Chemical Speciation and Transformation of Mercury in Contaminated Sediments

Andreas Drott
Faculty of Forest Sciences
Department of Forest Ecology and Management
Umeå

Doctoral Thesis
Swedish University of Agricultural Sciences

Acta Universitatis agriculturae Sueciae
2009:22

ISSN 1652-6880
ISBN 978-91-86195-69-4

Tryck: Arkitektkopia AB, Umeå 2009

Chemical Speciation and Transformation of Mercury in Contaminated Sediments

Abstract

Biomagnification of mercury (Hg) in aquatic food webs occurs almost exclusively as mono-methyl Hg (MeHg). In this thesis, the influence of chemical speciation and environmental conditions on transformations of inorganic Hg (Hg^{II}) and MeHg was studied at eight sites in Sweden with Hg contaminated sediments. The source of contamination was either Hg^0(l) or phenyl-Hg, and total Hg concentrations ranged between 1.0-1100 nmol g^{-1}. The environmental conditions, e.g. salinity, temperature climate, primary productivity, redox conditions and organic matter content and quality, varied substantially among sites. The results show that MeHg production (Hg^{II} methylation) is relatively more important than MeHg degradation (demethylation) and input-output for accumulation of MeHg in contaminated surface (0-20 cm) sediments. The total Hg concentration influences MeHg production, likely by a control of the concentration of bioavailable Hg^{II} species. The most important factor determining differences in accumulation of MeHg among sites is indicated to be the availability of electron donors to methylating organisms, as a result of differences in primary production and subsequent input of organic matter to sediments. In contrast, the availability of sulphate is not indicated to limit MeHg production in the sediments studied. Within sub-sets of sites with similar properties, a great proportion of the variation in MeHg concentration is explained by the concentration of dissolved neutral Hg^{II}-sulphides [$Hg(SH)_2^0$(aq) and possibly $HgOHSH^0$(aq)]. MeHg degradation is influenced by ambient concentrations of MeHg and/or Hg^{II}, but the effect appears to vary among sites. It is suggested that the rate of oxidative demethylation is positively related to the concentration of dissolved MeHg-sulphides [MeHgSH(aq) and $MeHgS^-$(aq)]. For improved risk assessment of Hg contaminated sediments, measurement of MeHg concentration and solubility is advised. It is shown that %MeHg (of total Hg) can be used as a proxy for MeHg production, across sites. It is also shown that filtration of pore water for analysis of MeHg concentrations must be done in an anoxic atmosphere to avoid oxidation artefacts.

Keywords: mercury, methyl mercury, contamination, sediment, methylation, demethylation, chemical speciation, biomagnification, transport

Author's address: Andreas Drott, Dept. of Forest Ecology and Management, SLU S-90183 Umeå, Sweden
E-mail: Andreas.Drott@sek.slu.se

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Drott A., Lambertsson L., Björn E., Skyllberg U. (2007). Effects of oxic and anoxic filtration on determined methyl mercury concentrations in sediment pore waters. *Marine Chemistry* 103, 76-83.

II Drott A., Lambertsson L., Björn E., Skyllberg U. (2008). Do Potential Methylation Rates Reflect Accumulated Methyl Mercury in Contaminated Sediments? *Environmental Science & Technology* 42, 153-158.

III Drott A., Lambertsson L., Björn E., Skyllberg U. (2007). Importance of Dissolved Neutral Mercury Sulfides for Methyl Mercury Production in Contaminated Sediments. *Environmental Science & Technology* 41, 2270-2276.

IV Drott A., Lambertsson L., Björn E., Skyllberg U. (2008). Potential demethylation rate determinations in relation to concentrations of MeHg, Hg and pore water speciation of MeHg in contaminated sediments. *Marine Chemistry* 112, 93-101.

Abbreviations

AAS	Atomic absorption spectrometry
DOC	Dissolved organic carbon
FeRB	Iron reducing bacteria
GC-ICPMS	Gas chromatography inductively coupled plasma mass spectrometry
Hg	Mercury
Hg^0	Elemental mercury
Hg^{II}	Inorganic mercury
MeHg	Mono-methyl mercury
NOM	Natural organic matter
SRB	Sulphate reducing bacteria

1 Introduction

1.1 Mercury in the Environment

Mercury [Hg, Hydrargyrum (Greek, "watery silver")] is well known as an environmental pollutant. Its electron configuration makes the properties of Hg unique, and it is the only metal that is a liquid at room temperature (Zumdahl, 1998). Because of its unique properties, Hg has been widely used for industrial applications. Hg is released into the environment from diffuse as well as local anthropogenic sources (Figure 1). The most important diffuse source is the combustion of fossil fuels (U.N., 2002). It is estimated that release of Hg into the atmosphere from diffuse anthropogenic sources has increased the deposition rate of Hg to lake sediments 3-5 times compared to pre-industrial times (Bindler *et al.*, 2001). In recent years, local contamination in general, including Hg, has gained interest. In Sweden, great resources are being invested in risk assessment and remediation of locally contaminated soils, buildings, waters and sediments, as a part of the Swedish environmental quality objective "a non-toxic environment" (Swedish government, 2000). In this process, Hg is among the most highly prioritized compounds (Börjesson, 2008).

There are numerous sites in Sweden with Hg contaminated sediments. The most important local Hg sources in Sweden are the pulp and paper- and the chlor-alkali industry. In the pulp and paper industry, phenyl Hg acetate has been used to prevent microbial growth in water systems and to impregnate pulp. The use of Hg in the pulp and paper industry in Sweden started during World War II and was banned in 1966-1967, (Länsstyrelsen Dalarna, 2000). In the chlor-alkali process, elemental Hg [$Hg^0(l)$] is used as cathode during electrolysis of sodium chloride (NaCl) and water. It is a

recommendation that the use of Hg in the chlor-alkali process should be replaced by other methods (e.g. diaphragm or membrane methods) within the European Union in 2010 (OSPAR, 1990). The products of the chlor-alkali industry, chlorine gas, $Cl_2(g)$, and sodium hydroxide, NaOH, are used in the pulp and paper industry. Therefore, pulp and paper- and chlor-alkali industries have often been situated close to each other, and Hg has in both cases been discharged to nearby waters, often associated to pulp fibre, ending up in pulp fibre enriched sediments.

In the environment, transformations between different chemical forms, or *species*, of Hg occur (Figure 1). A *chemical species* is defined as a "specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure", and *speciation* is defined as the "distribution of an element amongst defined chemical species in a system". For the analytical measurement of chemical species, the term *speciation analysis* should be used (Templeton *et al.*, 2000). Hg can form a number of species, with different properties. It occurs in three oxidation states, Hg^0, elemental Hg, Hg^I, mercurous Hg, and Hg^{II}, mercuric Hg. The monovalent oxidation state is not considered to be of quantitative importance in the environment, and Hg^0 is volatile (vapour pressure 0.17 Pa at 25°C), and can be oxidised to Hg^{II}. Thus, Hg^{II} is considered to be dominant in soils and sediments. Hg^{II} can also form a number of organometallic compounds by covalent bonding of Hg to short-chain alkyls and phenyls. Examples are phenyl Hg (PhHg), di-methyl Hg (Me_2Hg) and mono-methyl Hg (CH_3Hg^+, MeHg) (U.N., 2002). *Note that* in this thesis, Hg^{II}, or inorganic Hg, is used to denote Hg species with oxidation state +II, but *not* including organometallic compounds. Organometallic compounds are denoted by their abbreviations, e.g. MeHg for mono-methyl Hg. The speciation of Hg^{II}, the dominant Hg form, and MeHg, the dominant organometallic Hg form, in sediments is complex, and they both can form a number of different species, depending on the conditions. This will be discussed in more detail in sections 1.6 and 1.7.

Hg is toxic to higher organisms, its toxicity being caused by a strong association to sulphur (S) (Carty & Malone, 1979). However, toxicity differs among species. Because of their lipid solubility, organometallic Hg compounds can cross the blood-brain barrier, causing damage mainly to the central nervous system. This is also the case for Hg^0 vapour, but not for Hg^{II} compounds (Langford & Ferner, 1999). Gastro-intestinal absorption of Hg^0 as the liquid metal is less than 0.01 % of the ingested dose (Bornmann *et al.*,

1970), thus it rarely causes acute toxic effects (Langford & Ferner, 1999). Organometallic Hg compounds also have the ability to cross the placental barrier, causing foetal damage. It is well documented that the developing central nervous system is more sensitive to the toxicity of Hg than is the adult (W.H.O., 1990). Thus, organometallic Hg compounds, and quantitatively most important MeHg, pose the greatest toxicological risk.

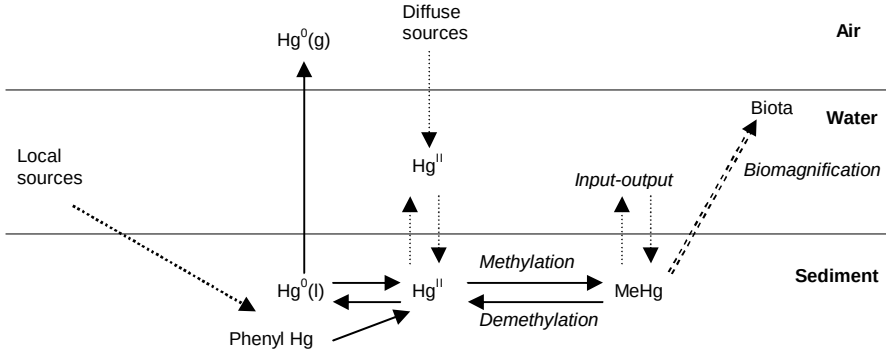

Figure 1. Principal transformation (unbroken arrows), transport (broken arrows) and biomagnification (double broken arrow) of Hg in a contaminated sediment.

In fish tissue, MeHg constitutes 80-95 % of total Hg (Bloom, 1992; Downs *et al.*, 1998; Grieb *et al.*, 1990), while in sediments the % MeHg of total Hg is less than a few % (Gilmour & Henry, 1991; Heyes *et al.*, 2006). The % MeHg of total Hg as well as the absolute total Hg concentration increases with trophic level in the aquatic food web, thus *biomagnification* of MeHg is taking place, Figure 1 (Downs *et al.*, 1998). The high concentration of Hg in fish from Swedish waters (Håkansson, 1996) is the reason for current recommendations to women in fertile age in Sweden to limit their intake of local fish (Petersson-Grawé *et al.*, 2007). The concentration of Hg in biota, mostly perch (*Perca fluviatilis*), at the eight contaminated sites studied in this thesis, was elevated compared to at nearby reference sites, demonstrating the importance of local contamination for Hg concentrations in fish (Skyllberg *et al.*, 2007). The quantitatively most important pathway for Hg into fish is likely via methylation in sediment or bottom water and subsequent biomagnification of MeHg through the food web (Downs *et al.*, 1998; Morel *et al.*, 1998). The concentration of MeHg in sediments that is potentially available for uptake in organisms is a net result of MeHg production (*methylation*), MeHg degradation (*demethylation*) and *input-output* processes (Figure 1). Thus, increased knowledge about methylation, demethylation and transport of MeHg in contaminated sediments is essential.

1.2 Hg Methylation

Methylation of Hg in sediments is predominantly biotic (Jensen & Jernelöv, 1969), even if abiotic Hg methylation, e.g. by humic substances, may occur (Nagase *et al.*, 1984; Nagase *et al.*, 1982). However, the biochemical mechanisms of biological Hg methylation have not been fully elucidated. Several studies, using inhibitors and stimulators (Compeau & Bartha, 1985; Gilmour *et al.*, 1992) as well as molecular techniques combined with Hg methylation and sulphate reduction rate measurements (Devereux *et al.*, 1996; King *et al.*, 2001), have demonstrated that sulphate reducing bacteria (SRB) are important Hg methylators in sediments. Recently, it was observed that addition of molybdate (MoO_4^{2-}), a known inhibitor of SRB activity, only inhibited about half of the Hg methylation in a freshwater sediment, and that an isolated iron reducing bacterium (FeRB) methylated Hg at a rate comparable to SRB (Fleming *et al.*, 2006). Several strains of FeRB have also been shown to methylate Hg in pure culture (Fleming *et al.*, 2006; Kerin *et al.*, 2006).

Two main groups of SRB exist: complete oxidisers, who oxidise acetate to carbon dioxide (CO_2), and incomplete oxidisers, who oxidise low molecular weight fatty acids (e.g. lactate, propionate and butyrate) and alcohols to acetate (Konhauser, 2007). Therefore, SRB require *i)* suitable organic substrate (electron donor), *ii)* sulphate (SO_4^{2-}, electron acceptor), and *iii)* optimum temperature, for their activity. In addition, many SRB are capable of oxidising inorganic substrates [e.g. sulphur compounds and hydrogen gas, $H_2(g)$], and are also able to switch to nitrate (NO_3^-) or oxygen (O_2) as electron acceptors (Dannenberg *et al.*, 1992). Most SRB that have been found to methylate Hg are found within the δ-subclass of the *Proteobacteria* (Fleming *et al.*, 2006; Kerin *et al.*, 2006), which are gram negative. To date, a biochemical mechanism of Hg methylation in SRB has only been reported for the strain *Desulfovibrio desulfuricans* LS. Combining a variety of methods, it was concluded that MeHg in *D. desulfuricans* LS is synthesised by enzymatic transfer of a methyl group to Hg^{II} via methylcobalamin (methylated vitamin B12) in the acetyl-coenzyme A (acetyl-CoA) pathway (Choi & Bartha, 1993; Choi *et al.*, 1994a; Choi *et al.*, 1994b). The acetyl-CoA pathway is a metabolic pathway used by acetogens, methanogens, and complete oxidising SRB. In the case of SRB, the pathway runs in the acetate-oxidising direction (Figure 2).

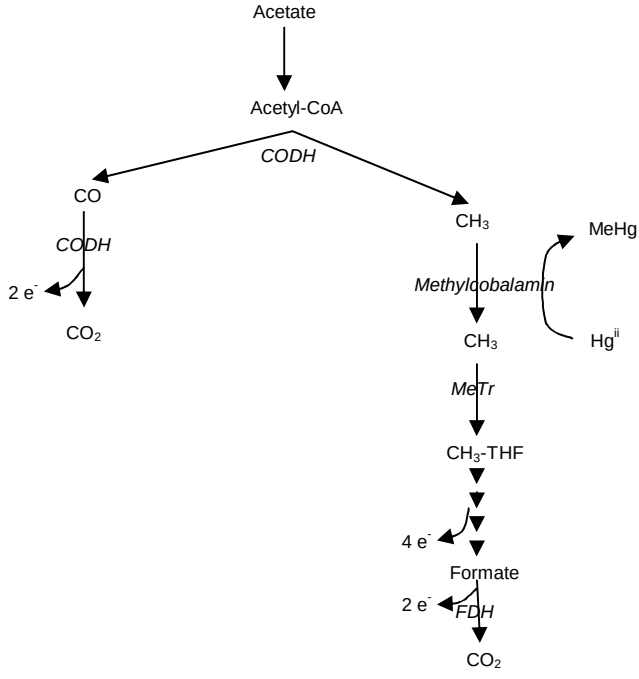

Figure 2. The acetyl-CoA pathway used for acetate oxidation in complete oxidising SRB. The possible methylation of Hg is indicated to the right. Modified from Ekstrom *et al.* (2003).

Since only complete oxidising SRB use the acetyl-CoA pathway in their metabolism, it is contradictory that *D. desulfuricans* LS was found to be an incomplete oxidiser (Compeau & Bartha, 1985). More recent work using chloroform, which inhibits the acetyl-CoA pathway, indicates that incomplete oxidising SRB are independent of the acetyl-CoA pathway both for their metabolism and for their ability to methylate Hg (Ekstrom *et al.*, 2003). Recent work also suggests that Hg methylation in complete oxidisers may be limited by the availability of cobalt (Co), which is the active center in methylcobalamin, whereas Hg methylation in incomplete oxidisers is not Co-limited (Ekstrom & Morel, 2008). This supports Hg methylation in complete oxidisers via methylcobalamin, but indicates a different mechanism for Hg methylation in incomplete oxidisers. To my knowledge, no mechanism for Hg methylation in FeRB has been proposed. It can be noted that certain FeRB, e.g. *Geobacter* sp., belong to the δ-*Proteobacteria* and thus are phylogenetically close to Hg methylating SRB (Fleming *et al.*, 2006).

1.3 MeHg Demethylation

Biotic as well as abiotic MeHg demethylation mechanisms have been described. Abiotic mechanisms include photodegradation (Sellers *et al.*, 1996) and degradation by reaction of MeHg with H_2S, eventually forming volatile Me_2Hg, and HgS (Deacon, 1978). Studies in estuarine sediment have indicated that abiotic demethylation mechanisms may be quantitatively important (RodriguezMartin-Doimeadios *et al.*, 2004), however, according to the authors, the sterilisation method applied may not have been effective enough, and therefore this result needs to be validated. Two main mechanisms for biotic MeHg demethylation have been described: reductive demethylation via the mercury resistance (*mer*) operon, and oxidative demethylation.

During reductive demethylation via the *mer* operon, two genes (*mer*B and *mer*A, respectively) code for two different enzymes, of which the first, organomercurial lyase, cleaves the bond between carbon and Hg, and the second, mercuric reductase, reduces Hg^{II} to Hg^0 (Robinson & Tuovinen, 1984). This is referred to as broad-spectrum resistance, in contrast to narrow-spectrum resistance, where only mercuric reductase is produced. Induction of the *mer* system is regulated by the *mer*R gene (Barkay *et al.*, 2003), which has been shown to be induced by Hg^{II} and phenyl mercury acetate (Nucifora *et al.*, 1989). However, despite a number of pure-culture studies, (e.g. Clark *et al.*, 1977; Schottel *et al.*, 1974) there is, to my knowledge, only one report that induction of the *mer* system by MeHg(Cl) has been tested (and supported) for a broad-spectrum resistant organism (Furakawa *et al.*, 1969). As has been pointed out by Selifonova *et al.* (1993), MeHgCl salts may contain traces of Hg^{II}. Thus, at this point we do not know if the *mer* system can be induced by MeHg. In contrast to biological methylation abilities, biological demethylation abilities are common among different groups of bacteria, and the *mer* operon is widespread in the environment, occurring for instance in soils (Pearson *et al.*, 1996) and marine waters (Dahlberg & Hermansson, 1995). In addition to reductive *mer*-mediated demethylation, another reductive mechanism where HS^-, produced by SRB, reacts with two MeHg to form HgS(s), MeHg and CH_4, has been proposed (Baldi *et al.*, 1993).

