{x}})^2(\mathrm{y}-\bar{\mathrm{y}})^2}} \qquad \textbf{Equation 9}$$

The range of r is between -1 and +1, with positive r values indicating a positive relationship between the two parameters, in other words when one increases the other also increases, and negative values of r indicating a negative relationship, in other words as one variable increases the other decreases. Categorisation schemes vary but the higher the values of r, the stronger the relationship, positive or negative, between the two variables. A value of $r = \pm 1$ indicates a perfect linear relationship. According to Dancy and Reidy (2004) an r value between 0.7 and 0.9 indicates a strong relationship, a value between 0.4 and 0.6 indicates a moderate relationship, and a value between 0.1 and 0.3 indicates a weak relationship. A value of $r = 0$ indicates no relationship.

The coefficient of determination, r^2, is obtained on squaring the correlation coefficient. This determination is useful since it expresses the proportion of variation in y that is associated with variations in x, the remaining variation therefore is a result of other causes (Haynes, 1996).

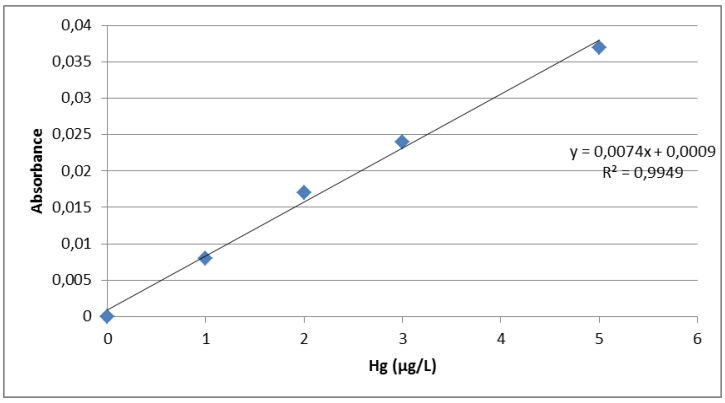
Wilcoxon signed rank test

To assess the difference between paired sets of observations, the Wilcoxon signed rank test was used. A test for non-parametric data, it can be performed when normal distribution cannot be assumed as occurs for example with small sample sets. With an assumption that there is no difference between the two sets of paired data (null hypothesis), the difference between each pair is calculated and sorted according to size and sign. The positive ranks and the negative ranks are summed and the smaller of the two values, Tcalc., is compared to the critical value Tcrit for *n* observations, available from statistical tables. If Tcalc is greater than Tcrit, the null hypothesis is accepted (Kanji, 2006).

Appendix B Naval boat used for sampling in Elefsina Bay, Greece

Appendix C Example calculation of limit of detection for Hg determination by CVAAS

Calibration curve for Hg standard solutions of concentrations 0, 1, 2, 3 and 5 *µ*g/L



Gradiant of calibration slope = 0.007

Absorbance measured for 10 replicates of 1 *µ*g/L Hg standard solution

Replicate	Absorbance of 1 *µ*g/L std
1	0.007
2	0.009
3	0.008
4	0.007
5	0.005
6	0.006
7	0.006
8	0.009
9	0.01
10	0.009

The SD on 10 readings = 0.0016

The LOD is then calculated by:

$$\text{LOD} = \frac{3 \times SD}{Gradient\ of\ the\ calibration\ slope} = 0.668$$

The limit of detection would be 0.668 *µ*g/L.

Appendix D Recovery of Hg in CRM BCR 320R using microwave digestion followed by CVAAS

	Target		Find	
Weight CRM used (g)	**mg Hg**	**µg/L**	**(µg/L)**	**recovery (%)**
0.129	0.000109	1.09	1.01	91
0.1379	0.000117	1.17	1.46	125
0.141	0.000120	1.20	1.55	130

Recovery of Hg from CRM BCR 320R (channel sediment containing 0.85 ± 0.09 mg/kg Hg) was 116 ± 20.3%.

Appendix E Flathead mullet from Elefsina Bay, Greece

Flathead mullet from Elefsina Bay, Greece.

Weight and length of each flathead mullet, weight used for Hg determination and Hg concentration are given in the table below.

