Remediation of mercury contaminated soil and biological mercury methylation in the landscape

JINGYING XU
Accumulation of mercury (Hg) in soil originating from both natural and anthropogenic sources poses a major hazard to environmental and human health. Inorganic Hg(II) in soil can be transformed to highly toxic methylmercury (MeHg) mainly via methylating microorganisms. Although MeHg constitutes less than 2% of total Hg in soil, it enters aquatic systems through runoff and can be subsequently bioaccumulated along the food chain, thereby causing severe harm to humans.

Current major remediation techniques to control soil Hg contamination were reviewed. Organic matter, clay/minerals and complexation ligands within soil are principal factors influencing Hg mobility that is crucial for evaluating and optimising remedial techniques. The potential of soil washing to treat soil Hg contamination was evaluated. The studied soil was fractionated from fine to coarse particles to assess the effectiveness of physical separation. Batch leaching and pH-static titration tests were performed using (1) water, (2) EDTA, (3) NaOH, (4) HCl, (5) acidic leachates from biodegradable wastes, and (6) alkaline leachates from fly/bottom ashes, to estimate the efficiency of chemical extraction. Less than 1.5% of the total Hg could be mobilised after combined treatments, implying very tight binding of Hg to soil particles, thereby hampering soil washing as a strategy for the studied soil.

Hg(II) methylation in boreal soils and lake sediments can have major consequences for MeHg inputs to downstream aquatic systems. It is therefore important to understand the biogeochemical mechanisms involved in MeHg formation in these landscapes. The microbes involved in Hg(II) methylation in sediments and boreal forests and wetlands were investigated by high-throughput 16S rRNA and hgcA sequencing with molecular barcoding. In all three environments, hgcA sequences were distributed among Proteobacteria, Firmicutes and Euryarchaeota, and Deltaproteobacteria, particularly Geobacteraceae, appeared to play a predominant role. Ruminococcaceae were also abundant Hg(II) methylators in soils from one forest and all the wetlands. The boreal forest survey provided some first insights about the possible link between MeHg formation and non-Hg(II) methylating bacterial communities that likely support the growth and activity of Hg(II) methylating members. Results from wetlands pointed out nutrient status as an important factor shaping Hg(II) methylating communities across the four wetlands, and highlighted a significant role of water content and iron in controlling the distribution of Hg(II) methylators within individual wetlands. Furthermore, the interactions between Hg(II) methylating groups revealed that the more anaerobic and productive conditions seemed to favour the activity of Methanoregulaceae and hamper the growth of Ruminococcaceae. Results from lake sediments supported that Geobacteraceae have an important role in Hg(II) methylation under ferruginous geochemical conditions. Our findings provide a better understanding of Hg(II) methylating communities in the landscape.

Keywords: Mercury contamination, Soil remediation, Methylmercury, Mercury methylation, hgcA, Community composition, Bacteria, Landscape

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<tr>
<td>16S rRNA</td>
<td>16S ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>CE</td>
<td>Chemical extraction</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>Inductively coupled plasma atomic emission spectrometry</td>
</tr>
<tr>
<td>ICP-SFMS</td>
<td>Inductively coupled plasma sector field mass spectrometry</td>
</tr>
<tr>
<td>ICPMS</td>
<td>Inductively coupled plasma mass spectrometry</td>
</tr>
<tr>
<td>IDA</td>
<td>Isotope dilution analysis</td>
</tr>
<tr>
<td>L/S</td>
<td>Liquid-to-solid ratio</td>
</tr>
<tr>
<td>MeHg</td>
<td>Methylmercury</td>
</tr>
<tr>
<td>nMDS</td>
<td>Nonmetric multidimensional scaling</td>
</tr>
<tr>
<td>OM</td>
<td>Organic matter</td>
</tr>
<tr>
<td>OTU</td>
<td>Operational taxonomic unit</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PS</td>
<td>Physical separation</td>
</tr>
<tr>
<td>SRB</td>
<td>Sulphate reducing bacteria</td>
</tr>
<tr>
<td>SW</td>
<td>Soil washing</td>
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</table>
1. Introduction

Mercury (Hg) is a naturally occurring heavy metal typically present at low concentrations in most environments (Ullrich et al., 2001). Currently, about 10% of total Hg released to the atmosphere comes from natural sources and 30% from anthropogenic sources, while re-emissions, most of which was originally from anthropogenic sources, constitute the third important source-class, accounting for about 60% of Hg emissions to air (UNEP, 2013). Hg cycling is therefore central for the re-distribution of emitted Hg.