Oxidative demethylation was first proposed when it was noticed that the end-product of demethylation from added isotope enriched $^{14}CH_3Hg^+$ was $^{14}CO_2$ and not, as expected from reductive *mer*-mediated demethylation, $^{14}CH_4$ (Oremland *et al.*, 1991). It was suggested that oxidative

demethylation was part of microbial metabolism, and that MeHg was used as electron-donor. Thus, oxidative demethylation is not an active detoxification mechanism. Mechanisms for oxidative demethylation have been proposed for sulfate reducers and methanogens (Marvin-DiPasquale & Oremland, 1998), but no organism has been isolated in pure culture (Benoit *et al.*, 2003).

In studies with isotopically labelled MeHg, the relative importance of reductive *mer*-mediated demethylation and oxidative demethylation varies with the level of Hg contamination. In highly Hg contaminated environments (total Hg concentration in sediments 22-106 nmol g^{-1}), reductive *mer*-mediated demethylation (as indicated by production of $^{14}CH_4$) was dominant, while in less contaminated environments, oxidative demethylation (as indicated by production of $^{14}CO_2$) was dominant (Marvin-DiPasquale *et al.*, 2000; Schaefer *et al.*, 2004).

1.4 Previous Work on Sample Treatment for Analysis of MeHg in Sediment Pore Water (Paper I)

Accurate determination of MeHg concentrations in sediment pore water is important for increased knowledge about Hg biogeochemistry in sediments. However, to my knowledge, previous work on sample treatment and its effects on determined concentrations of MeHg in sediment pore water is limited to one report (Mason *et al.*, 1998).

If, as often is the case, there are no possibilities to determine dissolved analyte concentrations in sediments directly by e.g. ion selective electrodes, the dissolved fraction is operationally defined and is usually obtained by filtration through a filter of certain pore size (often 0.45 μm), or by the use of dialysis membranes of certain pore size. Several potential pitfalls in sampling and sample treatment for analysis of trace metals in sediment pore water are known, e.g. oxidation, contamination, and temperature artefacts (Bufflap & Allen, 1995). The techniques applied in practice for pore water extraction for Hg and MeHg analysis vary, and include direct anoxic (N_2) filtration (Gilmour *et al.*, 1998), and anoxic (N_2) extrusion and centrifugation followed by anoxic filtration (Mikac *et al.*, 1999). Mason *et al.* (1998) compared several pore water sampling methods for Hg and MeHg analysis, namely: *i)* anoxic (N_2) extrusion and centrifugation followed by oxic filtration, *ii)* direct oxic vacuum filtration (without preceeding

centrifugation), *iii)* core squeezing using N_2 pressure, and *iv)* the use of dialysis membranes ("peepers"). It was concluded that centrifugation (*i*) was the most promising technique, in part because of the rather large volumes of pore water required for MeHg analysis. Direct oxic vacuum filtration (*ii*) was not considered to be reliable, mostly because of oxidation problems.

However, in the report by Mason *et al.*, the effects of oxic and anoxic filtration after anoxic centrifugation were not studied, as anoxic centrifugation combined with anoxic filtration was not included among the methods compared.

1.5 Previous Work on the Importance of Methylation, Demethylation and Transport for Accumulation of MeHg in Sediments (Papers II and IV)

Advances in analytical methodologies have enabled measurement of potential Hg methylation and MeHg demethylation rates in sediments using stable isotope tracers of Hg^{II} and MeHg (Hintelmann *et al.*, 2000; Lambertsson *et al.*, 2001), see section 2.6. In studies where such techniques have been applied, a positive relationship has been reported between the potential methylation rate, expressed either as the rate (e.g. mol $g^{-1}h^{-1}$), or as the specific potential methylation rate constant, k_m (day^{-1}), and the concentration of MeHg, in surface (0-15 cm) sediments from freshwaters (Benoit *et al.*, 2003), estuaries (Heyes *et al.*, 2006; Sunderland *et al.*, 2004) and marine environments (Hammerschmidt & Fitzgerald, 2006).

A positive relationship between Hg methylation rate and MeHg concentration indicates that methylation is relatively more important for MeHg accumulation than are demethylation and transport. The relationship has been improved by normalising the concentration of MeHg to total Hg, expressing the concentration as % MeHg of total Hg (Hammerschmidt & Fitzgerald, 2006). However, this relationship has not been evaluated in highly (> 1 nmol Hg g^{-1}) contaminated sediments. Nor has it been tested if it persists across a number of sites with different properties.

In $^{14}CH_3Hg^+$-labelling studies, the measured rate of demethylation increased with increasing total Hg concentration, i. e. a positive relationship was reported between total Hg concentration and specific potential demethylation rate constant, k_d (day^{-1}) (Marvin-DiPasquale *et al.*, 2000;

Schaefer *et al.*, 2004). Marvin-DiPasquale *et al.* also reported a positive relationship between total MeHg and k_d for one sub-set of data. These results indicate that demethylation may be relatively more important for the concentration of MeHg where total Hg and MeHg concentrations are higher.

1.6 Previous Work on Speciation of Hg^{II} and Its Influence on MeHg Production, with Emphasis on Anoxic Sediments (Papers II and III)

There are several reports of a positive relationship between total Hg and MeHg concentrations in sediments, both at sites that have been contaminated by local sources, and at sites that were contaminated by more diffuse sources (e.g. Benoit *et al.*, 2003; Benoit *et al.*, 1998; Hammerschmidt & Fitzgerald, 2004; Hammerschmidt & Fitzgerald, 2006; Sunderland *et al.*, 2006). However, there are also reports that this relationship is lacking (e.g. Benoit *et al.*, 2003; Lambertsson & Nilsson, 2006). A positive relationship between total Hg and MeHg concentrations may be explained by a linkage between total Hg and bioavailable Hg^{II} forms. The bioavailability is known to differ between Hg^{II} species, thus production of MeHg should be partly dependent on Hg^{II} speciation.

Being a soft Lewis acid, the speciation of Hg^{II} is to a great extent linked to sulphur chemistry (McBride, 1994). Spectroscopic studies have shown that Hg^{II} is coordinated linearly to two thiol groups in natural organic matter, NOM (Skyllberg *et al.*, 2006). The strong affinity of Hg^{II} for thiol groups results in a complete dominance of Hg-dissolved organic matter (DOM) complexes over complexes with other ligands (e.g. halides) in soil solutions and streams under oxidising conditions (Skyllberg *et al.*, 2003).

Under reducing conditions (Figure 3), as in many sediments, inorganic sulphides [$H_2S(aq)$, $HS^-(aq)$, $S^{2-}(aq)$] are also important ligands for Hg^{II} (Dyrssén & Wedborg, 1991). With inorganic sulphide, Hg^{II} can form the solid phases cinnabar (α-HgS(s), red, 2-coordinated) and metacinnabar (β-HgS(s), black, 4-coordinated). When iron (Fe) also is present in adequate concentration, iron sulphides, FeS(s), with varying degree of order, are formed. Hg^{II} is adsorbed to surfaces of FeS(s), and mixed Hg/FeS(s) phases may also form (Wolfenden *et al.*, 2005). In solution, Hg^{II} can form a number of inorganic sulphide complexes [e.g. $HgSH_2^0(aq)$, $HgS_2H^-(aq)$]. At intermediate redox conditions, elemental sulphur (S^0) may be present, and

Hg-polysulphide complexes [e.g. $Hg(S_x)(SH)^-$, where S_x denotes a polysulphide and x = 2-6] may form in solution (Paquette & Helz, 1995; Paquette & Helz, 1997). The knowledge about polysulphides is increasing, but it is still limited, and both the identity and stability constants of Hg-polysulphide species are uncertain (Jay *et al.*, 2000). In fundamental studies, the solubility of Hg^{II} in equilibrium with HgS(s) was explained with the species $HgSH_2^0$(aq), HgS_2H^-(aq) and HgS_2^{2-}(aq) at a constant total sulphide concentration of 0.2 M and pH 1-12 (Schwarzenbach & Widmer, 1963), as well as at a concentration of 1-100 mM S^{-II} and pH 1-12. It was also shown that polysulphides are important for Hg^{II} solubility if elemental S is present (Paquette & Helz, 1997). However, no experimental data on the solubility of HgS(s), with or without elemental S, below 10 µM S^{-II}, have been reported (Jay *et al.*, 2000).

Observed decreases in total MeHg concentrations with increasing pore water sulphide concentration in sediments (Benoit *et al.*, 1998), led to the hypothesis that the concentration of Hg^{II}-sulphides that are bioavailable to methylating bacteria decreases with increasing pore water sulphide concentration. As the described mechanism for Hg methylation in *Desulfovibrio desulfuricans* LS (section 1.2) is a side reaction, it was further hypothesised that the uptake of Hg^{II} in methylating bacteria occurs by passive diffusion and that dissolved neutral inorganic Hg-sulphides, which are small and uncharged, are the most important bioavailable Hg^{II} species in sediments with sulphidic conditions (Benoit *et al.*, 1999a), in line with work on bioavailability of Hg-halide complexes with varying charge (Barkay *et al.*, 1997; Mason *et al.*, 1996).

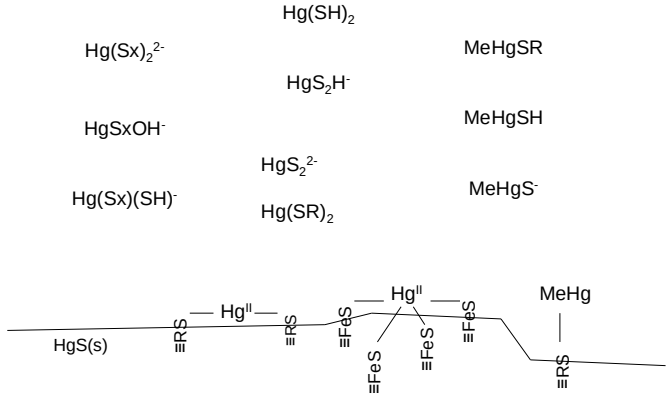

Figure 3. Example of the speciation of Hg^{II} and MeHg in a sediment with reducing conditions. Only species that may be quantitatively important are included. The symbol ≡ denotes a surface.

This hypothesis was tested by developing a chemical speciation model and fitting the output to measured data from the Patuxent river and the Everglades, U.S. (Benoit *et al.*, 1999a), by octanol-water partitioning experiments and speciation modelling over a sulphide gradient (0.001-16 mM S^{-II}) (Benoit *et al.*, 1999b), and by experiments with pure cultures of SRB over a sulphide gradient (0.03-1 mM S^{-II}) in equilibrium with a Hg-containing ore (Benoit *et al.*, 2001), and over a polysulphide gradient in equilibrium with HgS(s) (Jay *et al.*, 2002). In all this work, the importance of the neutral inorganic Hg-sulphide $HgOHSH^0(aq)$ was emphasised, as the chemical speciation models used predict dominance of this species [*Note that* in most of the previous reports, as well as in Paper III, this species is written as $HgS^0(aq)$, but quantum mechanical calculations indicate that the species is stable in aqueous solution in linear form, $HOHgSH^0(aq)$ (Tossell, 2001). In this thesis, this species is always denoted $HgOHSH^0(aq)$.].

Relationships between calculated concentrations of neutral Hg-sulphides and Hg methylation rates measured in environmental samples have not been reported. Also, in my opinion, there are several contradictions about the chemical speciation models used, and especially about the stability constant for $HgOHSH^0(aq)$, as shown by the great discrepancy between two constants for this species estimated by different approaches (Dyrssén & Wedborg, 1991).

1.7 Previous Work on Speciation of MeHg and its Influence on Demethylation (Paper IV)

The speciation of MeHg in sediments has several implications, being important for MeHg mobility, bioavailability, and possibly demethylation. Similar to Hg^{II}, MeHg has strong affinity for sulphur. The strong bonding of MeHg to thiol groups on NOM (Qian et al., 2002) results in complete dominance of MeHg-DOM complexes over dissolved complexes with other strong ligands (e.g. halides) under oxidising conditions (Karlsson & Skyllberg, 2003). Under reducing conditions (Figure 3), MeHg forms strong complexes with inorganic sulphides in solution, e.g. MeHgSH(aq). MeHg also adsorbs to surfaces of FeS(s) minerals, but does not form solid phases with sulphide. In a model seawater with 0.6 M Cl, but where organic thiols were not included, there was a complete dominance of MeHgSH over complexes with halides, e.g. MeHgCl, at 10 nM HS^- (Dyrssén & Wedborg, 1991).

To my knowledge, there are no reports on relationships between MeHg speciation and demethylation. Passive diffusion has been proposed as the uptake mechanism for MeHg during reductive *mer*-mediated demethylation (Barkay *et al.*, 2003), as the *mer*P and *mer*T transport system, which is responsible for transport of Hg^{II}, has been shown not to function as transporter for MeHg (Kiyono *et al.*, 1995). The knowledge about oxidative demethylation is limited, and no mechanism for MeHg uptake during oxidative demethylation has, as far as I know, been proposed.

1.8 Objectives

This thesis is focused on locally Hg contaminated sediments, with high (> 1 nmol g^{-1}) total Hg concentration. The purpose is to increase our mechanistic understanding of Hg biogeochemistry in sediments using a combination of field and laboratory methods. In short, the more specific objectives of this thesis are:

- To evaluate the effect of sample treatment, specifically filtration under oxic and anoxic conditions, on determined concentration of MeHg in sediment pore water (Paper **I**)

- To evaluate to what extent short-term potential methylation rates determined in the laboratory are related to the build-up of MeHg in sediments (Paper **II**)

- To determine the influence of speciation of Hg^{II} on production of MeHg in contaminated sediments, and to evaluate different chemical speciation models (Paper **III**)

- To evaluate relationships between concentrations of MeHg and Hg, and pore water speciation of MeHg, and demethylation rates (Paper **IV**)

- To determine the most important factors controlling accumulation of MeHg in contaminated sediments (Papers **II**, **III** and **IV**)

2 Materials and Methods

2.1 Site Descriptions and Sampling Occasions

Sampling of sediments was done in 2004-2006 at eight Hg contaminated sites in Sweden (Table 1, Figure 4). Four sites; Köpmanholmen (Köp), Skutskär (Sku), Ala Lombolo (Ala) and Marnästjärn (Mar) had been contaminated by Hg^0(l), and four sites; Karlshäll (Kar), Turingen (Tur), Övre Svartsjön (Sva), and Nötöfjärden (Nöt), had been contaminated by phenyl-Hg. The Hg^0 contamination had either been caused by the chlor-alkali industry (Köp and Sku) or by industrial activities related to the mining (Ala) or engineering (Mar) industry. The phenyl-Hg contamination had been caused by the pulp and paper industry. The ambient total Hg concentration ranged between 1.0-1100 nmol g^{-1} and was about one order of magnitude higher at the sites contaminated by Hg^0(l), Table 1. Phenyl-Hg is considered to be unstable and degrade to Hg^{II} in the environment. Thermo-desorption (TD) measurements (Biester & Scholz, 1997), section 2.5, showed that Hg^{II} was the dominant form of Hg at all sites, and traces of Hg^0 remained only at site Köp.

The sites Köp and Sku were situated in brackish water estuaries (chloride concentration 80-90 mM, Table 1), while the rest of the sites were freshwaters (chloride concentration 0.20-2.0 mM). The freshwaters were either estuaries that were sheltered from the sea (Kar, Nöt), or lakes (Ala, Mar, Tur, Sva). There was a wide range in climate among the sites, with average annual temperature sums (threshold 5° C) ranging from 520° C at the northernmost site Ala to 1500° C at the southernmost

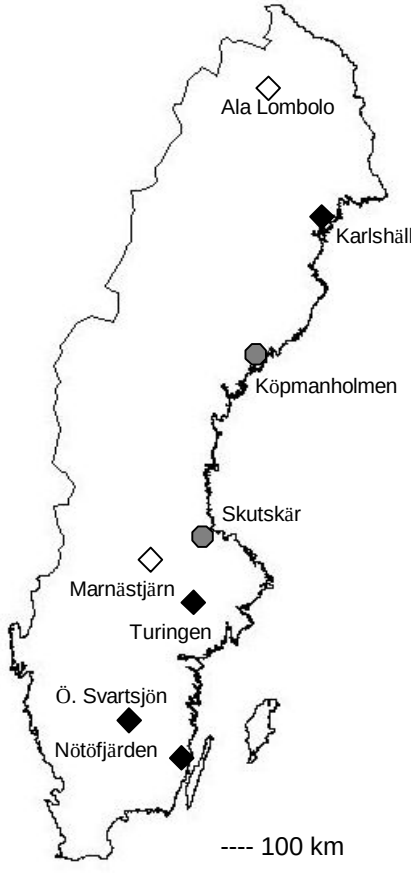

Figure 4. Map of Sweden with the sampled sites. Grey circles denote brackish waters contaminated by Hg^0(l), open diamonds denote freshwaters contaminated by Hg^0(l), and filled diamonds denote freshwaters contaminated by phenyl-Hg.

site Nöt (Table 1). There was also a wide range in organic matter content and quality among the sediments, related to the extent of pulp fibre contamination, and the input of organic matter to the sediment from primary production in the water column. All sites except Mar and Ala had been subjected to pulp fibre discharge. The sediments at Kar, Sva and Nöt were more or less dominated by pulp fibre, while the sediments at Köp and Sku were enriched in pulp fibre and the sediment at Tur was minerogenic and had the lowest content of organic carbon (C), although it contained pulp fibre (Table 1). Differences in Carbon/Nitrogen (C/N) ratios among sites reflect differences in organic matter quality and in primary productivity,

where a low C/N ratio in the sediment reflects a higher primary productivity, and a greater input of energy-rich organic matter from pelagic organisms, with a C/N ratio around 5-8, to the sediment. The C/N ratio in sediments, together with the average annual air temperature sum, was used to indicate differences in primary productivity among sites (Papers II and III). Sediments with a higher content of organic C were looser, while minerogenic sediments had a more firm consistency. Thus, the pulp fibre dominated sediments at Kar, Sva and Nöt were the loosest, and the rest of the sediments were more firm.