Fish identification number	Length (cm)	Weight (g)	Hg (mg/kg w/w)
1	34.2	415	< 0.267
2	35.4	420	< 0.267
3	36.6	423	< 0.267
4	37.3	520	< 0.267
5	30.2	402	< 0.267
6	31.7	414	< 0.267
7	36.9	461	< 0.267
8	38.2	549	< 0.267
9	39.4	573	< 0.267

Appendix F Concentrations of total, mobile, semimobile and non mobile Hg, and OM content in sediment from Elefsina Bay, Greece

	SUMMER							
Location	**Total Hg (mg/kg)**	**Mobile Hg (mg/kg)**	**Mobile Hg (%)**	**Semi-mobile Hg (mg/kg)**	**Semi-mobile Hg (%)**	**Nonmobile Hg (mg/kg)**	**Non mobile Hg (%)**	**OM (%)**
A1	1.93 (2.08, 1.77)	0.455 (0.426, 0.483)	23.6	< 0.067	nc	1.41	73.0	8.36 (8.66, 8.06)
A2	1.88 (1.73, 2.03)	1.62 (1.686, 1.558)	86.2	< 0.067	nc	0.192	10.2	8.96 (7.51, 10.4)
A5	2.96 (2.19, 3.73)	1.66 (1.750, 1.567)	56.1	0.107 (< 0.067, 0.156)	3.60	1.192	40.3	7.23 (6.76, 7.71)
A8	1.40 (1.66, 1.15)	0.465 (0.473, 0.456)	33.1	< 0.067	nc	0.871	62.1	8.09 (7.36, 8.81)
A4	1.50 (1.61, 1.38)	0.334 (0.345, 0.323)	22.4	< 0.067	nc	1.09	73.2	11.8 (11.0, 12.6)
A11	2.86 (3.51, 2.20)	< 0.067	nc	< 0.067	nc	2.74	95.9	9.08 (8.07, 10.1)
K1	0.380 (0.326, 0.434)	0.330 (0.322, 0.339)	86.8	< 0.067	nc	nc	nc	5.80 (6.28, 5.33)
K3	0.821 (0.748, 0.894)	0.442 (0.442, 0.442)	53.8	< 0.067	nc	0.313	38.1	6.88 (8.65, 5.11)
K5	0.312 (0.314, 0.310)	0.172 (0.178, 0.165)	55.0	< 0.067	nc	0.074	23.6	8.12 (5.40, 10.8)

WINTER								
A1	0.514 (0.532, 0.496)	0.376 (0.412, 0.339)	73.1	< 0.067	nc	0.072	13.9	6.06 (6.24, 5.89)
A2	2.07 (2.23, 1.90)	1.36 (1.37, 1.36)	65.9	< 0.067	nc	0.638	30.9	9.11 (8.81, 9.40)
A5	1.88 (1.90, 1.86)	0.572 (0.591, 0.553)	30.4	< 0.067	nc	1.24	66	5.61 (5.28, 5.95)
A8	0.605 (0.460, 0.749)	0.455 (0.451, 0.462)	75.2	< 0.067	nc	0.083	13.7	8.52 (8.65, 8.39)
A4	0.682 (0.687, 0.676)	0.366 (0.361, 0.375)	53.7	< 0.067	nc	0.249	36.5	10.1 (11.1, 9.19)
A11	0.220 (0.254, 0.187)	< 0.067	nc	< 0.067	nc	0.087	39.4	6.98 (6.82, 7.15)
K1	0.580 (0.556, 0.603)	0.356 (0.355, 0.357)	61.4	< 0.067	nc	0.157	27.1	2.26 (2.20, 2.32)
K3	0.461 (0.465, 0.456)	0.414 (0.419, 0.410)	89.9	< 0.067	nc	nc	nc	5.15 (5.79, 4.50)
K5	0.491 ± (0.492, 0.490)	0.371 (0.366, 0.375)	75.5	< 0.067	nc	0.053	10.9	8.31 (8.52, 8.10)

Results given as mean of two values (values in brackets)

nc = not calculated since concentrations were below LOD

Appendix G Total Hg concentration in sediments from the Union Canal, Scotland, U.K. as determined by CVAAS, TDAAS and pXRF