Hg is considered as a global contaminant because it can undergo long-range transport in the atmosphere, while it is also persistent in the environment, accumulating in the food web and posing severe adverse effects on both humans and ecosystem health. All forms of Hg are toxic, with methylmercury (MeHg), a neurotoxin, being the form of particular concern because of its ability to bioaccumulate and biomagnify. Acute Hg exposure can produce permanent damage to the nervous system; in addition, Hg can affect the lungs, kidneys, brain, and/or skin and cause adverse effects (Liu et al., 2011; Fitzgerald and Lamborg, 2013).

Human exposure has raised such concern that 128 countries have signed a global treaty, The Minamata Convention on Mercury, which entered into force in August 2017. This treaty has the explicit objectives to reduce Hg emissions and protect human health and the environment. Major highlights of the Minamata Convention include a ban on new Hg mines, phase-out of existing operations, and removal of Hg from a number of products and processes, including the regulation of artisanal and small-scale gold mining. The convention also entails control measures for Hg emissions to air and the release to land and water as well as for interim storage of Hg and its disposal once it becomes waste (UNEP, 2017).

Hg derived from both natural and anthropogenic sources enters the global Hg cycle and is ultimately wet or dry deposited into either aquatic or terrestrial ecosystems (Paper I and Fig. 1). Notably, Hg is very persistent in soils, lakes and oceans (Padmavathamma and Li, 2007; Tangahu et al., 2011) and its mobility depends on the chemical speciation, which is a function of several soil parameters and their interactions (Yin et al., 1997).

Hg in soil occurs in various forms: (i) dissolved (free ion or soluble complex), (ii) non-specifically adsorbed (binding mainly as a result of electrostatic forces), (iii) specifically adsorbed (strong binding owing to
covalent or coordinative forces), (iv) chelated (bound to organic substances) or (v) precipitated (as sulphide, carbonate, hydroxide, phosphate, etc. (Schuster, 1991). Hg transformations, e.g., methylation and demethylation, reduction and oxidation, might meanwhile modify Hg speciation (Hu, Lin, Zheng, Rao, et al., 2013; Hu, Lin, Zheng, Tomanicek, et al., 2013). Furthermore, geochemical parameters, such as temperature, pH, redox potential, sulphur content and dissolved organic matter of the soil, etc., strongly affect Hg mobility by changing its solubility and the biological process affecting Hg transformations (Ullrich et al., 2001; Lambertsson and Nilssons, 2006; Drott et al., 2007; Graham et al., 2012, 2013; Chiasson-Gould et al., 2014; Robles et al., 2014).

Figure 1. Hg cycle in the ecosystem including MeHg generation in aquatic environment, modified based on Roos et al. (2012)

Although soil has a natural capacity to attenuate heavy metals through various mechanisms, concentrations exceeding the attenuation capacity will inevitably lead to soil contamination (Hseu et al., 2010). Hg contamination in soil derives mainly from Hg mining and metallurgy, gold mines and Zn/Pb smelters, chemicals production facilities involved in the production of chlor–alkali, chloroethylene and acetaldehyde, landfills, military installations and wood/forestry impregnation sites (Wang et al., 2012). Accordingly, remediation techniques are needed to either remove Hg from
affected soils or to transform Hg into its most stable and least toxic forms in situ (Cui et al., 2011; Tangahu et al., 2011).

In brief, extraction procedures aim to separate Hg from the soil matrix or reduce the volume of contaminated soil, while immobilisation is based on encapsulation and stabilisation of Hg within the soil (Dermont, Bergeron, Mercier, and Richer-Laflèche, 2008; Wang et al., 2012). Various techniques, e.g., thermal treatment, vitrification, soil washing, biological techniques (e.g., phytoremediation), stabilisation/solidification, nanotechnology and electro-remediation etc., have previously been applied to counteract Hg contamination in soil (Randall and Chattopadhyay, 2004; Richter and Flachberger, 2010; Rodríguez et al., 2012). The advantages, disadvantages and experiences of using these technologies need to be considered prior to application under prevailing environmental conditions of the contaminated site at hand. Special attention should be given to the factors that affect Hg mobility in soil and in this way affect the efficiency of the selected treatments (Paper I). Meanwhile, properties of contaminated sites might differ substantially from those of natural soils. Therefore, soil characterisation is necessary to estimate the practical and economic feasibility of various treatments for individual remediation case (Paper II).