Table 1. *Locations, sources of Hg contamination, average annual air temperature sums, salinities, and selected chemical characteristics (±SD) for the sampled sites.*

Site	Location (Lat., Long.)	Hg source	Temp. sum[h]	Cl (mM)	C (%)	C/N	Hg (nmol g^{-1})
Köp[a]	63° 10',18° 36'	Hg^0	1100	83±2.1	11±7.9	190±290	220±320
Sku[b]	60° 39', 17° 23'	Hg^0	1300	80±18	15±3.3	28±10	160±110
Ala[c]	67° 50', 20° 15'	Hg^0	520	nd[i]	21±1.8	9.5±0.41	250±67
Mar[d]	60° 9', 15° 13'	Hg^0	1100	0.53±0.13	16±0.90	9.5±0.40	110±22
Kar[e]	65° 36', 22° 5'	Ph-Hg	940	0.68±0.56	34±4.8	49±13	16±9.5
Tur[f]	59° 13', 17° 27'	Ph-Hg	1300	0.62±0.030	5.3±0.41	11±1.1	12±7.9
Sva[d]	57° 27', 15° 33'	Ph-Hg	1400	0.22±0.013	28±1.4	16±0.4	3.6±1.2
Nöt[g]	57° 9', 16° 28'	Ph-Hg	1500	0.53±0.20	30±6.8	33±16	7.3±2.1

[a]n = 32, [b]n = 22, [c]n = 9, [d]n =4, [e]n =10, [f]n = 6, [g]n = 5, [h]average annual sum of air temp. exceeding 5° C 1961-1990, [i]not determined

Sampling at the brackish water site Köp was done in September (2005) and October (2004), Table 2, when the input of pelagic plankton and algae to the sediment, from primary production during summer, likely was greatest. Two organic profiles (profiles 1 and 3), enriched in pulp fibre, were sampled every 5 cm down to 25 and 20 cm, respectively. A more minerogenic profile (profile 2) was sampled every 5 cm down to 30 cm and then the last 10 cm down to 40 cm. All profiles were situated at accumulation bottoms. The water depth was 5-7 m where profiles 1 and 2 (organic and minerogenic) were sampled, and 2-3 m where profile 3 (organic) was sampled. Eight surface sediment profiles of 0-5 and 5-10 cm depth were also sampled, mostly across the organic accumulation bottom area (water depth 3-22 m).

At the other brackish water site Sku, sampling was done in December (2004) and in June (2005), thus reflecting seasonal changes in sediment properties and primary production (Table 2).

Table 2. *Sampling times, sampled sediment depths and number of samples (n).*

Site	Sampling time (Year-month)	Sampling depth (cm)	n
Köp	2004-10, 2005-09	0-40, 0-10	16, 16
Sku	2004-12, 2005-06	0-100, 0-5	17, 5
Ala	2006-09	0-15	9
Mar	2005-06	0-10	4
Kar	2005-03, 2005-09	0-20, 0-10	4, 6
Tur	2005-06	0-10	6
Sva	2005-06	0-10	4
Nöt	2005-06	0-10	5

In December, the water was still open and there was no ice-cover. One profile was sectioned at 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-23 cm, and 0-20, 20-40, 40-60, 60-80, 80-100 cm (profile 1). A second profile was sectioned every 5 cm down to 25 cm (profile 2). In June, five samples of 0-5 cm were taken. The sediment at Sku had a homogenous composition with quite similar organic content and enrichment in pulp fibre. The sediment had a black colour, indicating presence of FeS(s) minerals. All samples were taken at accumulation bottoms in a sheltered harbour basin where little relocation of sediment was taking place. The water depth at the sampling points was 4-7 m.

At site Ala, sampling was done in September (2006), thus similar to Köp there was likely a great input of pelagic plankton and algae to the sediment at the time of sampling (Table 2). At Ala, three profiles were sectioned every 5 cm down to 15 cm. Ala is a small, shallow (average water depth at sampling points 2 m) freshwater lake situated in the far north of Sweden.

At site Kar, which is a shallow bay in the Luleå river estuary in northern Sweden, sampling was done in March (2004) and in September (2005), thus similar to Sku reflecting seasonal differences (Table 2). In March, sampling was done under 80 cm of ice-cover. One profile was sectioned at 0-2, 2-6, 6-10 and 10-20 cm. In September, three profiles of 0-5 and 5-10 cm were sampled. Also at Kar, sampling was done at accumulation bottoms, with a water depth around 5 m.

At sites Mar, Tur, Sva and Nöt sampling was done in June (2005), which is before the peak in primary production during summer (Table 2). At Mar, two profiles of 0-5 and 5-10 cm were sampled at accumulation bottoms. The

lake is small and shallow (water depth at sampling points 2-3 m). At Tur, four samples of 0-5 cm and one profile of 0-5 and 5-10 cm were sampled at accumulation bottoms. The water depth at Tur was 4-9 m. At Sva, three profiles of 0-5, 0-5 and 5-10, and 0-10 cm were sampled at accumulation bottoms. Sva is a small, shallow forest lake (water depth at sampling points 3-8 m). At Nöt, three profiles of 0-5, 0-5 and 5-10, and 0-7 and 7-14 cm were sampled at accumulation bottoms. Nöt is a shallow (water depth at sampling points 1-3 m) freshwater estuary which only has contact with the sea at high water levels.

2.2 Sampling Methodology and Sample Treatment

At all sites, polycarbonate core samplers (GEMINI twin barrel core sampler, inner diameter 80 mm, Oy KART Ab, Finland and HTH sampler, inner diameter 70 mm, HTH-teknik, Sweden) were used for sediment sample collection. At each sampling point, sediment cores (n = 5-15) were repeatedly collected over a one by one meter area. The sediments were immediately sectioned by depth, and sections from the same depth were pooled in 1-5 L plastic buckets (Hammarplast, Sweden). Oxidation of sediments during sampling was minimised by flushing the buckets with N_2(g), and by filling the buckets to the top with sediment. pH and dissolved H_2S concentrations were measured in the pooled sediment samples on site, and in the laboratory when the pore water was extracted, using a pH-electrode (Mettler Toledo MA 130 ion meter, Mettler Toledo InLab 413 IP 67 electrode) and a H_2S microsensor (Unisense, Denmark). The buckets were kept sealed, cool and dark during transport to the laboratory.

In the laboratory, samples were mechanically homogenised under N_2(g). For samples with a firm consistency, a blender (Bosch, Germany) was used, while loose-consistency sediments were homogenised by a glass stick. Sub-samples were taken for determination of methylation and demethylation rates, total Hg and MeHg concentrations, and total C, N, S and Fe concentrations. Pore water was extracted by centrifugation of sub-samples in tightly capped 50 ml Falcon polypropylene tubes for 30 min at 4000 rpm (Centurion 1040 series, U.K.), and subsequent filtration of the supernatant using a 20 ml syringe, and polycarbonate disc filters (Millex AP20 glass fibre pre-filter and Millex HA mixed cellulose ester filter, 0.45 µm). Preparation of centrifugation tubes, collection of supernatant, filtration, and transfer of filtered pore water were performed in a glove-box, under N_2(g). A comparison was also made between filtration under N_2(g), and filtration in

ambient air for samples from sites Sku and Köp. The time required to obtain enough filtrate (about 50 ml) for the chemical analyses was about 5-15 min for each sample. The filtered pore water was analysed for total Hg, MeHg, Dissolved Organic Carbon (DOC), Dissolved Inorganic Carbon (DIC), Cl, Br, total S, Fe, Mn and Ca. Pore water to be analysed for total S, Fe, Mn and Ca was acidified to pH < 1 by addition of 1 M HCl immediately after filtration.

2.3 Methodology for Hg and MeHg Determination

Species specific isotope dilution (SSID) methods were applied for the determination of MeHg concentrations in pore water and sediment, as well as for the determination of potential methylation and demethylation in sediment. Isotope enriched internal standards (e.g. $Me^{202}HgCl$) were added to sediment and pore water, and gas chromatography hyphenated with inductively coupled plasma mass spectrometry (GC-ICPMS) was used for species separation and detection. Pre-concentration by purge and trap was used for pore water, and ionic compounds were derivatised to form volatile, neutral compounds prior to introduction on the GC. Species specific isotope dilution methods were not used for determination of Hg^{II} in sediment and pore water. Instead, total Hg in sediment and pore water was determined by Atomic Absorption Spectrometry (AAS) and the standard EPA method 1631 (modified by the use of an isotope enriched internal standard), respectively, and Hg^{II} was calculated as the difference between total Hg and MeHg. The reason for this is that it is difficult to get a sufficient recovery of Hg^{II} from complex matrices such as sediments and pore waters to obtain a reliable result with isotope dilution.

The use of isotope dilution techniques has several advantages compared to more traditional calibration techniques, e.g. external calibration and standard addition. Provided that the added isotope enriched internal standard is properly equilibrated with the sample matrix, it will behave similarly to the analyte, and thus transformations, losses and instrumental drift during sample treatment and analysis are compensated for. Isotope dilution is also a less laborious technique compared to e.g. standard addition. However, addition of the internal standard at accurate concentration requires that the approximate concentration of the analyte in the sample is known prior to addition, in order to achieve an acceptable ratio between added isotope and reference isotope. If this ratio is not within the acceptable range, the uncertainty of the result will increase.

The measured ratio between the added isotope and a reference isotope is used to calculate the analyte concentration in the sample according to the equation:

$$C_x = (C_s W_s / W_x) (A_s - R_m B_s / R_m B_x - A_x) \quad (1)$$

where C_x is the concentration of the analyte in the sample, C_s is the concentration of the isotope standard added, W_x is the weight of the sample, W_s is the weight of the isotope standard added, A_x and B_x are the fractions of the enriched and reference isotopes in the sample (before addition of internal standard), A_s and B_s are the fractions of the enriched and reference isotopes in the added standard, and R_m is the measured ratio between added and reference isotope after addition of internal standard (Fassett & Paulsen, 1989).

2.4 Chemical Analyses of Pore Water

For determination of pore water MeHg, an isotope enriched (97.7 %) $Me^{202}Hg$- standard (Snell *et al.*, 2004) was added to the pore water samples and was left to equilibrate with the sample matrix for at least 24 h. During equilibration, the samples were stored in tightly sealed Falcon polypropylene tubes, at 4°C. The samples were then ethylated using $NaB(C_2H_5)_4$, and derivatised MeHg was purged and trapped on Tenax adsorbent columns as described by Lambertsson and Björn (2004), with the exception that the reaction vessel was purged for 10 min with He at 50 ml/min. A comparison was made between direct ethylation and triple liquid-liquid extraction using dichloromethane, as described by Qvarnström *et al.* (2000). In all cases, ethylated MeHg was desorbed onto a GC-ICPMS system (Agilent 6890N GC, Agilent 7500 ICPMS) (Larsson *et al.*, 2005).

For determination of total Hg in pore water, a $^{201}Hg^{II}$-enriched standard (98.11 %) was added to the pore water samples and was equilibrated as described for MeHg. The sample was then oxidised using BrCl for a minimum of 12 h at 25°C, sequentially reduced using hydroxylamine hydrochloride and $SnCl_2$, and purged onto a gold adsorbent column as described in US EPA method 1631 (EPA, 2002). Hg^0 was desorbed from the gold adsorbent onto the GC-ICPMS system as described above for MeHg.

Concentrations of pore water MeHg and total Hg were calculated according to equation 1 (Fassett & Paulsen, 1989). A series of blanks (n=3-11) were analysed in connection to sample analysis and the results were blank corrected. Both MeHg and total Hg blanks corresponded to about 1-10 % of the sample concentrations. The concentrations of the isotope standards used were controlled by reverse isotope dilution, using natural isotope abundance MeHg (MeHgCl, Pestanal grade, Riedel-de Haen) and Hg^{II} ($HgCl_2$, 99.999 %, Sigma-Aldrich) standards. For validation of the total Hg analysis, samples were spiked with the natural isotope abundance Hg^{II} standard (matrix spikes), and were analysed in connection to sample analysis. With one exception (70 %), the apparent recovery of added Hg^{II} was within the EPA method 1631 acceptance criteria, 71-125 % (EPA, 2002). The concentration of Hg^{II} in the pore water was calculated by subtracting the MeHg concentration from the total Hg concentration.

Pore water DOC and DIC were analysed with a Shimadzu TOC-5000 analyser. Pore water Cl and Br were analysed using anion-exchange HPLC with conductivity detection (Dionex 4000i), and pore water S, Fe, Mn and Ca were analysed by ICP-MS (PerkinElmer Elan 6100 DRC), using external calibration. Added Rh and Sc (Referensmaterial AB, Ulricehamn, Sweden) were used as internal standards to correct for ICP-MS drift, and a certified reference material (1640, natural water, NIST, USA) was analysed to assure accuracy of the ICP-MS measurements.

2.5 Chemical Analyses of Sediment

Total Hg concentrations in homogenised sediment samples were measured by Solid combustion Atomic Absorption Spectrometry using a LECO AMA 254 mercury analyzer. The accuracy of the measurements was continuously verified by analysing marine sediment certified reference materials MESS-2 (National Research Council of Canada) and IAEA-356 (International Atomic Energy Agency), at random positions in the sample queue. Total C and N concentrations in dried, grinded, homogenized sediment samples were measured on a PerkinElmer 2400 CHN elemental analyzer. Total S and Fe concentrations in sediments were determined after complete digestion in a closed system (EPA 3052). 300 mg of fresh sediment was digested for 15 minutes at 180 ºC two times in 10 mL concentrated HNO_3 and 3 ml HF and a third time after addition of 4 ml HNO_3 and 2 ml H_2O_2. Total S and Fe were determined by ICP-AES (Perkin Elmer). As references river sediment

(CRM 320), bush branches (NCS DC73348) and apple leaves (SRM 1515) were used.

X-ray photoelectron spectroscopy (XPS) was used to quantify different sulfur species. Spectra were collected with an electron spectrometer (Kratos Axis Ultra) using a monochromated Al Kα source operated at 180 W. Wet samples were analyzed using a pre-cooling technique. To compensate for surface charging, a low-energy electron gun was used. The binding energy (BE) scale was referenced to the C 1s line of aliphatic carbon, set at 285.0 eV. The spectra were processed using Kratos software. The S2p peak was fitted with three well resolved doublets: inorganic S with oxidation state -II at BE = 161.1 – 161.5 eV, organic thiol + sulphide and disulfide at BE = 163.3 – 163.8 eV and organic/inorganic sulfate at binding energy (BE) = 168.7 – 169.2 eV (Urban *et al.*, 1999). The precision of atomic concentration determinations was approximately 6-10 relative %. Thermo-desorption (TD) analysis was performed on sediments from Köp and Sku using pyrolysis. The sediment was heated gradually and the released Hg was transformed to Hg^0 through thermal reduction in a heated quartz tube, and was detected by AAS. TD analysis can distinguish between Hg^0, organically bound Hg^{II}, and Hg^{II} occurring as HgS(s) (Biester & Scholz, 1997).

2.6 Determination of Potential Methylation and Demethylation, and Ambient MeHg

Potential Hg methylation and MeHg demethylation rates were determined from a single analysis of the same sample by spiking with aqueous solutions of isotope-enriched $^{201}Hg(NO_3)_2$ (98.11%) and $Me^{204}HgCl$ (98.11%) to 40% and 0.2% of total Hg, respectively. For accurate determination of the (spiked) $Me^{204}Hg$ concentration at the start of the incubation period, all (spiked) samples were divided into two parts, of which one was immediately frozen at -20 °C, representing t = 0 days (t_0). The other part was incubated in darkness at 23 °C for 48 h in a glovebox under N_2(g), representing t = 2 days (t_2). The incubation time was based on a linear response of produced $Me^{201}Hg$ as a function of time. After 48 h, the incubation was stopped by freezing at -20 °C. Prior to sample preparation, thawed sediment samples were spiked with an aqueous solution of $Me^{200}Hg$ to 0.5% of the total Hg concentration as a species-specific isotope standard for isotope dilution calibration. In addition, $^{199}Hg^{II}$ was added to the samples at 40% of the total Hg concentration to correct for possible MeHg formation during sample treatment and analysis.

On the basis of the determined ambient MeHg concentration, the concentration of formed $Me^{201}Hg$ in the t_2 samples (methylated from the $^{201}Hg^{II}$ spike during the incubation) was calculated from the measured 202/201 MeHg isotope ratio by reverse isotope dilution calculation. Correspondingly, demethylation of the added $Me^{204}Hg$ during incubation was calculated as the difference between the t_0 and t_2 $Me^{204}Hg$ concentrations, derived from the measured 202/204 MeHg isotope ratio, by reverse isotope dilution calculation.

MeHg was solid-liquid extracted from thawed sediment samples using $KBr/CuSO_4/H_2SO_4/CH_2Cl_2$, derivatised with $NaB(C_2H_5)_4$ (Lambertsson *et al.*, 2001) and analysed by GC-ICPMS (Agilent 6890N GC, 7500a ICPMS) (Larsson *et al.*, 2005). The concentrations of all isotope standards used were controlled by reverse isotope dilution, using natural isotope abundance MeHgCl and $HgCl_2$ aqueous standards (MeHgCl Pestanal grade, Riedel-de Haen and $HgCl_2$ 99.999%, Sigma-Aldrich). The method precision for ambient MeHg and Hg methylation determinations was 3% relative standard deviation (RSD), based on replicate subsample incubations and analyses (n = 9). The method detection limit for MeHg measurements was calculated to be 0.02 ng g^{-1}. The accuracy of MeHg determinations was controlled by analysing marine sediment reference materials BCR 580 and IAEA 356.