Location	Total Hg by CVAAS (mg/kg)	Total Hg by TDAAS* (mg/kg)	Total Hg by pXRF** (mg/kg)
1	21.9 ± 0.61	25.1 ± 0.12	< 10
2	80.2 ± 6.1	100 ± 4.8	31.5 ± 8.5
4	410 ± 47	451 ± 6.6	173 ± 16
5	565 ± 48	542 ± 27	218 ± 19
6	423 ± 110	433 ± 5.6	155 ± 24
7	199 ± 23	189 ± 2.1	91.3 ± 16
8	300 ± 30	274 ± 18	106 ± 21
10	30.3 ± 3.1	31.3 ± 1.9	< 10

Results given as mean ± SD, n=3

*Data provided by Reis, A. T., (2013)

**Data provided by Aspray, T., (2013)

Appendix H Total Hg content, species mobility and OM content in sediment of the Union Canal, Scotland U.K.

Location	Total Hg (mg/kg)	Mobile Hg (mg/kg)	Mobile Hg (%)	Semimobile Hg (mg/kg)	semi-mobile Hg (%)	Nonmobile Hg (mg/kg)	Nonmobile Hg (%)	OM (%)
1	21.9 ± 0.61	19.0 ± 1.5	86.3	2.63 ± 0.38	12.0	0.268	1.2	6.84 ± 1.6
2	80.2 ± 6.1	66.2 ± 6.3	82.5	1.64 ± 0.37	2.0	12.4	15.4	14.6 ± 3.2
4	410 ± 47	374 ± 28	91.2	6.17 ± 0.87	1.5	29.8	7.3	18.1 ± 1.1
5	565 ± 48	429 ± 44	75.9	6.75 ± 0.66	1.2	129	23	15.6 ± 1.5
6	423 ± 110	339 ± 28	80.1	5.81 ± 1.1	1.4	78.2	18.5	10.9 ± 0.93
7	199 ± 23	187 ± 14	94.0	3.71 ± 0.45	1.9	8.29	4.22	6.12 ± 1.4
8	300 ± 30	232 ± 34	77.3	3.25 ± 0.52	1.1	64.8	21.6	13.7 ± 0.48
10	30.3 ± 3.1	26.3 ± 1.3	86.3	0.399 ± 0.06	1.3	3.6	11.9	8.47 ± 0.16

Results given as mean ± SD (n =3)

Appendix I Hg speciation by TDAAS in sediments of the Union Canal, Scotland, U.K.

Location	Total Hg (mg/kg)	$HgCl_2$/HgFe (mg/kg)	% Hg as $HgCl_2$/HgFe	HgHA/HgS (mg/kg)	% Hg as HgHA/HgS (mg/kg)	HgO* (mg/kg)	% Hg as HgO	% total Hg
1	26.9	19.7	73.2	6.18	23.0			96
2	106.7	102	95					95
4	438	297	67.9	136	31.1	23.8	5.4	104
5	709	677	95					95
6	453	426	94.0					94.0
7	198	193	97					97
8	275	248	90.3			4.11	1.50	91.8
10	35.3	28.6	81.1	4.95	14.0			95

Results given as mean ± SD (n =3)

Data provided by Reis, A. T., (2013)