With the implementation of the Minamata Convention to reduce the products and processes involving metallic and inorganic Hg, the majority of the contemporary human exposure to Hg comes from organic Hg, particularly MeHg, through the consumption of marine food and rice (Council National Research, 2000; UNEP, 2013). Soil environments are particularly important for the conversion of inorganic Hg (Hg(II)) to MeHg that subsequently bioaccumulates and biomagnifies in food webs (Fitzgerald and Clarkson, 1991; Gabriel and Williamson, 2004). This is because Hg(II) methylation is mainly a biotic process taking place under oxygen limiting conditions in water saturated soils, wetlands or sediments (Watras et al., 1995; Ullrich et al., 2001).

Transformation of Hg(II) to MeHg is mediated by microorganisms. A number of factors are known to affect Hg(II) methylation, by e.g., controlling bacterial activity (Bravo et al., 2017) and/or the geochemical speciation of Hg(II) (Jonsson et al., 2014; Bravo, Sylvain Bouchet, et al., 2017). For example:

- Increases in temperature might lead to increases in biological activity and subsequently in Hg(II) methylation rates (Ullrich et al., 2001).
- Redox potential seems to be a key factor and it is clear that suboxic and mildly reducing conditions (e.g. conducive for sulphate reduction) are prone to feature high Hg(II) methylation rates, whereas anoxic and strongly reducing conditions where sulphide may build up could prevent Hg(II) from being available for methylation (Bigham et al., 2017).
• Sulphur plays a major role in influencing Hg\(^{\text{II}}\) methylation by affecting the activity of methylating bacteria (e.g. sulphate reducing bacteria) and by modulating the availability of Hg\(^{\text{II}}\) for the methylation process (Drott et al., 2007).
• High contents of dissolved organic matter (DOM) can promote Hg\(^{\text{II}}\) methylation by enhancing the overall bacterial activity including the Hg\(^{\text{II}}\) methylating heterotrophic bacteria (Ullrich et al., 2001; Lambertsson and Nilssons, 2006). Low molecular mass DOM can also facilitate Hg\(^{\text{II}}\) methylation by inhibiting HgS\((\text{s})\) precipitation or enhancing HgS\((\text{s})\) dissolution, thereby providing available Hg\(^{\text{II}}\) for methylating microorganisms (Graham et al., 2013). High molecular mass DOM might however decrease Hg\(^{\text{II}}\) methylation by forming large compounds that hamper Hg\(^{\text{II}}\) availability (Chiasson-Gould et al., 2014).

Recently it has been concluded that the availability of soil Hg\(^{\text{II}}\) to methylating microorganisms depends heavily on the S\(^{\text{II}}\) concentrations in porewater and the molar ratio of dissolved and solid/adsorbed thiols in DOM (Liem-Nguyen et al., 2015).

Sulphate-reducing bacteria (SRB) are among the first identified bacteria implicated in Hg\(^{\text{II}}\) methylation (Compeau and Bartha, 1985; Gilmour and Henry, 1991; Gilmour et al., 1992). SRB is a functional guild with lineages that differ in their metabolic traits, including lineages capable of syntrophic fermentation of simple organic acids in the absence of sulphate as the terminal electron acceptor (McInerney et al., 2008; Plugge et al., 2011). Syntrophic SRB have been revealed as central Hg\(^{\text{II}}\) methylators in soils and sediments low in sulphate (Pak and Bartha, 1998; Han et al., 2010; Bae et al., 2014).

Iron-reducing bacteria within Deltaproteobacteria have also been seen to have the capacity for Hg\(^{\text{II}}\) methylation (Fleming et al., 2006; Kerin et al., 2006; Yu et al., 2012). Certain Geobacter species affiliated to family Geobacteraceae were found to be particularly efficient at MeHg formation under controlled laboratory conditions (Kerin et al., 2006). Essentially, every single organism tested within family Geobacteraceae seems to be capable of high MeHg production rates (Fleming et al., 2006; Kerin et al., 2006; Schaefer and Morel, 2009).