First-order reactions were used to describe the potential methylation and demethylation rates (Hintelmann *et al.*, 2000; RodriguezMartin-Doimeadios *et al.*, 2004). Assuming that the contribution from demethylation of newly produced $Me^{201}Hg$ [from the $^{201}Hg^{II}$ spike] is negligible within 48 h of spike addition (due to a very low initial $Me^{201}Hg$ concentration), the specific methylation rate constant (k_m) was calculated from the first-order equation:

$$k_m = [Me^{201}Hg]/ ([^{201}Hg]t) \qquad (2)$$

where t = 2 days. Similarly, assuming that methylation of demethylated $^{204}Hg^{II}$ is very low during the 48 h incubation, the specific demethylation rate constant (k_d) was calculated from the first-order equation:

$$k_d = \ln([Me^{204}Hgt_0]/[Me^{204}Hgt_2])/t \qquad (3)$$

where [$Me^{204}Hgt_2$] is the concentration of $Me^{204}Hg$ at the end of the incubation,[$Me^{204}Hgt_0$] is the concentration of $Me^{204}Hg$ at the start of the incubation, and t = 2 days.

For demethylation, two different experiments were carried out. In one experiment the variation in demethylation rates was determined among samples taken at different depths and at different places within the two brackish water sites Köp and Sku (three depth profiles of 0-20, 0-25 and 0-40 cm at site Köp and two depth profiles of 0-100 and 0-25 cm at site Sku). In this experiment, a fairly constant concentration of isotope enriched $Me^{204}HgCl$ was added to the samples at each site (300-900 pmol g^{-1} at Sku and 50-200 pmol g^{-1} at Köp). Because of substantial variations in ambient concentrations of MeHg and Hg within and among the sediment profiles, the ratio between added $Me^{204}Hg$ tracer and ambient MeHg varied between 95 and 5800 % at site Sku and between 53 and 1500 % at site Köp. It was found that both the demethylation rate (ppb d^{-1}) and the k_d were dependent on the added tracer expressed as % of ambient MeHg (and ambient Hg) in this experiment. In another experiment, demethylation rates were determined in surface sediments (0-10 cm) across all sites. The aim was to add $Me^{204}HgCl$ corresponding to 40% of ambient MeHg. However, since ambient concentrations of Hg were used as predictors, the additions of $Me^{204}HgCl$ fell within the range of 1-170 % of ambient MeHg (20-60% for most samples). In this experiment, demethylation rates and k_d were indicated not to be related to the added quantity of MeHg (and Hg) tracer (expressed as % of ambient MeHg).

2.7 Chemical Speciation Calculations

Chemical equilibrium speciation calculations were carried out using the softwares MinteqA2 (Allison & Brown, 1995) (paper I), and Microsoft Excel (Papers I, III and IV). All calculations were performed for 25°C. The ionic strength differed between sites and chemical activities were calculated using the Davies equation. To estimate organic thiol group (RSH) concentrations, it was assumed that 0.5 mass % of DOC were reduced S and that 30 mol % of reduced S was RSH, as indicated by combined Hg EXAFS and S XANES studies of organic soils and DOM from organic soils (Qian *et al.*, 2002; Skyllberg *et al.*, 2006). Concentrations of sulphate were calculated as [total S]-[H_2S+HS^-+S^{2-}]-[total organic S], where total organic S was estimated to be 0.7 mass % of DOC (Qian *et al.*, 2002). For H_2S and RSH, pK_a values of 7.0 (Stumm & Morgan, 1996) and 9.96 (Karlsson &

Skyllberg, 2003), were used. Concentrations of HCO_3^- and CO_3^{2-} (Paper I) were calculated from DIC and pH. Saturation indices (SI, Paper I) were calculated based on data after filtration under N_2, as SI = log ion activity product - log solubility product, using log K_{sp} $FeS_{am}(s)$ = -2.95 (Morse *et al.*, 1987), log K_{sp} $FeCO_3(s)$ = -10.89, log K_{sp} $CaCO_3(s)$ =-8.48 and log K_{sp} $CaSO_4(s)$ =-4.62 (Nordstrom *et al.*, 1990). In order to consider the uncertainty in using SI in environmental samples, a SI between -1 and 0 was used to indicate saturation, i.e. the solution was considered to be saturated if the ion activity product was $\geq$ 0.1 solubility product (Essington, 2004). The pe values (Paper I) were calculated from the reaction $HS^- + 4H_20 = SO_4^{2-} + 9H^+ + 8e^-$, log K = -34 (Stumm & Morgan, 1996).

The speciation of MeHg (Papers I and IV) and Hg^{II} (Paper III) in pore water was modelled considering complexes with hydroxides, halides, inorganic sulphides and bisulphides, and organic thiols (Table 3). Measured concentrations of MeHg, Hg^{II}, halides, H_2S, DOC and pH in pore water were used as input data. For samples in which the concentration of H_2S was below the detection limit, the detection limit concentration, 0.3 μM, was used. Values of pH and $[H_2S]$ measured in the laboratory were used, in order to match the laboratory measurements of methylation and demethylation rates as closely as possible. *Note that* in Paper III, the species $HgOHSH^0(aq)$ is written as $HgS^0(aq)$, while in Table 3 it is denoted $HgOHSH^0$ and the reactions have been rewritten accordingly.

For Hg^{II}, four different chemical speciation models were used, in order to consider uncertainties in the stability constant for $HgOHSH^0(aq)$, and differences between sites in whether or not HgS(s) was present. Solid HgS(s) was used to limit the concentration of $HgOHSH^0(aq)$ (reactions 15a and b, Table 3), but the HgS(s), as a solid phase, was not considered part of the solution. Model A included reactions 6-13, model B reactions 6-13 and 14a, model C reactions 6-13, 14b and 15a, and model D reactions 6-13, 14b and 15b.

Table 3. *Reactions and stability constants used in speciation modelling for MeHg (reactions 1-5) and Hg^{II} (reactions 6-15) at 25° C and I = 0.*

	Reaction	Log K	References
1	$MeHg^+ + OH^- = MeHgOH$	9.4	(Schwarzenbach & Schellenberg, 1965)
2	$MeHg^+ + Cl^- = MeHgCl$	5.3	(Schwarzenbach & Schellenberg, 1965)
3	$MeHg^+ + HS^- = MeHgSH$	14.5	(Dyrssén & Wedborg, 1991)
4	$MeHg^+ + RS^- = MeHgSR$	16.5, 17.0[a]	(Karlsson & Skyllberg, 2003)
5	$MeHgSH = MeHgS^- + H^+$	-7.5	(Dyrssén & Wedborg, 1991)
6	$Hg^{2+} + nCl^- = HgCl_n^{2-n}$	7.3,[b] 14.0[c]	(Smith & Martell, 1976)
7	$Hg^{2+} + nBr^- = HgBr_n^{2-n}$	9.0,[b] 17.5[c]	(Smith & Martell, 1976)
8	$Hg^{2+} + nOH^- = Hg(OH)_n^{2-n}$	10.6,[b] 21.8[c]	(Smith & Martell, 1976)
9	$Hg^{2+} + HS^- = HgSH^+$	20.0	(Dyrssén & Wedborg, 1991)
10	$Hg^{2+} + 2HS^- = Hg(SH)_2^0$	37.5	(Benoit *et al.*, 1999a; Dyrssén & Wedborg, 1991)
11	$Hg^{2+} + 2HS^- = HgS_2H^- + H^+$	31.3	(Benoit *et al.*, 1999a; Dyrssén & Wedborg, 1991)
12	$Hg^{2+} + 2HS^- = HgS_2^{2-} + 2H^+$	23.0	(Benoit *et al.*, 1999a; Dyrssén & Wedborg, 1991)
13	$Hg^{2+} + 2RSH = Hg(SR)_2 + 2H^+$	22.1	(Khwaja *et al.*, 2006; Skyllberg *et al.*, 2000)
		Log K	
14a	$Hg^{2+} + OH^- + HS^- = HgOHSH^0$	38.2	
14b	$Hg^{2+} + OH^- + HS^- = HgOHSH^0$	40.2	(Benoit *et al.*, 1999a)
		Log K	
15a	$HgS(s) + H_2O = HgOHSH^0$	-10.0	(Dyrssén & Wedborg, 1991)
15b	$HgS(s) + H_2O = HgOHSH^0$	-9.3	(Jay *et al.*, 2000)

[a]paper I, [b]n =1, [c]n =2

Dyrssén and Wedborg (1991) reported two values for the stability constant for $HgOHSH^0$ in equilibrium with HgS(s). From a statistical relationship for mixed complexes, a log K of -22.3 was estimated for the reaction $HgS(s) + H_2O = HgOHSH^0(aq)$. From solubilites of ZnS(s) and CdS(s) determined by Gübeli and Ste-Marie (1967), a log K of -10 was instead estimated. Daskalakis and Helz (1993) determined significantly lower solubilities for ZnS(s) and CdS(s) than Gübeli and Ste-Marie, and concluded that the higher solubility determined by the latter likely was caused by colloidal contamination. This was also the comment of Dyrssén and Wedborg to the great discrepancy (12 orders of magnitude) between the two constants. However, the larger log K of -10 has been extensively used (Benoit *et al.*, 2001; Benoit *et al.*, 1999a; Benoit *et al.*, 1999b; Jay *et al.*, 2000; Jay *et al.*, 2002). Benoit *et al.* estimated a log K of 26.5 for the reaction $Hg^{2+} + HS^- = HgS^0 + H^+$, by combining the log K of -10 with a log solubility product of -36.5 for HgS(s) (Benoit *et al.*, 1999a). If this constant is combined with the ionic product of water ($pK_w = 13.7$), a log K of 40.2 is

obtained for the reaction $Hg^{2+} + OH^- + SH^- = HgOHSH^0$ (reaction 14b, Table 3), which is analogous to the formation reactions for $Hg(OH)_2^0$ (reaction 8) and $Hg(SH)_2^0$ (reaction 10) in Table 3. As can be seen, the log K for formation of $HgOHSH^0$ is about three orders of magnitude larger than the log K for formation of $Hg(SH)_2^0$. It is well established that Hg has a higher affinity for sulphide than for oxygen ligands. Thus, the log K for reaction 14b is unreasonably large.

In the chemical modelling, model A (reactions 6-13, Table 3), not including formation of $HgOHSH^0$, is equivalent to deriving a constant for formation of $HgOHSH^0$ in solution from the smaller log K of -22.3 estimated by Dyrssén and Wedborg. If this log K is used, the contribution from $HgOHSH^0$ is negligible compared to other species (Skyllberg, 2008). In model B (reactions 6-13, 14a), a constant two orders of magnitude smaller than the log K of 26.5 estimated by Benoit *et al.* is used for formation of $HgOHSH^0$ in solution. In model C (reactions 6-13, 14b, 15a), the log K of -10 for $HgOHSH^0$ in equilibrium with HgS(s) is used to limit the concentration of $HgOHSH^0$, and in model D (reactions 6-13, 14b, 15b), a log K of -9.3, suggested by Jay *et al.* (2000), is used to limit the concentration of $HgOHSH^0$. Thus, in models C and D, it is assumed that HgS(s) is present in the solid phase.

Polysulphides were not included in the chemical speciation models. In addition to uncertainties concerning identities and stability constants for Hg-polysulphide species (Jay *et al.*, 2000), the main constraint if polysulphides would be included would be to assign a correct activity to elemental S, as this was not measured in the present study. If elemental S is assigned an activity of unity, i. e. solid phase $S_8(s)$ is assumed present, constants from Jay et al. (2000) are used, and HgS_xOH^- and $Hg(S_x)_2^{2-}$ are included, the total Hg^{II} concentration in solution will exceed 2 nM at pH 7. Only three sediment pore waters in this thesis had Hg^{II} concentrations above 1 nM. Thus this is not a reasonable approach in the present case.

3 Results and Discussion

3.1 Effects of Oxic and Anoxic Filtration on MeHg Concentrations in Sediment Pore Waters (Paper I)

The effects of oxic and anoxic filtration, after anoxic centrifugation, on determined pore water concentrations of MeHg, Fe, Mn, S and DOC were evaluated for one sample from Sku and 12 samples from Köp. For all samples, determined concentrations of MeHg (Figure 5), Fe and Mn in pore water were greater after filtration under N_2(g) than after filtration in ambient air. For MeHg, the difference between the treatments, i.e. the ratio between pore water MeHg concentration after filtration under N_2 and pore water MeHg concentration after filtration in ambient air, varied substantially among samples (Figure 5). There were no differences between the treatments in determined concentrations of DOC, while the treatment effect on determined S concentrations varied among samples (Paper I).

In previous work, 10-15 times lower MeHg concentration was determined in pore water from sediments that had been maintained under "oxic laboratory conditions" than under "anoxic laboratory conditions" (Mason *et al.*, 1998). In the present work, the ratio between pore water MeHg concentrations in the anoxic and oxic treatment varied between 3.4 and 340 (Paper I). Thus, for the samples studied, a greater error was introduced by the filtration alone, if this was performed in ambient air. This indicates that filtration is an oxidation sensitive step in sample treatment, and that filtration needs to be carried out under anoxic atmosphere if accurate pore water MeHg concentrations are to be determined.

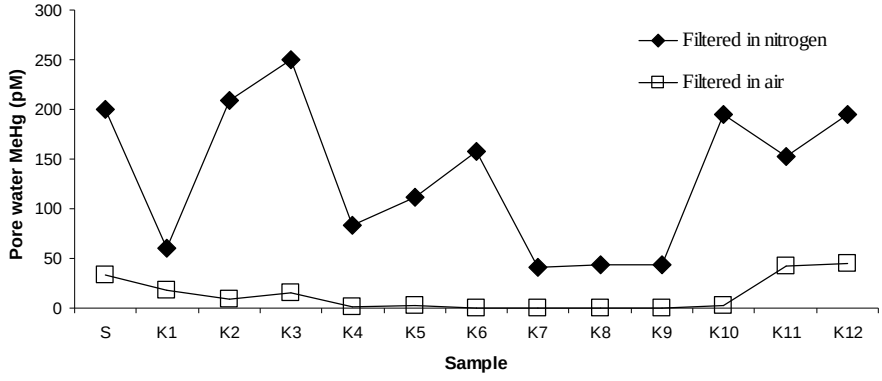

Figure 5. Determined concentrations of MeHg (pM) in sediment pore waters from Sku (sample S) and Köp (samples K1-K12) after anoxic (N_2(g), filled diamonds), and oxic (air, open squares) filtration.

It is proposed that adsorption of MeHg to newly formed $Fe^{III}/Mn^{III/IV}$-oxy/hydroxide surfaces is the main mechanism responsible for MeHg removal during oxic filtration (Paper I). The proposed mechanism is supported indirectly by decreases in pore water Fe and Mn concentrations after oxic filtration, and by decreases in pore water SO_4^{2-} concentrations after oxic filtration in the samples with the greatest treatment effect on MeHg. Likely, SO_4^{2-} was removed by the same mechanism as suggested for MeHg, as shown in previous work (Geelhoed *et al.*, 1997; Rietra *et al.*, 1999). Chemical speciation calculations showed that MeHgSH, $MeHgS^-$ and MeHgSR all were affected by the oxic treatment. $MeHgS^-$ is attracted to the newly formed surfaces because of its negative charge, as pH was around 7 and surfaces of Fe^{III}-oxy/hydroxides have a point of zero charge (pzc) of 8.8 (Essington, 2004). MeHgSH and MeHgSR could be adsorbed to these surfaces as a consequence of their hydrophobic properties.

The greatest treatment effect for MeHg occurred in samples with relatively less reducing conditions (but pe was below -3). This indicates that a strict anoxic handling of samples during filtration may be most important for samples with an intermediate redox potential. Interestingly, DOC was unaffected by the oxic treatment. The fact that MeHgSR was affected by oxic filtration while DOC was not, may suggest that MeHg associates to specific organic molecules containing thiol groups, e.g. small peptides or amino acids, in line with previous work (Han *et al.*, 2006).

3.2 Importance of Methylation, Demethylation and Transport for Accumulation of MeHg in Contaminated Sediments (Papers II and IV)

With the exception of site Sku, average specific potential demethylation rate constants, k_d (day^{-1}, equation 3, section 2.6) were of a similar magnitude at all sites, while average specific potential methylation rate constants, k_m (day^{-1}, equation 2, section 2.6) varied by two orders of magnitude among sites (Table 4 and Paper II). This indicates that differences in MeHg accumulation among sites are to a greater extent determined by Hg methylation than by MeHg demethylation reactions. At the sites where both depth profiles and surface sediments were sampled, Köp and Sku, k_m was about one order of magnitude lower below the top 10-20 cm in the sediment (Table 4 and Paper II). This shows that methylation rates are highest in surface sediments, in line with previous reports (Lambertsson *et al.*, 2001; Lambertsson & Nilsson, 2006). The pattern is likely explained by a peak in the activity of SRB, and FeRB, at the oxic/anoxic interface in sediments, where these bacteria are able to compete with respect to electron acceptors (e.g. O_2), and have access to energy-rich organic matter, which mainly is deposited at the sediment surface and is depleted further down.