*Results based on literature value (Biester *et al.,* 1999) for HgO desorption

Appendix J Tuning parameters for ICP-MS

Tune Report

Tune File : ATUNE.U
Comment : Hg speciation

Tuning Parameters

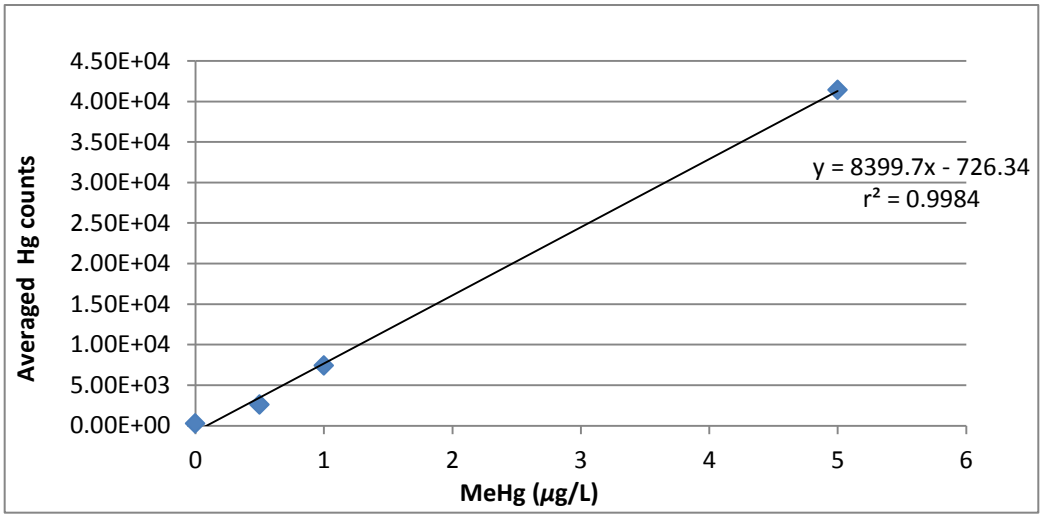
===Plasma Condition===			===Ion Lenses===			===Q-Pole Parameters===		
RF Power	:	1360 W	Extract	:	2.5 V	AMU Gain	:	130
RF Matching	:	1.53 V	Einzel 1,3	:	-70 V	AMU Offset	:	127
Smpl Depth	:	7.5 mm	Einzel 2	:	15 V	Axis Gain	:	1.0004
Torch-H	:	1.3 mm	Cell Entrance	:	-23 V	Axis Offset	:	-0.07
Torch-V	:	0.6 mm	Cell Exit	:	-37 V	QP Bias	:	-6.5 V
Carrier Gas	:	0.78 L/min	Plate Bias	:	-49 V			
Makeup Gas	:	0.37 L/min				===Detector Parameters===		
Optional Gas	:	0 %				Discriminator	:	8 mV
Nebulizer Pump	:	0.2 rps	===Octopole Parameters===			Analog HV	:	2910 V
Sample Pump	:	--- rps	OctP RF	:	191 V	Pulse HV	:	1880 V
S/C Temp	:	--- degC	OctP Bias	:	-3 V			

===Reaction Cell===

Reaction Mode	:	OFF						
H2 Gas	:	0 mL/min	He Gas	:	0 mL/min	Optional Gas	:	0 %

m/z	Range	Count	Mean	RSD%	Background
7	50,000	28337.0	28921.8	1.97	0.30
89	100,000	65827.0	66291.7	1.84	0.60
205	100,000	72699.0	72825.5	1.87	1.00
156/140	5	3.697%	3.564%	5.53	
70/140	5	1.887%	1.914%	5.38	

Appendix K Calibration curve obtained using MeHg standard solutions of concentration 0, 0.5, 1.0 and 5.0 µg/L



Appendix L Total Hg, MeHg and EtHg concentration, %MeHg and %EtHg, OM content and pH in sediments from the Union Canal, Scotland, U.K.

Location	Total Hg (mg/kg)	MeHg (*μ*g/kg)	%MeHg	EtHg	%EtHg	OM (%)	pH
1	35.3 ± 7.3	8.17 ± 2.1	0.023	< 1.16	NC	7.6 ± 0.8	6.94
5	1200 ± 180	10.8 ± 2.9	0.001	6.12 ± 1.0	0.0005	13.9 ± 2.5	5.71
6	571 ± 70	6.11 ± 2.1	0.001	2.49 ± 1.1	0.0004	5.10 ± 2.0	6.11
7	742 ± 94	18.6 ± 4.2	0.003	4.11 ± 1.0	0.0006	11.4 ± 0.8	6.40
8	787 ± 220	9.93 ± 1.2	0.001	3.73 ± 0.6	0.0005	13.7 ± 1.5	5.92
10	71.7 ± 8.2	6.02 ± 2.0	0.008	< 1.16	NC	7.6 ± 0.8	6.43

Results for total Hg, MeHg and EtHg concentration and OM content are given as mean ± SD (n=3).