Methanogens were suspected to be Hg\(^{\text{II}}\) methylators very early on (Wood, 1975), but it was not until recently that they were verified as significant Hg\(^{\text{II}}\) methylators in various environments (Gilmour et al., 2013; Yu et al., 2013; Schaefer et al., 2014). They may even be the primary methylators in some special environments such as in lake periphyton (Hamelin et al., 2011).

Recently, the identification of two functional genes, hgcA and hgcB, that are both required for Hg\(^{\text{II}}\) methylation (Parks et al., 2013), has provided
means to directly characterise Hg(II) methylating communities in various environments such as marshes, sediments, wetlands, paddy soils and water conservation areas (Bae et al., 2014; Schaefer et al., 2014; Y. R. Liu, Wang, et al., 2014; Y. R. Liu, Yu, et al., 2014). The discovery of these genes has enabled researchers to identify Hg(II) methylating capacity of both historically known methylators, including SRB and iron-reducing bacteria, certain methanogens (Gilmour et al., 2013; Parks et al., 2013; Podar et al., 2015), and new branches of Hg(II) methylators, including Firmicutes and Chloroflexi (Parks et al., 2013; Yu et al., 2013; Bae et al., 2014; Schaefer et al., 2014). The presence of the hgcAB genes in a wide range of microorganisms has significantly broadened our view on environments that may be conducive for Hg(II) methylation beyond those systems where sulphate or iron reduction are the dominant processes (Paranjape and Hall, 2017). Potential new environments for Hg(II) methylation include thawing permafrost soils, coastal dead zones, Antarctic sea ice and other extreme environmental conditions (Podar et al., 2015; Gionfriddo et al., 2016).

Boreal regions are dominated by forests and wetlands with soils enriched in OM. Soil OM has been identified as a main vector for Hg and MeHg transport from catchments to adjacent surface waters in boreal regions (Grigal, 2002; Bravo, Sylvain Bouchet, et al., 2017). Indeed, the mobilisation of Hg and the more harmful MeHg from soils by means of OM-mediated transport has been linked to MeHg accumulation in fish (Hongve et al., 2012). High Hg levels in fish in many boreal regions have caused substantial concern over the past decades (Åkerblom et al., 2012; Gandhi et al., 2014). Since soils and lake sediments are important sites for MeHg formation, it is crucial to understand the processes and the organisms involved in MeHg formation in boreal soils and lake sediments. However, very little research has been conducted to elucidate the composition and spatial variation of Hg(II) methylating communities in boreal soils (Schaefer et al., 2014; Eklöf et al., 2018), and in freshwater lakes (Isidorova et al., 2016; Bravo, S. Bouchet, et al., 2017). This could have resulted from difficulties and high costs in using traditional sequencing methods with datasets that often ended up with insufficient coverage to robustly describe and compare microbial communities (Liu et al., 1997; Fisher and Triplett, 1999; Curtis et al., 2006).

High-throughput next generation sequencing in combination with the application of barcode indexing has now emerged as an economical and powerful alternative that enables deeper insights into the microbial community composition by simultaneous analyses of large amount of samples at a fraction of the previous cost (Andersson et al., 2008; Hamady et al., 2008; Sinclair et al., 2015). Therefore, studies using such new sequencing approaches for hgcA genes could be very useful for understanding and predicting MeHg production in the landscape.
2. The Scope of the Thesis

The overall goals of this thesis are to study the Hg contamination in soil and the microbial communities that convert Hg$^{(II)}$ to MeHg in the landscape. The research focuses on (1) evaluating the feasibility of soil washing technique to remediate a soil contaminated by Hg and other trace elements and (2) describing the total and Hg$^{(II)}$ methylating microbial communities in various landscape components and (3) identifying environmental factors important for shaping these communities and the production of MeHg.

The specific aims are:

1. To provide an overview of promising remediation techniques and their applications to control soil Hg contamination. The focus is on soil washing, as it is one of the most used techniques for soil contamination. The development stage, applicability and limitations of this technique are compared to three other well-established soil remedial techniques (Paper I).