Table 4. *Average rate constants (±SD) for potential methylation (k_m, day^{-1}) and demethylation (k_d, day^{-1}) for all sampling occasions.*

Site	**Depth (cm)**	**k_m (day^{-1})**	**k_d (day^{-1})**
Köp	0-10	0.002±0.003[a]	0.07±0.08[b]
	10-40	0.0002±0.0001[c]	*0.09±0.06*[i, b]
Sku	0-20	0.004±0.006[b]	0.5±0.3[d]
	20-100	0.0004±0.00004[e]	*0.1±0.09*[i, b]
Kar[c]	0-20	0.0004±0.0006[c]	0.05±0.06[e]
Ala[f]	0-15	0.002±0.002	0.02±0.006
Mar[h]	0-10	0.02±0.01	0.1±0.02
Tur[e]	0-10	0.01±0.007	0.09±0.06
Sva[h]	0-10	0.05±0.02	0.1±0.08
Nöt[d]	0-10	0.01±0.008	0.1±0.1

[a]n = 22, [b]n = 16, [c]n =10, [d]n = 5, [e]n = 6, [f]n = 9, [g]n = 19, [h]n = 4, [i]k_d dependent on added isotope tracer, depth at Köp 0-40 and at Sku 0-100 cm

Note that the pattern with sediment depth for total Hg and MeHg was not consistent, but that k_m was always highest in the top five cm of the sediment, for the sites sampled to a greater sediment depth than 10 cm (Köp, Sku, Kar and Ala, Figure 6). If methylation is more important than demethylation and

input-output for accumulation of MeHg in sediments, a positive relationship between k_m and MeHg concentration is expected. However, there was also a significant ($p \leq 0$ .001) positive relationship between total Hg and MeHg, for all sites, and for all individual sites with $n \geq 10$ (Paper II). Thus, in order to observe a positive relationship between MeHg production (k_m) and concentration, MeHg needs to be normalised to total Hg, expressed as %MeHg (of total Hg).

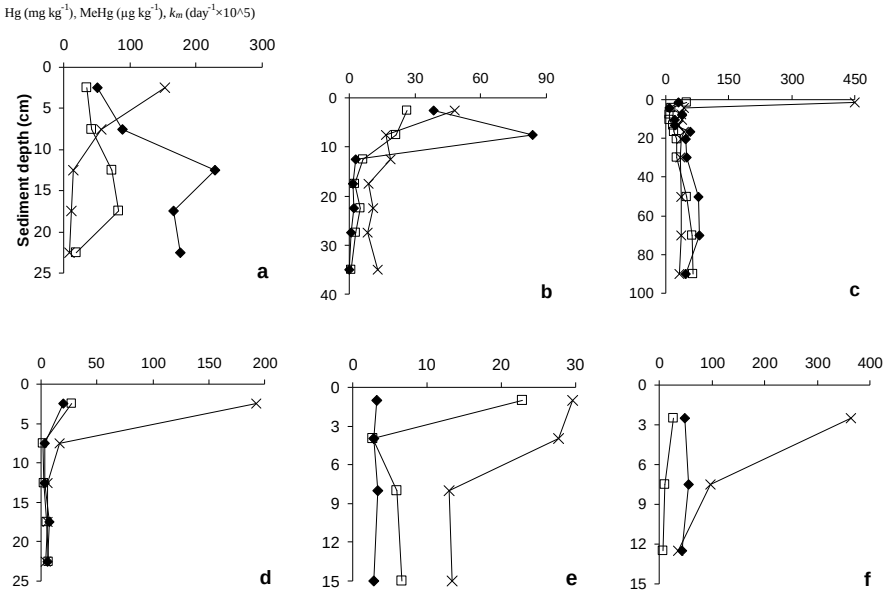

Figure 6. Sediment depth (cm) profiles of total Hg (filled diamonds, mg kg-1), total MeHg (open squares, µg kg-1) and specific potential methylation rate constant, k_m (crosses, day-1×10^5, in Figure 2d day-1×10^4) for a) Köp, profile 1, b) Köp, profile 2, c) Sku, profile 1, d) Sku, profile 2, e) Kar, and f) Ala (average for 3 profiles).

Consequently, a significant positive relationship was observed between %MeHg and k_m for all surface (0-20 cm) sediments (Figure 7a), as well as for sub-sets of loose, organic freshwater surface sediments (Sva, Nöt, Kar,, Figure 7b), firm, minerogenic freshwater surface sediments (Mar, Tur, Ala, Figure 7c), and firm, pulp-fibre enriched brackish water surface sediments (Köp and Sku, Figure 7d).

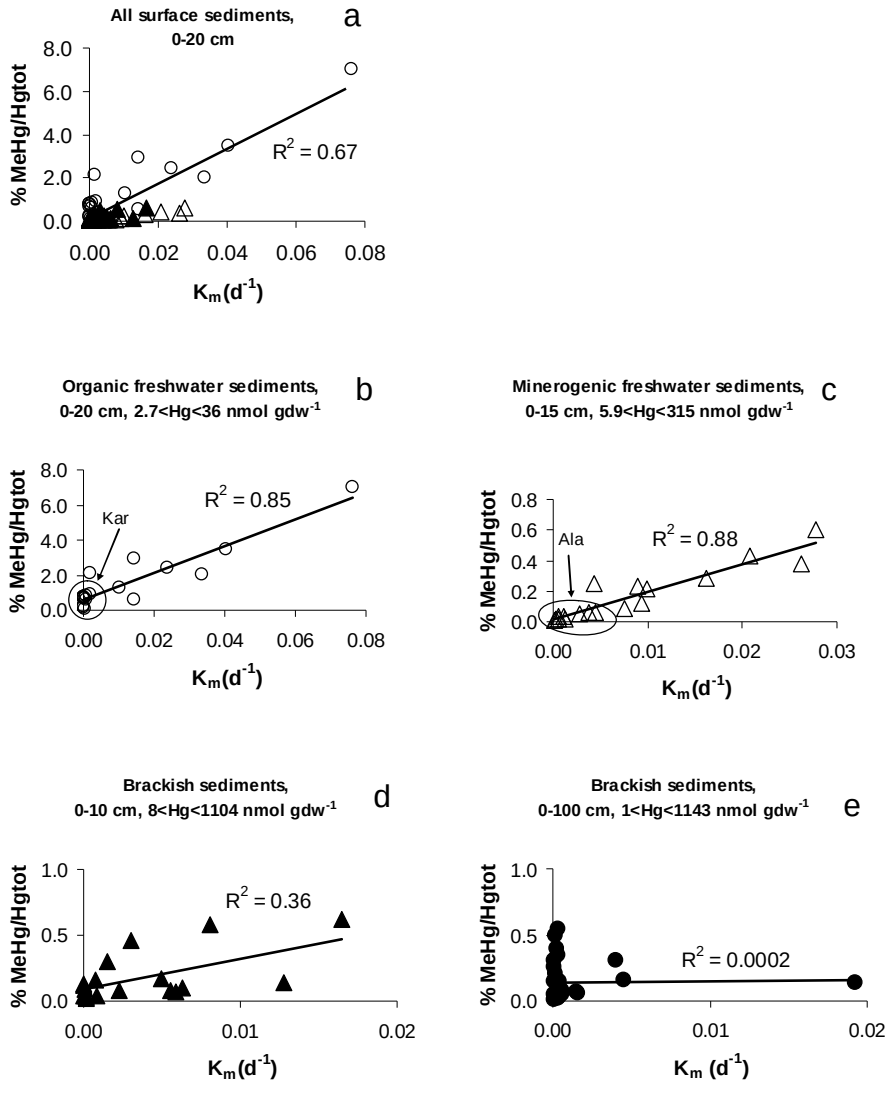

Figure 7. Relationship between k_m (day^{-1}) and % MeHg (of total Hg) for (a) all surface sediments (open circles=organic freshwater sediments, open triangles=minerogenic freshwater sediments, filled triangles=brackish sediments, n=59 $p < 0.001$) (b) loose, organic, pulp-fibre dominated freshwater sediments (Sva+Nöt+Kar, n=19, $p < 0.001$), (c) firm minerogenic freshwater sediments (Mar+Tur+Ala, n=19, $p < 0.001$), (d) firm, pulp-fibre enriched brackish water surface sediments (Köp+Sku, n=21, $p = 0.006$), and (e) firm, pulp-fibre enriched brackish water sediment depth profiles (Köp+Sku, n=33, $p = 0.95$). From Paper II.

In contrast, the relationship between k_m and MeHg concentration (not normalised for variations in total Hg) was only significant ($p = 0.004$) for the sub-set of firm, minerogenic freshwater surface sediments (Paper II). For brackish water sediment depth profiles (Köp and Sku, 0-100 cm, Figure 7e) there was no significant ($p > 0.05$) relationship between k_m and %MeHg.

A significant positive relationship between k_m and %MeHg in surface (0-20 cm) sediments (Figures 7a-d) has previously been reported (Benoit *et al.*, 2003; Hammerschmidt & Fitzgerald, 2006; Sunderland *et al.*, 2004), although not in highly (> 1 nmol Hg g^{-1}) contaminated environments. This relationship shows that, in surface sediments, variation in methylation is more important for the accumulation of MeHg than variation in demethylation and input-output, in line with the greater variation in k_m than k_d among sites (Table 4). At greater sediment depth (i.e. below 20 cm, Figure 7e), demethylation and net transport are of greater relative importance, in line with the observed decrease in k_m with sediment depth (Figure 6). The less strongly significant relationship between k_m and %MeHg for brackish water surface sediments (Figure 7d) than for surface sediments from other sites may be explained by a higher rate of demethylation at site Sku, which was the only site with a markedly higher k_d (Table 4). The relationship between k_m and %MeHg in surface sediments remained strong across a range of sites with different properties (Figure 7a). This has previously not been reported, and implies that %MeHg can be used as a proxy for MeHg production, across sites. %MeHg is a suitable parameter to use for risk assessment purposes, as measurement of total Hg and MeHg is less complicated than measurement of Hg methylation.

Among factors that are known to influence bacterial activity (section 1.2), the most important factor determining differences between sites in %MeHg and k_m was indicated to be the availability of electron donors (suitable organic substrate) to methylating bacteria, as a result of differences among sites in primary production and subsequent input of energy-rich organic matter to the sediment surface. Both the %MeHg and k_m were lower at the northerly (Figure 4) situated sites Kar and Ala (Figures 7b and c, highlighted) than at more southerly situated sites with similar properties. At Kar and Ala, average annual air temperature sums are low (Table 1), and Kar is oligotrophic. The C/N ratio, used in the present work to indicate differences in input of energy-rich organic matter to sediments (section 2.1), was higher at Kar (49), than at Sva and Nöt (16 and 33, respectively), in line with lower MeHg production at the former site (Figure 7b). Similarly,

the C/N ratio was high at the brackish water site Köp, and low at the freshwater sites Mar, Tur, Sva and Nöt (Table 1). However, the C/N ratio at all minerogenic freshwater sites (Figure 7c) was quite similar, thus the lower MeHg production at site Ala is likely mainly an effect of lower temperature.

There were no significant relationships between calculated (section 2.7) concentrations of sulphate, $[SO_4^{2-}]$, and MeHg production ($p > 0.05$, Paper II), indicating that SO_4^{2-} did not limit MeHg production. The availability of SO_4^{2-} was higher at the brackish water sites and lower at the freshwater sites (Table 5), but both %MeHg and k_m were highest at freshwater sites (Figures 7b, c and d).

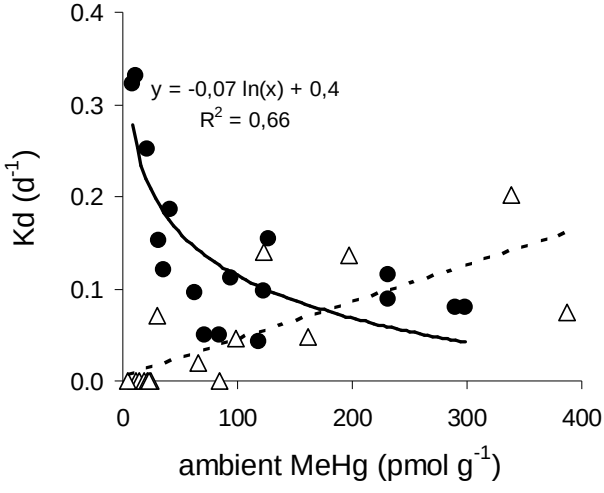

Figure 8. Relationship between ambient MeHg (pmol g^{-1}) and k_d (day^{-1}) for Köp (open triangles, dotted line n = 16, $p < 0.001$) and Sku (filled circles, solid line, n = 17, $p < 0.001$), in experiment one. Modified from Paper IV.

For demethylation, two experiments were carried out (Paper IV and section 2.6). In the first experiment, isotope enriched MeHg tracer was added to depth profiles from Köp (0-40 cm) and Sku (0-100 cm), at a constant concentration to each sample. This resulted in ratios between added MeHg tracer and ambient MeHg ranging between 53-1500% at Köp and 95-5800% at Sku. The response to this was different at the two sites; at Köp the relationship between added tracer, expressed as % of ambient MeHg, and k_d was log-negative and no demethylation was detected at tracer additions above 500% of ambient MeHg, while at Sku there was a linear positive relationship between added tracer, expressed as % of ambient MeHg, and k_d, up to the tracer addition maximum of 5800% of ambient MeHg (Paper IV). Hg^{II} tracer was also added to the samples, for simultaneous determination of

k_m (section 2.6), and similar, but slightly less significant, relationships were observed between added Hg^{II} tracer expressed as % of ambient Hg^{II}, and k_d (Paper IV). Thus, in this experiment, determined k_d were negatively related to ambient Hg and MeHg at Sku, and positively related to ambient Hg and MeHg at Köp (Figure 8). In Table 4, k_d from this experiment are shown in italics.

In the second experiment, MeHg tracer was added to surface sediments (0-10 cm) from all sites, at a final concentration of 1-170% of ambient MeHg. In this experiment, there was no relationship between added tracer, expressed as % of ambient MeHg, and k_d. Using data from experiment two, a significant ($p < 0.001$) positive relationship was observed between ambient MeHg (pmol g^{-1}) and k_d for all sites, Figure 9. However, there was no significant positive relationship between ambient total Hg and k_d (Paper IV).

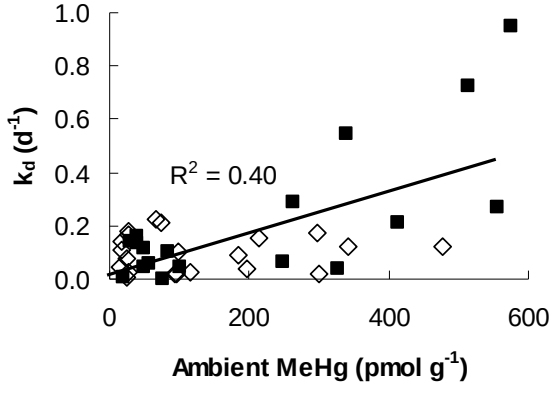

Figure 9. Relationship between ambient MeHg (pmol g^{-1}) and k_d (day^{-1}) for all surface sediments (experiment two). Filled squares are brackish water sites (Köp and Sku) and open diamonds are freshwaters (Mar, Tur, Sva, Nöt and Kar), $p < 0.001$. Modified from Paper IV.

The results from the demethylation experiments indicate that demethylation rates are influenced by ambient Hg and/or MeHg concentrations, in line with previous reports (Marvin-DiPasquale *et al.*, 2000). It appears, however, that the relationship between ambient MeHg and k_d may be both positive (Köp, all sites), indicating that demethylation is stimulated by ambient MeHg concentrations, and negative (Sku), perhaps indicating that low ambient MeHg concentration is a result of a high rate of demethylation. It can also be noted that the k_d at site Sku was higher than at all other sites, which may suggest that demethylation at Sku occurred by a different and more efficient mechanism. The interpretation of the results with

respect to induction and demethylation mechanisms is complicated by the simultaneous addition of both Hg^{II} and MeHg tracers to each sample in experiment one, and the significant positive relationship between total Hg and MeHg (Paper II) in experiment two.

3.3 Influence of Hg^{II} Speciation on MeHg Production in Contaminated Sediments (Papers II and III)

Pore water speciation of Hg^{II} was calculated using four different chemical models (section 2.7). The distribution among major Hg^{II} species in pore water differed among those (Table 6 and Paper III). Complexes with halides and hydroxides, free Hg^{2+}, and the positively charged $HgSH^{+}$(aq) species, were not quantitatively important with any of the models (Paper III). With model A, where $HgOHSH^{0}$(aq) was not included, organic thiol complexes, $Hg(SR)_2$(aq), constituted 10-20% of pore water Hg^{II} at all sites except Kar, where the concentration of DOC in relation to H_2S was lowest (Table 5). In models B-D, the importance of $HgOHSH^{0}$ was increased (section 2.7). At the freshwater sites Mar, Tur and Kar, with low concentrations of H_2S, $HgOHSH^{0}$ dominated over $Hg(SH)_2^{0}$ with any of the models B-D. At the brackish water site Köp, with the highest concentration of H_2S (Table 5), $HgOHSH^{0}$ was only dominant over $Hg(SH)_2^{0}$ with model D (Table 6).

Relationships between calculated concentrations of pore water Hg^{II} species and k_m or MeHg concentration were evaluated at all sites and with all chemical models (Paper III). Significant ($p < 0.05$), linear positive relationships between calculated concentrations of dissolved neutral Hg-sulphides [$Hg(SH)_2^{0}$ + $HgOHSH^{0}$] and k_m or MeHg concentration were observed for several combinations of sites and chemical models. This supports the hypothesis that neutral Hg-sulphides are the most important bioavailable Hg^{II} species in sediments with sulphidic conditions, and are taken up by a passive diffusion mechanism (e.g. Figures 10 and 11). For the species $Hg(SR)_2$, HgS_2H^{-} and HgS_2^{2-}, and for total pore water Hg^{II}, the relationship with k_m or MeHg concentration was only significant ($p < 0.05$) positive for the few sub-sets (e.g. Kar, and Mar and Tur) where the concentration of these species was positively correlated with the concentration of neutral Hg-sulphides.

In the following discussion, relationships between dissolved neutral Hg-sulphides [$Hg(SH)_2^{0}$ + $HgOHSH^{0}$] and k_m or MeHg concentration will be

shown for a few important sub-sets of data, in order to highlight the most important aspects of site properties and chemical models used. The results will also be discussed in relation to previous work, with special concern to the relationship between pore water sulphide and neutral Hg-sulphide concentration.

Table 5. *Selected chemical characteristics (average±SD) of sediments and pore waters.*

Site	S (%)	Fe (%)	S inorg. (%)[h]	pH	DOC (mM)	SO_4^{2-} (µM)[j]	H_2S (µM)	Hg^{II} (pM)	MeHg (pM)
Köp[a]	0.55±0.18	1.0±0.53	0.080	6.9±0.40	7.0±18	940±710	250±220	740±570	100±100
Sku[b]	2.6±0.92	3.3±1.3	1.5	7.3±0.57	6.7±2.5	1400±1500	28±37	400±280	110±88
Ala[c]	5.2±0.91	7.2±2.0	nd[i]	7.1±0.17	3.9±1.0	380±270	13±12	380±270	2.9±1.7
Mar[d]	2.1±0.35	4.1±0.082	nd	6.6±0.11	1.3±0.21	47±18	1.0±1.1	38±10	9.4±8.1
Tur[e]	0.36±0.051	3.7±0.46	nd	6.8±0.12	1.2±0.27	240±87	2.5±3.0	31±5.0	5.6±1.9
Kar[f]	0.62±0.029	1.7±0.43	nd	5.9±0.12	1.1±0.23	20±23	5.9±9.3	92±66	24±22
Sva[d]	1.1±0.17	3.3±0.81	nd	6.7±0.34	2.3±0.23	39±17	1.8±1.2	42±5.1	7.6±4.4
Nöt[g]	1.8±0.33	1.6±0.67	nd	6.3±0.35	2.1±0.64	80±44	23±28	100±130	19±7.3

[a]n = 32. [b]n = 22. [c]n = 9. [d]n = 4. [e]n = 6. [f]n = 10. [g]n = 5. [h]determined by XPS. [i]not determined. [J]calculated as [total S]-[H2S+HS-+S2-]-[total organic S], where total organic S was estimated to be 0.7 mass % of DOC (Qian et al., 2002)

Table 6. *Distribution (%) of major Hg^{II} species in pore water calculated using the chemical speciation models A-D. Modified from Paper III.*

Site	**Model (A) reactions 6-13**			**Model (B) reactions 6-13, 14a**			**Model (C) reactions 6-13, 14b, 15a**			**Model (D) reactions 6-13, 14b, 15b**		
	$Hg(SR)_2$	$Hg(SH)_2^0$	Σneg Hg-S	$Hg(SR)_2$	$Hg(SH)_2^0$ + $HgOHSH^0$	Σneg Hg-S	$Hg(SR)_2$	$Hg(SH)_2^0$ + $HgOHSH^0$	Σneg Hg-S	$Hg(SR)_2$	$Hg(SH)_2^0$ + $HgOHSH^0$	Σneg Hg-S
Köp	14	9	77	13	13 (3)[a]	74	9	29(11)[a]	62	1	52(34)[a]	47
Sku	20	9	71	16	14(5)[a]	69	15	33(21)[a]	52	1	57(49)[a]	42
Ala	12	9	79	9	16(7)[a]	75	1	51(43)[a]	48	0	61(56)[a]	39
Mar	16	25	59	6	56(44)[a]	38	0	98(97)[a]	2	0	98(97)[a]	2
Tur	13	16	71	5	43(31)[a]	51	0	90(88)[a]	10	0	90(88)[a]	10
Kar	3	64	33	1	74(37)[a]	25	0	96(92)[a]	4	0	96(92)[a]	4
Nöt	11	25	64	6	41(18)[a]	53	0	96(94)[a]	4	0	96(94)[a]	4
Sva	13	35	52	4	56(17)[a]	40	0	91(80)[a]	8	0	91(80)[a]	8

[a] % $HgOHSH^0$ of total Hg in pore water

For sediment profiles at the brackish water site Köp, significant ($p < 0.05$) positive relationships were observed between the concentration of neutral Hg-sulphides and k_m (5-40 cm), or MeHg concentration (0-40 cm), with all four chemical speciation models, but most highly significant ($p < 0.001$) with model A or B (Paper III). For sediment profiles at the other brackish water site Sku (0-100 cm), however, no significant ($p > 0.05$) positive relationships between neutral Hg-sulphides and k_m were observed with any of the speciation models, and a significant ($p < 0.05$) positive relationship between neutral Hg-sulphides and MeHg concentration was observed only with model D.

A possible explanation for the lack of relationship between neutral Hg-sulphides and k_m or MeHg at Sku is that none of the chemical speciation models reflect the chemistry of the sediment accurately. Independent measurements (e.g. XPS, Table 5) indicate that FeS(s) was present at Sku, but this, or solid solution Hg/FeS(s), was not included in any of the chemical models. Another possibility is that there are bioavailable Hg^{II} species present at Sku that have not been included in the models. Polysulphides were not included, but were indicated not to contribute to uptake of Hg^{II} in methylating bacteria in previous reports (Jay *et al.*, 2002). In a recent study, however, both uptake and methylation of Hg by the *δ-Proteobacterium Geobacter sulfurreducens* was shown to be substantially enhanced in the presence of the amino acid glutathione (Schaefer & Morel, 2008). Glutathione has, in one of the few studies where this was adressed, been indicated to be an important ligand for Hg^{II} in estuarine water (Han *et al.*, 2006), but Hg-amino acid complexes were not included in the present work. Thus, it is possible that the lack of significant positive relationship between neutral Hg-sulphides and MeHg production at Sku is explained by the presence of Hg-amino acid complexes.

For a combination of the profiles from Köp and Sku, the most highly significant ($p < 0.001$) positive relationship between neutral Hg-sulphides and k_m (Figure 10) or MeHg (Figure 11) was observed if model A was used for Köp and model C or D, respectively, was used for Sku. This seems reasonable given the properties of the sediments, where independent methods (TD analysis) indicated that HgS(s) was present at Sku, and $Hg(SR)_2$ was indicated to contribute to Hg^{II} solubility at Köp (Paper III). Note the lack of significant relationship with $Hg(SR)_2$, HgS_2H^-, HgS_2^{2-}, and total pore water Hg^{II} (Figures 10 a-c). Note also that for the relationship with k_m (Figure 10),

the uppermost part (0-5 and 0-3 cm, respectively) of the sediment was not included, while for the relationship with MeHg (Figure 11), all data were included.

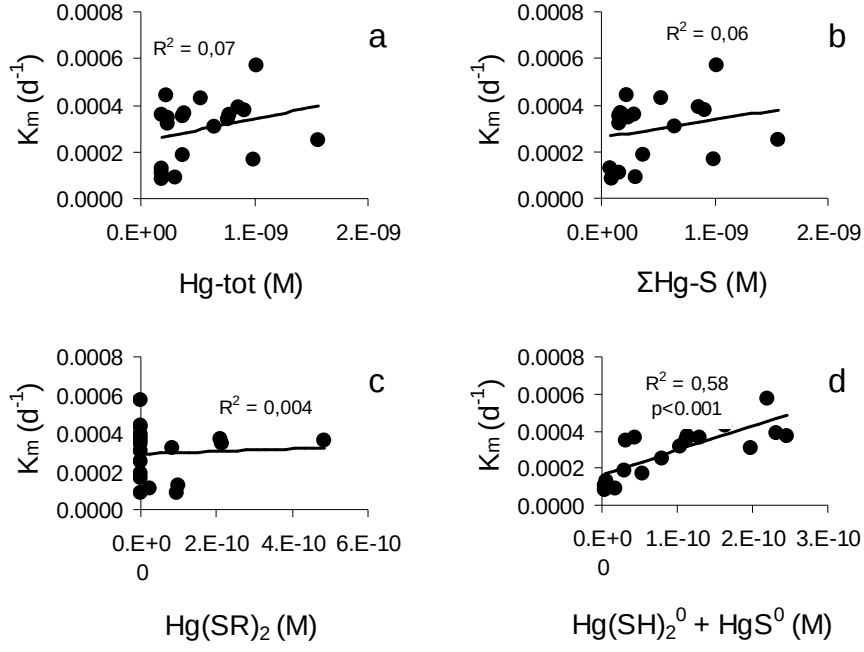

Figure 10. Relationship between (a) Hg^{II} in pore water (M) and k_m (day^{-1}), (b) inorganic Hg^{II}-sulphides in pore water (M) and k_m, (c) Hg^{II}-organic thiol complexes (M) and k_m, and (d) neutral Hg-sulphides (M) and k_m, for the sub-set Köp (5-40 cm) and Sku (3-100 cm). Model A was used for Köp and model C for Sku. From Paper III.

Relationships between concentrations of neutral Hg-sulphides and MeHg are summarized in Figure 11 for the sub-sets of brackish water sediments (Köp and Sku), northern freshwater sediment (Kar), and southern freshwater sediments (Mar, Tur, Sva, Nöt). In Figure 11, model C was used at Mar, model D at Sku, and model B at the rest of the sites. Within each sub-set, a great proportion of the variation in MeHg concentration in the sediment is apparently explained by the concentration of neutral Hg-sulphides in the sediment pore water. Given the uncertainties in measurements as well as in chemical modelling, the relationships appear surprisingly strong. There are substantial differences in slopes of the relationships among the different sub-sets, the slope being greatest for the southern freshwaters and smallest for the brackish waters. This difference in slope may be explained by differences in primary productivity and subsequent availability of suitable organic substrate to methylating organisms, as indicated by a lower C/N ratio (used

as a proxy for input of energy-rich organic matter from pelagic organisms to the sediment surface, section 2.1) and higher annual average temperature sum for the southern freshwaters than for the other sites. In contrast, sulphate is indicated not to be a limiting factor for MeHg production, as the slope is greatest for the southern freshwaters and smallest for the brackish waters, with the highest availability of sulphate. Note that in Figure 11, the entire sediment profiles are included; surface sediments have not been removed, in contrast to e.g. Figure 10. This indicates that spatial variations in electron donor availability, in the long term (as reflected by total accumulated MeHg) may be less important.

The fact that model A or B gave the most highly significant relationship between neutral Hg-sulphides and MeHg concentration for all sites except Sku and Mar may be taken as an indication that the species $Hg(SH)_2^0$ is quantitatively more important than the species $HgOHSH^0$ for uptake in methylating organisms (e.g. Table 6). Even if there are differences in molar volumes and diffusional uptake rates between $Hg(SH)_2^0$ and $HgOHSH^0$, giving a higher uptake rate across the bacterial membrane for $HgOHSH^0$ (Jay *et al.*, 2002), $HgOHSH^0$ will be negligible if model A is used. Given the uncertainties in stability constants for $HgOHSH^0$, discussed in section 2.7, in my opinion, the more conservative log K of -22.3 for $HgOHSH^0$ in equilibrium with HgS(s), e.g. as in model A, is most reasonable to use.

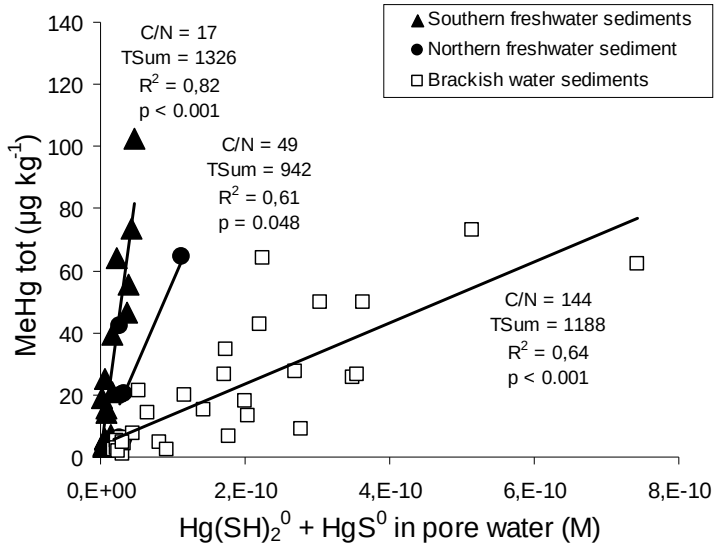

Figure 11. Relationship between concentration of neutral Hg-sulphides ($Hg(SH)_2^0$ + $HgOHSH^0$, M) and MeHg (µg kg^{-1}) for southern freshwaters (filled triangles, Mar, Tur, Sva, Nöt 0-10 cm), northern freshwater (filled circles, Kar 0-10 cm), and brackish waters (open squares, Köp and Sku 0-100 cm). C/N ratios, average annual air temperature sums (threshold 5° C), and levels of significance are given in the figure. Model C was used for speciation modeling at Mar, model D at Sku, and model B at the rest of the sites. From Paper III.

Benoit *et al.*, (1998 and 1999a) reported a negative relationship between the concentration of sulphide in pore water and the concentration of MeHg in sediments, in the Florida Everglades and the Patuxent River, U.S. This observation was explained by a model which predicted a decrease in the concentration of bioavailable, dissolved neutral Hg-sulphides with increasing pore water sulphide concentration (Benoit *et al.*, 1999a). Thus, it was assumed that lower MeHg concentrations in sediments with high concentration of sulfide were due to less uptake (and transformation) of Hg by methylating bacteria. In contrast, we did not observe any significant ($p > 0.05$) relationships between pore water sulphide and neutral Hg-sulphide concentration, nor between pore water sulphide and sediment MeHg concentration in our sediments (Paper III). The lack of relationship between pore water sulphide and neutral Hg-sulphide concentration is likely explained by the fact that the calculated concentration of neutral Hg-sulphides is a result of competition among ligands (mainly organic and inorganic sulphides) for Hg^{II} in solution and that input variables for the chemical modeling, such as DOC and pH, either may covary with, or

counteract the differences among samples in pore water sulphide concentration.

Under what conditions is a negative relationship between the concentrations of sulphide and neutral Hg-sulphides in pore water then expected? In the most straightforward case, if all other factors (e.g. DOC and pH) remain constant, the concentration of neutral Hg-sulphides in pore water is expected to *increase* with increasing concentration of pore water sulphide, regardless if it is controlled by surface complexation reactions (Skyllberg, 2008), with e.g. organic thiols, or by solid HgS(s) (Benoit *et al.*, 1999a). A *decrease* in the concentration of neutral Hg-sulphides with increasing pore water sulphide concentration may only be obtained if the concentration of Hg^{II} species in solution is controlled by surface complexation reactions, and the competition for Hg between the surface and the ligands in solution is increasingly in favour of the surface complex with increasing sulphide concentrations. A model with such properties was developed by Benoit *et al.* (1999a) and was also used in a following paper (Benoit *et al.*, 2001). In addition to true thermodynamic reactions, a diagenetic incorporation of HS^- into the solid phase was included in this model. Because no data are available for this proposed diagenetic reaction, the usefulness of this model, in my opinion, is very limited.

It can be noted that a negative relationship between dissolved sulphide and MeHg production may be explained by factors other than a shift in chemical speciation. For instance, increasing sulphide concentration is paralleled by decreasing sulphate and Fe^{III} concentration, which may limit the activity of methylating bacteria. Also, at high (mM) H_2S concentration, H_2S has a direct negative effect on SRB activity, perhaps via inhibition of cytochromes (Reis *et al.*, 1992). In my opinion, this needs to be considered as alternative explanations for the observed negative relationship between pore water sulphide and sediment MeHg concentration.

3.4 Pore Water Speciation of MeHg and Its Influence on Demethylation in Contaminated Sediments (Paper IV)

Pore water speciation of MeHg was calculated including complexes with halides, hydroxides, inorganic sulphides, and organic thiols (reaction 1-5, Table 3). Complexes with halides and hydroxides, and free $MeHg^+$, were quantitatively negligible for all samples, thus complexes with inorganic

sulphides, MeHgSH(aq) and $MeHgS^-$(aq), and organic thiols, MeHgSR(aq) were dominant. This shows that the solubility of MeHg mainly is controlled by the concentrations of DOC and S^{-II} in the sediment pore water, in sediments with sulphidic conditions. This also becomes apparent when the similar magnitude of the stability constants for formation of MeHgSH and MeHgSR is considered (Table 3, reactions 3 and 4).

Table 7. *Average pore water species distribution (%) of MeHg for sites and sub-sets of sites. Where several profiles were sampled, sampling depths are given for each profile. Modified from Paper IV.*

Site and depth (cm)	**% MeHgSR**	**% MeHgSH**	**% $MeHgS^-$**
Köp 0-20, 0-25, 0-40	17	54	29
Köp 0-10	0	88	11
Sku 0-25, 0-100	18	55	27
Sku 0-10	0	41	59
Kar 0-20	8	90	2
Southern freshwaters[a] 0-10	17	73	10

[a]sites Mar+Tur+Sva+Nöt

It can be noted that MeHg-sulphide species (MeHgSH or $MeHgS^-$) were dominant in all surface (0-10 cm) sediments, while at greater sediment depth, there was greater contribution from MeHgSR (Table 7).

The influence of pore water MeHg speciation on MeHg demethylation rate (k_d, day^{-1}), was evaluated in surface sediments (0-10 cm, sites Köp, Sku, Mar, Kar, Tur, Sva, Nöt), for which k_d was indicated to be independent of tracer additions, section 2.6 (Paper IV). Based on differences in total Hg concentration, the sites were divided into two sub-sets: sites contaminated by Hg^0, with high total Hg concentration (Köp, Sku, Mar), and sites contaminated by phenyl-Hg, with lower total Hg concentration (Kar, Tur, Sva, Nöt). Compared to previous work (Marvin-DiPasquale *et al.*, 2000), the average total Hg concentration for the highly contaminated sub-set (190 nmol g^{-1}) was above the maximum Hg concentration at sites dominated by reductive *mer*-mediated demethylation, while the average total Hg concentration for the low contaminated sub-set was an order of magnitude lower (8.2 nmol g^{-1}).

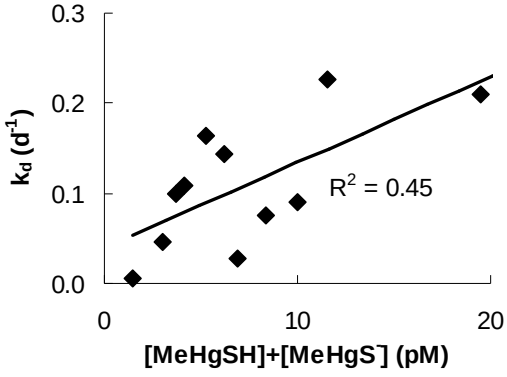

Figure 12. Relationship between calculated concentration of MeHg-sulphides (MeHgSH + $MeHgS^-$, pM) and k_d (day^{-1}) for southern low contaminated surface sediments (Tur, Sva, Nöt, p = 0.02). Modified from Paper IV.