2. To study the potential of soil washing to treat an Hg contaminated soil. We hypothesised that the efficiency of this treatment for Hg contaminated soil could be largely dependent on the behaviour of Hg contaminants in the soil matrix. To test this, the feasibility of soil washing to remediate an Hg contaminated soil was experimentally evaluated by determining the efficiency of physical separation based on particle size separation and chemical extraction by acidic/alkaline leachates derived from various types of waste. Additionally the influence of dissolved organic matter, pH and chlorides on Hg mobilisation was studied to reveal the factors affecting the treatment effectiveness (Paper II).

3. To map the composition of bacterial communities and, more specifically, the microbial communities capable of mediating MeHg formation in soils from boreal forests and wetlands, and in lake sediments, all being major landscape components (Papers III, IV and V). The rationale is the increasing concern of elevated MeHg levels observed in fish over the past decades, and the studies suggesting MeHg formation in soils and sediments might be one of the major causes. We hypothesised that not only the Hg$^{(II)}$ methylators, but also the
composition of the broader bacterial community influences Hg\(^{\text{II}}\) methylation and thereby the formation of MeHg in soils and sediments.

4. To describe in what ways geochemical factors contribute to shaping the assembly of the above-mentioned communities (Papers III, IV and V). We postulated an association between soil/sediment geochemistry and the composition of Hg\(^{\text{II}}\) methylating microorganisms. This is also crucial information that is needed for understanding and predicting the processes and the organisms involved in MeHg production in soils and sediments. By linking microbial community composition and environmental variables to MeHg formation in representative and abundant landscapes such as forest soils, boreal wetlands and lake sediments, we will be in a better position to manage our natural resources in a way that minimises the Hg problem.
3. Material and Methods

3.1 Remedial technique study: soil washing

3.1.1 Contaminated soils

Soil samples (Paper II, Table 1) were collected from a padding area about 10 km upstream of Göta River, Sweden. The Hg contamination in this soil has resulted from inappropriate waste disposal, industrial chlor alkali processing and harbour activities. The soil had also been polluted by other metals, such as zinc, copper and lead.

The waste products used to generate acidic leachates consisted of food-waste from the campus restaurant (Luleå University) and sludge from a paper mill in Piteå. The ashes used to produce alkaline leachates originated from (a) fly ash from municipal solid waste incineration (Högdalen), (b) fly ash from coal burning (Värtan), (c) fly ash from biofuel burning (Brista), and (d) bottom ash from phosphorus-containing slags (Uddevalla). None of the above materials contains detectable Hg.

Table 1. Properties of Hg-contaminated soil from the padding area upstream of Göta River (± SD, n=3)

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>6.5 ± 0.0</td>
</tr>
<tr>
<td>Electrical conductivity (EC)</td>
<td>mS/cm</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Redox potential</td>
<td>mV</td>
<td>109.4 ± 2.1</td>
</tr>
<tr>
<td>Total organic carbon (TOC)</td>
<td>%</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>(n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved organic carbon (DOC)</td>
<td>mg/kg</td>
<td>209.2 ± 14.2</td>
</tr>
<tr>
<td>Hg concentration (n=6)</td>
<td>mg/kg</td>
<td>34.5 ± 14.1</td>
</tr>
</tbody>
</table>

3.1.2 Remediation tests

The soil was sieved into eleven particle-size fractions from < 0.063 mm to > 25 mm to test dry and wet sieving as a treatment strategy. In parallel, pH-dependent dissolution of Hg was performed in the range of pH 3 to 11, obtained by adding acid (0.1 M HNO₃) or base (1 M or 0.1 M NaOH) directly to the soil. HCl was additionally used to compare the effect of chlorides to nitrate for Hg mobilisation. The influence of chlorides on Hg mobilisation was further studied using batch-leaching tests lasting 24 h and 48 h (Paper II).
Mobilisation of Hg by various extractants, i.e. acidic leachates from biodegradable wastes, alkaline leachates from ashes, 0.1 M and 0.4 M NaOH solutions and 0.01 M EDTA (ethylenediaminetetraacetic acid), was studied by batch leaching tests. The extraction was performed at L/S 2 with acidic leachates and EDTA solution, and at L/S 10 with alkaline leachates and NaOH solutions in closed glass bottles and rotated for 24 h ± 1 h (Xu, 2013).