For the sub-set of highly contaminated sites (Köp, Sku, Mar), no significant ($p > 0.05$) relationships between pore water MeHg species and k_d were observed (Paper IV). Similarly, no significant relationships between pore water MeHg species and k_d were observed for all low contaminated sites (Kar, Tur, Sva, Nöt). However, when only the southern low contaminated sites (Tur, Sva, Nöt) were evaluated, a significant ($p = 0.02$) positive relationship was observed between the concentration of MeHg-sulphides ($MeHgSH+MeHgS^-$) and k_d, regardless of whether the concentration of the individual species, or the sum of both species, was considered (Figure 12). The relationship between MeHgSR and k_d was negative for this sub-set (Paper IV).

The significant positive relationship in Figure 12 suggests an influence of pore water MeHg speciation on demethylation. To my knowledge, this has previously not been reported. If oxidative demethylation is assumed for the sub-set of Tur, Sva, Nöt, because of lower total Hg concentration, the relationship suggests an influence of pore water MeHg speciation on oxidative demethylation. The knowledge about oxidative demethylation is limited, thus it is not possible to draw strong conclusions about uptake mechanisms from the relationship. A positive linear relationship is expected if the mechanism is passive diffusion, but if the concentration of the species taken up is low, and only the lower region of the uptake curve is reached, a positive linear relationship may also be observed if carrier-mediated uptake is occurring (e.g. Michaelis-Menten kinetics). The uncertainty in the pK_a for MeHgSH, reaction 5, Table 3 (Dyrssén & Wedborg, 1991), may suggest that the uptake occurs by passive diffusion, because the pK_a may be higher,

and then MeHgSH, which is neutral, may be dominant. It has been suggested that the degradation of MeHg during oxidative demethylation may be analogous to the degradation of monomethylamine ($CH_3NH_3^+$) by methanogens, and acetate by SRB (Marvin-DiPasquale & Oremland, 1998). It can be noted that for the uptake of MeHg in algae, both active (Moye *et al.*, 2002) and passive diffusion (Mason *et al.*, 1996) mechanisms have been suggested.

3.5 Conclusions

The results in this thesis suggest in general that MeHg production (Hg^{II} methylation) is more important than MeHg degradation (demethylation) and input-output processes, for the build-up of MeHg in surface (0-20 cm) sediments.

Total Hg concentrations are shown to have a positive effect on the accumulation of MeHg in sediments. At the same time it is shown that the influence of total Hg and MeHg concentrations on MeHg degradation is complex, and may be both positive and negative.

The positive effect of total Hg on MeHg accumulation is likely explained by a link between the total Hg concentration, and the concentration of Hg^{II} species that are bioavailable to Hg methylating organisms. It can be noted that if the concentration of Hg^{II} species in solution is strictly controlled by HgS(s), no such relationsip is expected, because the dissolved concentration is predicted to be unrelated to the total Hg concentration. A positive relationship between total Hg and MeHg concentrations is likely most frequently observed in sediments contaminated by a local source, where the variation in total Hg is greater than in environments contaminated by more diffuse sources.

The concentration of MeHg in surface sediments is a net result of Hg methylation – MeHg demethylation + MeHg input – MeHg output. The single most important process is Hg methylation. Thus, it is likely safe to use the concentration of MeHg for risk assessment purposes, as a measure of MeHg production in the sediment during a longer time period (likely months-years). In the present work, %MeHg (of total Hg) is shown to be a proxy for MeHg production which is useful also across sites with different properties.

Differences in MeHg production among sediments are often assumed to be controlled by factors influencing bacterial activity, and by the chemical speciation of Hg^{II}. In the present work, the availability of organic substrate (electron donor) to methylating bacteria, as a result of differences in primary production and subsequent input of organic matter to sediments, is indicated to be the most important factor behind differences in MeHg production among sediments with different redox and primary productivity conditions. In previous work, the availability of sulphate (electron acceptor), which is not indicated to limit MeHg production in the present work, has instead been emphasised. In my opinion, the influence of electron donor availability on MeHg production is understudied to date. Perhaps, the influence of electron donor availability on Hg methylation is most apparent in cold climates with strong seasonality, because of greater variability.

Chemical speciation is indicated to be a controlling factor both for MeHg production and degradation. The hypothesis that neutral Hg-sulphides [$Hg(SH)_2^0$(aq) and $HgOHSH^0$(aq)] are the most important bioavailable Hg^{II} species in sediments with anoxic conditions, and are taken up by a passive diffusion mechanism by methylating bacteria, is for the first time supported by a relationship with MeHg production measured in environmental samples. Previous work has put much emphasis on $HgOHSH^0$, but the results indicate that $Hg(SH)_2^0$ may be quantitatively more important. In the present work, there is no support for the hypothesis that the concentration of neutral Hg-sulphides decreases with increasing pore water sulphide concentration. Therefore, other explanations are required for observed negative relationships between concentrations of pore water sulphide and sediment MeHg.

For the first time, relationships between pore water MeHg speciation and demethylation rates have been evaluated. The results suggest an influence of MeHg-sulphides [MeHgSH(aq) and $MeHgS^-$(aq)] on demethylation rates. However, because of limited knowledge, e.g. about the pK_a of MeHgSH and demethylation mechanisms, this relationship should be interpreted with caution. The measured rate of demethylation at the site Sku, which is a brackish water site, was markedly higher than at all other sites. Demethylation rates at Sku increased with concentrations of added MeHg in relation to ambient MeHg, suggesting a stimulation of demethylating organisms. This result is intriguing and merits further study.

Sample treatment is known to be important for the determined concentration of chemical species in environmental samples. In the present work, it is shown that oxidation during filtration of pore water in ambient air causes significantly lower determined pore water MeHg concentrations, as compared to filtration in anoxic atmosphere. The results indicate that filtration is a particularly oxidation sensitive step in sample pre-treatment, and that samples with intermediate redox conditions may be most sensitive. This likely has implications for determination of MeHg in solution in all matrices with at least slightly reducing conditions.

3.6 Implications

The importance of total Hg for MeHg production, reported in this thesis, shows that remediation of locally contaminated sediments likely is beneficial in order to decrease Hg concentrations in biota. However, it should be borne in mind that it is not possible to remediate the entire area that has been subjected to more diffuse Hg contamination. Thus, obtaining "a non-toxic environment" with respect to Hg concentrations in biota is, in my opinion, indeed a challenge, if not impossible.

Risk assessment of contaminated sites in Sweden is mainly based on total concentrations of the compounds of interest, via the use of generic or site-specific guidance values. However, it is well established that it is MeHg that biomagnifies in aquatic food webs, which causes effects on aquatic organisms, and leads to exposure of humans to Hg via consumption of fish. Thus, the risk of Hg contaminated sediments is linked to MeHg rather than to total Hg, and risk assessment of Hg contaminated areas can be refined by including MeHg concentration and mobility. Most obviously, when comparing sites with similar total Hg concentration, the risk will be higher where MeHg concentration and mobility is higher, all other factors equal. The results reported in this thesis show that %MeHg (of total Hg) is a robust estimate of MeHg production, which can be used across sites with different properties. MeHg solubility, and thus mobility and bioavailability, is mainly controlled by the concentrations of dissolved organic matter and inorganic sulphides. To more accurately evaluate the risk at Hg contaminated sites, differences in environmental conditions, most importantly primary production and redox, should also be considered.

3.7 Future Research Needs

Further studies are needed within a number of areas of Hg biogeochemistry in sediments. The points listed below are of particular scientific interest and practical significance:

- The influence of primary production on Hg methylation needs to be studied further.

- Experimental data are needed on the stability constant for $HgOHSH^0(aq)$ in equilibrium with HgS(s).

- Measurements of concentrations of dissolved neutral Hg-sulphides as well as their uptake in organisms are needed.

- The potential bioavailability of Hg^{II}-amino acid complexes to methylating organisms needs to be further examined.

- Increased knowledge is needed on Hg methylation mechanisms. For instance, is the mechanism similar among different phylogenetic groups of bacteria?

- Pure-culture experiments are needed on induction of reductive *mer*-mediated demethylation by MeHg, and the proposed mechanisms of oxidative demethylation need to be verified in pure culture.

- The quantitative importance of different factors for accumulated MeHg concentration and uptake in biota in different systems remains to be determined.

References

Allison, J.D. & Brown, D.S. (1995). MINTEQA2/PRODEFA2-a geochemical speciation model and interactive pre-processor. In: Loeppert, P.H. (Ed.) *Chemical equilibrium and reaction models.* Madison, Wisconsin, U.S.: SSSA. p. 241-252.

Baldi, F., Pepi, M. & Filipelli, M. (1993). Methylmercury resistance in *Desulfovibrio desulfuricans* strains in relation to methylmercury degradation. *Applied and environmental microbiology* 59, 2479-2485.

Barkay, T., Gillman, M. & Turner, R.R. (1997). Effects of dissolved organic carbon and salinity on bioavailability of mercury. *Applied and environmental microbiology* 63, 4267-4271.

Barkay, T., Miller, S.M. & Summers, A.O. (2003). Bacterial mercury resistance from atoms to ecosystems. *FEMS microbiology reviews* 27, 355-384.

Benoit, J.M., Gilmour, C.C., Heyes, A., Mason, R.P. & Miller, C.L. (2003). Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. *ACS symposium series* 835, 262-297.

Benoit, J.M., Gilmour, C.C. & Mason, R.P. (2001). The influence of sulfide on solid-phase mercury bioavailability for methylation by pure cultures of *Desulfobulbus propionicus* (1pr3). *Environmental science and technology* 35, 127-132.

Benoit, J.M., Gilmour, C.C., Mason, R.P. & Heyes, A. (1999a). Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. *Environmental science and technology* 33, 951-957.

Benoit, J.M., Gilmour, C.C., Mason, R.P., Riedel, G.S. & Riedel, G.F. (1998). Behaviour of mercury in the Patuxent river estuary. *Biogeochemistry* 40, 249-265.

Benoit, J.M., Mason, R.P. & Gilmour, C.C. (1999b). Estimation of mercury-sulfide speciation in sediment pore waters using octanol-water partitioning and implications for availability to methylating bacteria. *Environmental toxicology and chemistry* 18, 2138-2141.

Biester, H. & Scholz, C. (1997). Determination of mercury binding forms in contaminated soil: mercury pyrolysis versus sequential extraction. *Environmental science and technology* 31, 233-239.

Bindler, R., Renberg, I., Appleby, P.G., Andersson, N.J. & Rose, N.L. (2001). Mercury accumulation rates and spatial patterns in lake sediments from west greenland: a coast to ice margin transect. *Environmental science and technology* 35, 1736-1741.

Bloom, N.S. (1992). On the chemical form of mercury in edible fish and marine invertebrate tissue. *Canadian journal of fisheries and aquatic sciences* 49, 1010-1017.

Börjesson, E. (2008). Lägesbeskrivning av efterbehandlingsarbetet i landet 2007. Swedish EPA, Dnr 642-516-08 Rf.

Bornmann, G., Henke, G., Alfes, H. & Möllmann, H. (1970). Über die enterale resorption von metallischem quecksilber. *Archives in toxicology* 26, 203-209.

Bufflap, S.E. & Allen, H.E. (1995). Sediment pore water collection methods for trace metal analysis: a review. *Water research* 29, 165-177.

Carty, A.J. & Malone, S.F. (1979). The chemistry of mercury in biological systems. In: Nriagu, J.O. (Ed.) *The biogeochemistry of mercury in the environment.* New York: Elsevier/North Holland biomedical press. p. 433-479.

Choi, S.-C. & Bartha, R. (1993). Cobalamin-mediated mercury methylation by *Desulfovibrio desulfuricans* LS. *Applied and environmental microbiology* 59, 290-295.

Choi, S.-C., Jr, T.C. & Bartha, R. (1994a). Enzymatic catalysis of mercury methylation by *Desulfovibrio desulfuricans* LS. *Applied and environmental microbiology* 60, 1342-1346.

Choi, S.-C., Jr, T.C. & Bartha, R. (1994b). Metabolic pathways leading to mercury methylation in *Desulfovibrio desulfuricans* LS. *Applied and environmental microbiology* 60, 4072-4077.

Clark, D.L., Weiss, A.A. & Silver, S. (1977). Mercury and organomercurial resistances determined by plasmids in *Pseudomonas*. *Journal of bacteriology* 132, 186-196.

Compeau, G.C. & Bartha, R. (1985). Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. *Applied and environmental microbiology* 50, 498-502.

Dahlberg, C. & Hermansson, M. (1995). Abundance of Tn*3*, Tn*21*, and Tn*501* transposase (*tnpA*) sequences in bacterial community DNA from marine environments. *Applied and environmental microbiology* 61, 3051-3056.

Dannenberg, S., Kroder, M., Dilling, W. & Cypionka, H. (1992). Oxidation of H_2, organic compounds and inorganic sulfur compounds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria. *Archives in microbiology* 158, 93-99.

Daskalakis, K.D. & Helz, G.R. (1993). The solubility of sphalerite (ZnS) in sulfidic solutions at 25° C and 1 atm pressure. *Geochimica et cosmochimica acta* 57, 4923-4931.

Deacon, G.B. (1978). Volatilisation of methyl-mercuric chloride by hydrogen sulphide. *Nature* 275, 344.

Devereux, R., Winfrey, M.R., Winfrey, J. & Stahl, D.A. (1996). Depth profile of sulfate reducing bacterial ribosomal RNA and mercury methylation in an estuarine sediment. *FEMS microbiology ecology* 20, 23-31.

Downs, S.G., Macleod, C.L. & Lester, J.N. (1998). Mercury in precipitation and its relation to bioaccumulation in fish: a literature review. *Water, air and soil pollution* 108, 149-187.

Dyrssén, D. & Wedborg, M. (1991). The sulphur-mercury(II) system in natural waters. *Water, air and soil pollution* 56, 507-519.

Ekstrom, E.B. & Morel, F.M.M. (2008). Cobalt limitation of growth and mercury methylation in sulfate-reducing bacteria. *Environmental science and technology* 42, 93-99.

Ekstrom, E.B., Morel, F.M.M. & Benoit, J.M. (2003). Mercury methylation independent of the acetyl-coenzyme a pathway in sulfate-reducing bacteria. *Applied and environmental microbiology* 69, 5414-5422.

EPA, U.S. (2002). Method 1631, revision E: mercury in water by oxidation, purge and trap, and cold vapour atomic fluorescence spectrometry.

Essington, M.E. (2004). *Soil and water chemistry-an integrative approach.* Boca Raton: CRC press.

Fassett, J.D. & Paulsen, P.J. (1989). Isotope dilution mass spectrometry for accurate elemental analysis. *Analytical chemistry* 61, 643A-649A.

Fleming, E.J., Mack, E.E., Green, P.G. & Nelson, D.C. (2006). Mercury methylation from unexpected sources: molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Applied and environmental microbiology* 72, 457-464.

Furakawa, K., Suzuki, T. & Tonomura, K. (1969). Decomposition of organic mercurial compounds by mercury-resistant bacteria. *Agricultural and biological chemistry* 33, 128-130.

Geelhoed, J.S., Hiemstra, T. & Riemsdijk, W.H.v. (1997). Phosphate and sulfate adsorption on goethite: single anion and competitive adsorption. *Geochimica et cosmochimica acta* 61, 2389-2396.

Gilmour, C.C. & Henry, E.A. (1991). Mercury methylation in aquatic systems affected by acid deposition. *Environmental pollution* 71, 131-169.

Gilmour, C.C., Henry, E.A. & Mitchell, R. (1992). Sulfate stimulation of mercury methylation in freshwater sediments. *Environmental science and technology* 26, 2281-2287.

Gilmour, C.C., Riedel, G.S., Ederington, M.C., Bell, J.T., Benoit, J.M., Gill, G.A. & Stordal, M.C. (1998). Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. *Biogeochemistry* 40, 327-345.

Grieb, T.M., Driscoll, C.T., Gloss, S.P., Schofield, C.L., Bowie, G.L. & Porcella, D.B. (1990). Factors affecting mercury accumulation in fish in the upper Michigan peninsula. *Environmental toxicology and chemistry* 9, 919-930.

Gübeli, A.O. & Ste-Marie, J. (1967). Constantes de stabilité de thiocomplexes et produits de solubilité de sulfures de meteux. II. Sulfure de zinc. *Canadian journal of chemistry* 45, 2101-2108.

Håkansson, L. (1996). A simple model to predict the duration of the mercury problem in Sweden. *Ecological modelling* 93, 251-262.

Hammerschmidt, C.R. & Fitzgerald, W.F. (2004). Geochemical controls on the production and distribution of methylmecuiry in near-shore marine sediments. *Environmental science and technology* 38, 1487-1495.

Hammerschmidt, C.R. & Fitzgerald, W.F. (2006). Methylmercury cycling in sediments on the continental shelf of southern New England. *Geochimica et cosmochimica acta* 70, 918-930.

Han, S., Gill, G.A., Lehman, R.D. & Choe, K.-Y. (2006). Complexation of mercury by dissolved organic matter in surface waters of Galveston Bay, Texas. *Marine chemistry* 98, 156-166.

Heyes, A., Mason, R.P., Kim, E.-H. & Sunderland, E. (2006). Mercury methylation in estuaries: insights from using measuring rates using stable mercury isotopes. *Marine chemistry* 102, 134-147.

Hintelmann, H., Keppel-Jones, K. & Evans, R.D. (2000). Constants of mercury methylation and demethylation rates in sediments and comparison of tracer and ambient mercury availability. *Environmental toxicology and chemistry* 19, 2204-2211.

Jay, J.A., Morel, F.M.M. & Hemond, H.F. (2000). Mercury speciation in the presence of polysulfides. *Environmental science and technology* 34, 2196-2200.