Total organic carbon was assessed for bulk soil and particle size fractions. Additional elements of the soil and leachates were measured by ICP-AES and ICP-SFMS. Elements in the waste used for producing acidic leachate were tested by X-ray fluorescence. Dissolved organic carbon was measured to find out the correlation between dissolved organic matter and soluble Hg. Analyses of thermal Hg desorption were carried out to identify the changes in Hg species using HCl for titrations (Papers II). Pearson’s correlation coefficient was assessed to reveal linear relationships between variables.

3.2 Microbial community composition surveys

3.2.1 Soil and porewater samples

In Paper III, 200 soil samples were collected from eight catchments across three geographical regions in Sweden (Fig. 2). Among these, 34 samples with high soil MeHg concentrations and %MeHg > 1% were defined as “MeHg hotspots”. In Paper IV, 81 soil and porewater samples were collected from seventeen sampling sites across four Swedish boreal wetlands (Fig. 2). More details on the studied regions and sampling sites are provided in Löfgren et al., (2009), Eklöf et al., (2013), Tjerngren et al., (2012) and Liem-Nguyen et al., (2017). The sediment study (Paper V) was conducted in the Vidy Bay of Lake Geneva, one of the largest freshwater reservoirs in Europe. Samples were collected at a site close to the outlet pipe of the waste water treatment plant (CP, 46°30'52.89” N, 6°35'21.25” E), and detailed information is referred in Bravo et al. (2015).
3.2.2 Geochemical analyses

3.2.2.1 Boreal forests
Total Hg in soils was measured using a Perkin Elmer SMS100 total Hg analyser while analyses of MeHg were done by using GC-ICPMS on fresh samples immediately after thawing (Lambertsson and Lundberg, 2001). Soil C, N and S were analysed by high temperature catalytic oxidation with a COTECH ECS 4010 elemental analyser (Paper III).

3.2.2.2 Boreal wetlands

Soil
Total Hg was determined by solid combustion atomic absorption spectrometry using an AMA-254 Advanced Mercury Analyzer (LECO),
while MeHg was acid extracted and determined by isotope dilution analysis (IDA) using GC-ICPMS (Lambertsson and Lundberg, 2001) (Paper IV).

**Porewater**

THg was measured by IDA using Hg cold-vapour generation coupled to ICPMS. MeHg was analysed by IDA using purge and trap thermal desorption coupled to gas chromatography ICPMS (Lambertsson and Björn, 2004). Dissolved organic carbon was measured using a TOC-VCPH (Shimadzu), and FeII and FeIII were determined by UV absorption spectrophotometry (Viollier et al., 2000). The concentrations of SO$_4^{2-}$, Cl$^{-}$, PO$_4^{3-}$, NH$_4^{+}$ and NO$_3^{-}$ in porewaters were measured by ion chromatography. Total thiols in freeze-dried porewater were determined using Sulphur K-edge X-ray absorption near edge structure spectroscopy (S K-edge XANES) and Hg LIII-edge EXAFS (X-ray extended absorption fine structure) spectroscopy as described by Liem-Nguyen et al. (2017). Concentrations of low molecular mass thiols were determined in porewater using solid phase extraction online pre-concentration hyphenated to liquid chromatography tandem mass spectrometry (SPE/LC-MS/MS) (Liem-Nguyen et al., 2015) (Paper IV).

### 3.2.2.3 Lake Geneva

**Sediments**

Total C, organic C, inorganic C and total N were measured with an elemental analyzer Perkin Elmer 2400. Organic matter content was determined by loss on ignition (Walter E. Dean, Jr., 1974). Total Fe and S were measured by ICP-AOS. Total Hg was quantified using an Advanced Mercury Analyser (254 Altec). MeHg was measured by species-specific isotope dilution and GC-ICPMS (Bravo et al., 2015). Poorly crystalline FeIII-oxides were quantified with the ferrozine method (Thamdrup et al., 1994). Elemental S was analysed by RP-HPLC and UV detection (Zopfi et al., 2008) (Paper V).

**Porewater**

Total Hg was measured by CV-AFS (Bloom and Fitzgerald, 1988) while MeHg was measured by species-specific isotope dilution and GC-ICPMS (Rodríguez Martin-Doimeadios et al., 2003). Concentrations of SO$_4^{2-}$ and NO$_3^{-}$ were analysed by ion-chromatography (Dionex ICS-3000) (Paper V).
3.2.3 Microbiological analyses

16S rRNA gene

Microbial DNA was extracted from soil samples and amplification of bacterial 16S rRNA gene was performed to study the composition of microbial communities (Olsen et al., 1986). For soils from boreal forests and sediments from Lake Geneva, 16S rRNA gene were amplified in two-step PCR assays according to the protocol described in Sinclair et al. (2015). Non-barcoded bacterial domain primers (Bakt_341F and Bakt_805R) were used for the 1st PCR amplification. The resulting PCR products were diluted 100 times before use as the template in the 2nd stage PCR with similar primers carrying 7 bp sample-specific DNA barcodes. The details of PCR reactions were described in Papers III and V.

Sequencing of purified amplicons was carried out following the protocol described in Sinclair et al. (2015), using an Illumina MiSeq instrument at the SNP/SEQ SciLifeLab facility hosted by Uppsala University. Chimera identification and OTU (Operational Taxonomic Unit) clustering of sequences at 3% sequence dissimilarity were done using UPARSE. A SILVA database was used with CREST for taxonomic annotation of OTUs (Papers III and V).

HgcA gene

Amplicon analyses of the protein coding gene hgcA were carried out to reveal Hg\(^{\text{II}}\) methylating communities in hotspot samples from the forest soils and all the samples from the wetlands and sediments (Papers III, IV and V). In my work, previously published hgcA primers (hgcA_261F and hgcA_912R) (Schaefer et al., 2014) were adapted for high-throughput Illumina sequencing. HPLC-purified primers linked to adaptors were used for a 1st stage PCR. In the 2nd PCR, standard Illumina handles and barcode primers were utilised to include all the 1st PCR products into one pool run on an Illumina sequencing. The details of PCR reactions and thermal programs for the two PCR steps were described in Papers III, IV and V.

After each PCR step, hgcA amplicons were purified to remove remaining primers and other assay components. Purified amplicons were sequenced using the same method as for 16S rRNA. For the sequence analyses, only forward read sequences were used because of the excessive length of the PCR products. Low quality sequences were filtered out using SICKLE and adaptors were trimmed by using CUTADAPT. Subsequent processing of reads were performed by USEARCH version 8.0 and reads were clustered at 60% identity cutoff using cd-hit-est. HMMER search was used for taxonomical annotation with manually curated database of Proteobacteria and sequences of Podar et al. (2015) (Papers III, IV and V).
3.2.4 Statistical analyses

Microbial community compositions were compared at family- and OTU-levels using non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarities via software PRIMER E (Clarke and Gorley, 2015). Relative dissimilarities (or distances) among the samples were computed according to the resemblance matrix. In brief, the closer data points are to each other, the more similar are their community compositions. Information on the common set of samples from community composition based on Bray-Curtis similarities and that from geochemical variables based on Euclidean distance was presented in one single ordination. A combined nMDS plot with bubble and vector plots of geochemical factors projected on the same ordination of community composition was constructed to reveal the relationship between community compositions and potentially explanatory geochemical variables (Clarke and Gorley, 2015). Pearson's correlation coefficients (R) between variables were tested using a significance level of p < 0.05.
4. Results and Discussion

4.1 Remediation of Hg contaminated soil

4.1.1 Remedial technologies for Hg contamination in soil

Historically, thermal treatment, vitrification, soil washing, biological techniques (e.g., phytoremediation), stabilisation/solidification and several other methods have been used to deal with Hg contamination in soil (Randall and Chattopadhyay, 2004; Dermont et al., 2008a; Rodriguez et al., 2012). An overview of the development stage, applicability, limitations, and secondary waste of four major technologies that are most widely used and implemented in the field is given in Table 2.
Table 2. Overview of selected Hg treatment technologies (Paper I).