Jay, J.A., Murray, K.J., Gilmour, C.C., Mason, R.P., Morel, F.M.M., Roberts, A.L. & Hemond, H.F. (2002). Mercury methylation by *Desulfovibrio desulfuricans* ND132 in the presence of polysulfides. *Applied and environmental microbiology* 68, 5741-5745.

Jensen, S. & Jernelöv, A. (1969). Biological methylation of mercury in aquatic organisms. *Nature* 223, 753-754.

Karlsson, T. & Skyllberg, U. (2003). Bonding of ppb levels of methyl mercury to reduced sulfur groups in soil organic matter. *Environmental science and technology* 37, 4912-4918.

Kerin, E.J., Gilmour, C.C., Roden, E., Suzuki, M.T., Coates, J.D. & Mason, R.P. (2006). Mercury methylation by dissimilatory iron-reducing bacteria. *Applied and environmental microbiology* 72, 7919-7921.

Khwaja, A.R., Bloom, P.R. & Brezonik, P.L. (2006). Binding constants of divalent mercury (Hg^{2+}) in soil humic acids and soil organic matter. *Environmental science and technology* 40, 844-849.

King, J.K., Kostka, J.E., Frischer, M.E., Saunders, F.M. & Jahnke, R.A. (2001). A quantitative relationship that demonstrates mercury methylation rates in marine sediments are based on the community composition and activity of sulfate-reducing bacteria. *Environmental science and technology* 35, 2491-2496.

Kiyono, M., Omura, T., Fujimori, H. & Pan-Hou, H. (1995). Lack of involvement of *mer*T and *mer*P in methylmercury transport in mercury resistant *Pseudomonas* K 62. *FEMS microbiology letters* 128, 301-306.

Konhauser, K. (2007). *Introduction to geomicrobiology.* Oxford U. K.: Blackwell publishing.

Lambertsson, L. & Björn, E. (2004). Validation of a simplified field-adapted procedure for routine determinations of methyl mercury at trace levels in natural water samples using species-specific isotope dilution mass spectrometry. *Analytical and bioanalytical chemistry* 380, 871-875.

Lambertsson, L., Lundberg, E., Nilsson, M. & Frech, W. (2001). Applications of enriched stable isotope tracers in combination with GC-ICP-MS to study mercury species

transformation in sea sediments during *in situ* ethylation and determination. *Journal of analytical atomic spectrometry* 16, 1296-1301.

Lambertsson, L. & Nilsson, M. (2006). Organic material: the primary control on mercury methylation and ambient methyl mercury concentrations in estuarine sediments. *Environmental science and technology* 40, 1822-1829.

Langford, N.J. & Ferner, R.E. (1999). Toxicity of mercury. *Journal of human hypertension* 13, 651-656.

Länsstyrelsen Dalarna (2000). Pappersindustrin i Dalarna-industrihistorisk kartläggning med avseende på förorenad mark, Rapport 2000:14.

Larsson, T., Björn, E. & Frech, W. (2005). Species specific isotope dilution with on line derivatisation for determination of gaseous mercury species. *Journal of analytical atomic spectrometry* 20, 1232-1239.

Marvin-DiPasquale, M., Agee, J., McGowan, C., Oremland, R.S., Thomas, M., Krabbenhoft, D. & Gilmour, C.C. (2000). Methyl-mercury degradation pathways: a comparison among three mercury-impacted ecosystems. *Environmental science and technology* 34, 4908-4916.

Marvin-DiPasquale, M.C. & Oremland, R.S. (1998). Bacterial methylmercury degradation in Florida Everglades peat sediment. *Environmental science and technology* 32, 2556-2563.

Mason, R., Bloom, N., Capellino, S., Gill, G., Benoit, J. & Dobbs, C. (1998). Investigation of porewater sampling methods for mercury and methylmercury. *Environmental science and technology* 32, 4031-4040.

Mason, R.P., Reinfelder, J.R. & Morel, F.M.M. (1996). Uptake, toxicity, and trophic transfer of mercury in a coastal daitom. *Environmental science and technology* 30, 1835-1845.

McBride, M.B. (1994). *Environmental chemistry of soils.* Oxford U.K.: Oxford university press.

Mikac, N., Niessen, S., Ouddane, B. & Wartel, M. (1999). Speciation of mercury in sediments of the Seine estuary (France). *Applied organometallic chemistry* 13, 715-725.

Morel, F.M.M., Kraepiel, A.M.L. & Amyot, M. (1998). The chemical cycle and bioaccumulation of mercury. *Annual review of ecology and systematics* 29, 543-566.

Morse, J.W., Millero, F.J., Cornwell, J.C. & Rickard, D. (1987). The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. *Earth-science reviews* 24, 1-42.

Moye, H.A., Miles, C.J., Philips, E.J., Sargent, B. & Merritt, K.K. (2002). Kinetics and uptake mechanisms for monomethylmercury between freshwater algae and water. *Environmental science and technology* 36, 3550-3555.

Nagase, H., Ose, Y., Sato, T. & Ishakawa, T. (1984). Mercury methylation by compounds in humic material. *The science of the total environment* 32, 147-156.

Nagase, H., Ose, Y., Sato, T. & Ishikawa, T. (1982). Methylation of mercury by humic substances in an aquatic environment. *The science of the total environment* 24, 133-142.

Nordstrom, D.K., Plummer, L.N., Langmuir, D., Busenberg, E., May, H.M., Jones, B.F. & Parkhurst, D.L. (1990). Revised chemical equilibrium data for major water-mineral reactions and their limitations. In: Melchior, D.C., *et al.* (Eds.) *Chemical modelling of aqueous systems: II. ACS ser. volume 416.* Washington DC: American chemical society. p. 398-413.

Nucifora, G., Chu, L., Silver, S. & Misra, T.K. (1989). Mercury operon regulation by the MerR gene of the organomercurial resistance system of plasmid pDU1358. *Journal of bacteriology* 171, 4241-4247.

Oremland, R.S., Culbertson, C.W. & Winfrey, M.R. (1991). Methylmercury decomposition in sediments and bacterial cultures: involvement of methanogens and sulfate reducers in oxidative demethylation. *Applied and environmental microbiology* 57, 130-137.

OSPAR (1990). OSPAR PARCOM decision 1990:3.

Paquette, K. & Helz, G. (1995). Solubility of cinnabar (red HgS) and implications for mercury speciation in sulfidic waters. *Water, air and soil pollution* 80, 1053-1056.

Paquette, K.E. & Helz, G.R. (1997). Inorganic speciation of mercury in sulfidic waters: the importance of zerovalent sulfur. *Environmental science and technology* 31, 2148-2153.

Pearson, A.J., Bruce, K.D., Osborn, A.M., Ritchie, D.A. & Strike, P. (1996). Distribution of class II transposase and resolvase genes in soil bacteria and their association with *mer* genes. *Applied and environmental microbiology* 62, 2961-2965.

Petersson-Grawé, K., Concha, G. & Ankarberg, E. (2007). *Riskvärdering av metylkvicksilver i fisk*. Livsmedelsverket rapport 10 2007.

Qian, J., Skyllberg, U., Frech, W., Bleam, W.F., Bloom, P.R. & Petit, P.E. (2002). Bonding of methyl mercury to reduced sulfur groups in soil and stream organic matter as determined by x-ray absorption spectroscopy and binding affinity studies. *Geochimica et cosmochimica acta* 66, 3873-3885.

Qvarnström, J., Tu, Q., Frech, W. & Lüdke, W. (2000). Flow injection-liquid chromatography-cold vapour atomic absorption spectrometry for rapid determination of methyl and inorganic mercury. *The analyst* 125, 1193-1197.

Reis, M.A.M., Almeida, J.S., Lemos, P.C. & Carrondo, M.J.T. (1992). Effect of hydrogen sulfide on growth of sulfate reducing bacteria. *Biotechnology and bioengineering* 40, 593-600.

Rietra, R.P.J.J., Hiemstra, T. & Riemsdijk, W.H.v. (1999). Sulfate adsorption on goethite. *Journal of colloid and interface science* 218, 511-521.

Robinson, J.B. & Tuovinen, O.H. (1984). Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical, and genetic analyses. *Microbiological reviews* 48, 95-124.

RodriguezMartin-Doimeadios, R.C., Tessier, E., Amouroux, D., Guyoneaud, R., Duran, R., Caumette, P. & Donard, O.F.X. (2004). Mercury methylation and volatilisation pathways in estuarine sediment slurries using species-specific enriched stable isotopes. *Marine chemistry* 90, 107-123.

Schaefer, J.K. & Morel, F.M.M. (2008). High methylation rates of mercury bound to cysteine by *Geobacter sulfurreducens*. *Nature geoscience* In press.

Schaefer, J.K., Yagi, J., Reinfelder, J.R., Cardona, T., Ellickson, K.M., Tel-Or, S. & Barkay, T. (2004). Role of the bacterial organomercury lyase (MerB) in controlling

methylmercury accumulation in mercury-contaminated natural waters. *Environmental science and technology* 38, 4304-4311.

Schottel, J., Mandal, A., Clark, D., Silver, S. & Hedges, R.W. (1974). Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. *Nature* 251, 335-337.

Schwarzenbach, G. & Schellenberg, M. (1965). Die komplexchemie des metylquecksilberkations. *Helvetica chimica acta* 48, 28-46.

Schwarzenbach, G. & Widmer, M. (1963). Die löslichkeit von metallsulfiden I. schwarzes quecksilbersulfid. *Helvetica chimica acta* 46, 2613-2628.

Selifonova, O., Burlage, R. & Barkay, T. (1993). Bioluminescent sensors for detection of bioavailable Hg(II) in the environment. *Applied and environmental microbiology* 59, 3083-3090.

Sellers, P., Kelly, C.A., Rudd, J.W.M. & Machutchon, A.R. (1996). Photodegradation of methylmercury in lakes. *Nature* 380, 694-697.

Skyllberg, U. (2008). Competition among thiols, inorganic sulfides and polysulfides for Hg and MeHg in wetland soils and sediments under suboxic conditions-illumination of controversies and implications for MeHg net production. *Journal of geophysical research* In press.

Skyllberg, U., Bloom, P.R., Qian, J., Lin, C.-M. & Bleam, W.F. (2006). Complexation of mercury(II) in soil organic matter: EXAFS evidence for linear two-coordination with reduced sulfur groups. *Environmental science and technology* 40, 4174-4180.

Skyllberg, U., Drott, A., Lambertsson, L., Björn, E., Karlsson, T., Jonsson, T., Heinemo, S.-Å. & Holmström, H. (2007). Net methylmercury production as a basis for improved risk assessment of mercury-contaminated sediments. *Ambio* 36, 437-442.

Skyllberg, U., Qian, J., Frech, W., Xia, K. & Bleam, W.F. (2003). Distribution of mercury, methyl mercury and organic sulphur species in soil, soil solution and stream of a boreal forest catchment. *Biogeochemistry* 64, 53-76.

Skyllberg, U., Xia, K., Bloom, P.R., Nater, E.A. & Bleam, W.F. (2000). Binding of mercury(II) to reduced sulfur in soil organic matter along upland-peat soil transects. *Journal of environmental quality* 29, 855-865.

Smith, R.M. & Martell, A.E. (1976). *Critical stability constants: inorganic complexes* New York: Plenum press.

Snell, J.P., Quétel, C.R., Lambertsson, L. & Qvarnström, J. (2004). Preparation and certification of ERM-AE670, a ^{202}Hg enriched methylmercury isotopic reference material. *Journal of analytical atomic spectrometry* 19, 1315-1324.

Stumm, W. & Morgan, J.J. (1996). *Aquatic Chemistry*. 3rd edition. New York U.S.: John Wiley and Sons.

Sunderland, E.M., Gobas, F.A.P.C., Branfireun, B.A. & Heyes, A. (2006). Environmental controls on the speciation and distribution of mercury in coastal sediments. *Marine chemistry* 102, 111-123.

Sunderland, E.M., Gobas, F.A.P.C., Heyes, A., Branfireun, B.A., Bayer, A.K., Cranston, R.E. & Parsons, M.B. (2004). Speciation and bioavailability of mercury in well-mixed estuarine sediments. *Marine chemistry* 90, 91-105.

Swedish government (2000). Svenska miljömål – delmål och åtgärdsstrategier, Prop. 2000/01:130.

Templeton, D.M., Ariese, F., Cornelis, R., Danielsson, L.-G., Muntau, H., Leeuwen, H.P.v. & Lobinski, R. (2000). Guidelines for terms related to chemical speciation and fractionation of elements, definitions, structural aspects, and methodological approaches. *Pure and applied chemistry* 72, 1453-1470.

Tossell, J.A. (2001). Calculation of the structures, stabilities, and properties of mercury sulfide species in aqueous solution. *Journal of physical chemistry* 105, 935-941.

U.N. (2002). Global mercury assessment. Geneva,Switzerland: United nations environment program.

Urban, N.R., Ernst, K. & Bernasconi, S. (1999). Addition of sulfur to organic matter during early diagenesis of lake sediments. *Geochimica et cosmochimica acta* 63, 837-853.

W.H.O. (1990). Environmental health criteria 101, methylmercury. World health organisation.

Wolfenden, S., Charnock, J.M., Hilton, J., Livens, F.R. & Vaughan, D.J. (2005). Sulfide species as a sink for mercury in lake sediments. *Environmental science and technology* 39, 6644-6648.

Zumdahl, S.S. (1998). *Chemical principles*. 3rd edition. Boston U.S.: Houghton Mifflin.

Acknowledgements

I would like to express my gratitude to those who contributed to this work. I think I have been very lucky to have supervisors that have believed in me, and had a genuine interest in the project. I have also been surrounded, both in the field, in laboratories, and during coffee breaks, by colleagues that have made the hard moments easier and the pleasant ones even more pleasant. You know who you are, but a few people need to be mentioned by name:

Ulf Skyllberg, main supervisor: Thank you for sharing your great knowledge on Hg as well as on birds, and for infinite energy and enthusiasm. Thank you for all the time you have invested.

Erik Björn, co-supervisor: Thank you for excellent guidance through the world of isotope dilution, and for insightful comments on the manuscripts.

Lars Lambertsson, Tom Larsson, Bengt Andersson, Birgitta Olsson, Andrei Shchukarev, Harald Biester, Torbjörn Karlsson: Thank you for assistance in the laboratory. A special thanks to Lars and Tom for enduring my questions.

Henning Holmström, Sven-Åke Heinemo, Christin Jonasson, Lisbeth Drugge, Nils Ivarsson, Anders Hellman, Göran Eriksson, Ronald Bergman, Jonny Skarp, Anders Helgée, Jens Nilsson, Anna Aleljung, Per Olsson, Veronica Östman, Jonatan Klaminder: Thank you for assistance with planning and practical issues before and during sampling.

Thanks to Wolfgang Frech for initial guidance in the laboratory, and for a good time in Karlsruhe.

The Centre for Environmental Research (CMF) in Umeå, the Kempe Foundation, Swedish EPA, European Union Structural Funds and New Objective 1, and the Knut and Alice Wallenberg Foundation are greatly acknowledged for providing funding.

Finally, I would like to thank the evaluation committee for valuable and constructive comments on the thesis during pre-evaluation.

Sammanfattning

Biomagnifikation av kvicksilver (Hg) i akvatiska näringskedjor sker nästan uteslutande i form av monometylkvicksilver (MeHg). I avhandlingen har inverkan av kemisk form och miljöfaktorer på omvandlingar mellan MeHg och oorganiskt kvicksilver (Hg^{II}), som är den dominerande formen av Hg i sediment, studerats på åtta platser i Sverige med lokalt Hg-förorenade sediment. Källan till Hg-förorening var antingen Hg^0(l) eller fenylkvicksilver och totalhalten Hg varierade mellan 1,0-1100 nmol g^{-1}. Miljöfaktorer, tex salinitet, temperaturklimat, primärproduktion, redoxförhållanden och innehåll och kvalitet av organiskt material varierade påtagligt mellan platser. Resultaten visar att produktion av MeHg (metylering av Hg^{II}) är en viktigare faktor för ackumulation av MeHg i ytsediment (0-20 cm) än nedbrytning (demetylering) och nettotransport av MeHg. Totalhalten Hg påverkar MeHg-produktionen, sannolikt genom dess inverkan på koncentrationen av biotillgängliga former av Hg^{II}. Resultaten indikerar att den viktigaste faktorn för variation i MeHg-produktion mellan platser är tillgången på elektrondonatorer (organiskt material) för metylerande bakterier, som ett resultat av skillnader mellan platser i primärproduktion och därmed följande tillskott av organiskt material till sedimenten. Däremot finns inga indikationer på att tillgång på sulfat (elektronacceptor) är begränsande för MeHg-produktionen i de sediment som studerats. Inom delpopulationer av platser med likartade förhållanden förklaras en stor del av variationen i totalhalt MeHg av koncentrationen av lösta, oladdade Hg^{II}-sulfider [$Hg(SH)_2^0$(aq) och möjligen $HgOHSH^0$(aq)], vilket styrker hypotesen att dessa är de viktigaste biotillgängliga Hg^{II}-formerna i sediment med reducerande förhållanden. Nedbrytning av MeHg påverkas av totalhalterna av Hg och MeHg i sedimenten, men effekterna varierar mellan platser. Det finns indikationer på att oxidativ nedbrytning av MeHg är positivt relaterad till koncentrationen av lösta MeHg-sulfider [MeHgSH(aq) och $MeHgS^-$(aq)]. För en förbättrad riskbedömning av Hg-förorenade sediment rekommenderas att koncentration och löslighet av MeHg kvantifieras. Resultaten visar att %MeHg (av totalt Hg) ger ett robust mått på MeHg-produktion som kan användas för jämförelse både inom och mellan platser. Lösligheten av MeHg i sediment med reducerande förhållanden (sulfidbildning) styrs framför allt av halterna av löst organiskt material och oorganiska sulfider [H_2S(aq), HS^-(aq)]. En delstudie visar att filtrering av porvatten måste ske i syrefri atmosfär för att få riktiga data på koncentrationen MeHg i porvattnet.