<table>
<thead>
<tr>
<th>Technology</th>
<th>Soil Washing</th>
<th>Stabilisation/Solidification</th>
<th>Thermal treatment</th>
<th>Biological Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Physical separation concentrates Hg into a smaller volume; chemical extraction solubilizes Hg in soil with chemical reagents.</td>
<td>Stabilisation is to put Hg into stable and highly insoluble forms over wide ranges of pH and oxidizing conditions; solidification is to trap the stabilized Hg in a rigid and durable matrix.</td>
<td>Using heat and reduced pressure to volatilize Hg, followed by condensing Hg vapours into liquid elemental Hg.</td>
<td>Using Hg resistant microorganisms or plants to remove Hg from soil; biosolids, microbes or plants to reduce Hg toxicity or bioavailability to the environment</td>
</tr>
<tr>
<td><strong>Development status</strong></td>
<td>Full scale; not extensively used in the United States; commonly used in Europe.</td>
<td>Full scale; widely used in the United States; not extensively used in Europe.</td>
<td>Full scale; specific applications.</td>
<td>Pilot scale; in development phase; used more in the United States than in Europe.</td>
</tr>
<tr>
<td><strong>Applicability</strong></td>
<td>On/off site</td>
<td>On/off site</td>
<td>On/off site</td>
<td>On site</td>
</tr>
<tr>
<td><strong>Limitation</strong></td>
<td>Physical separation is difficult with soils containing high levels of clay/insoluble humic substances, soils of high viscosity; for chemical extraction, use of chemical agents is both expensive and hazardous.</td>
<td>Increased volume of the treated material; compounds such as organic matter can interfere; long-term monitoring is needed.</td>
<td>Requires gas emission control and specialized facilities, pre-treatment to assist melting is needed; the capital cost is very high.</td>
<td>Requires more pilot studies to evaluate the efficiency. Remaining liability issues, including maintenance for an indefinite period of time.</td>
</tr>
<tr>
<td><strong>Secondary waste</strong></td>
<td>Sludge/water containing chemicals and contaminants.</td>
<td>None</td>
<td>Off-gas, waste water if pre-treatment is included.</td>
<td>Contaminated plants; Hg vapour emission from microbial volatilisation</td>
</tr>
</tbody>
</table>
Thermal treatment can be applied to soils with very high concentrations of Hg and its remedial efficiency can reach up to 99%. Extended high vacuum-rotary conditions and renewable energy sources have been proposed to counteract the disadvantages of altered soil properties resulting from high temperature and the very high capital and operational costs. Stabilisation/solidification (S/S) can remediate sites with a wide range of mixed contaminants and soil types. In-situ S/S technology has been proposed as a promising alternative to ex situ S/S because of its cost advantage and the option of site re-vegetation, but significant monitoring is required for the long-term stability and integrity of the stabilised and solidified product (Dermont et al., 2008a; Paper I). Phytoremediation is environmentally friendly; nevertheless, there are few suitable plant species for Hg immobilisation or uptake. Therefore, a number of current studies aim at enhancing the tolerance of plants to Hg using genetic modification. However, their techno-economic perspective and environmental safety need to be taken into account (Tangahu et al., 2011; Paper I).

Soil washing is primarily a physical separation process and the principle is based on the concept that most contaminants tend to bind to fine (clay and silt) rather than coarse particles (sand and gravel) in soil (FRTR, 1995; USEPA, 1997). Strategies of gravity concentration, attrition scrubbing and froth flotation, etc., have been developed to enhance the treatment efficiency (Paper I). In general, the volume of soil to be further treated or disposed of could be considerably reduced. In addition, the treatment systems are easily modular, and some full-scale mobile units are available for on-site remediation. However, although soil washing is commercially available, low liberation degree of Hg or high contents of clay/silt and organic matter remains challenging. Hence feasibility studies are needed for individual soils prior to field implementation.

4.1.2 Soil washing based on particle size separation

Usually, soil washing based on particle size separation is appropriate for soils with sand/gravel content in excess of 50–70% (ITRC, 1997; Dermont et al., 2008b). The contaminated soil studied here consisted of more than 98% of the coarse-grained fraction (Jury and Horton, 2004) (Table 3). Although total Hg concentrations decreased with increasing particle sizes, the least contaminated fraction (Table 3) exceeded the Swedish generic guideline value for Hg in soils with less sensitive use (2.5 mg/kg) (Swedish EPA, 2009), suggesting that particle separation via dry sieving was apparently insufficient to treat the soil contamination (Paper II).
Table 3. Distribution of particle size fractions; total Hg (HgT) in solid soil samples after dry sieving and soluble Hg (HgS) in leachates of different particle size fractions after wet sieving (± SD, n=3).

<table>
<thead>
<tr>
<th>Texture</th>
<th>Particle size, mm</th>
<th>Percentage passing</th>
<th>HgT, mg/kg</th>
<th>HgS, µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay/Silt</td>
<td>&lt; 0.063</td>
<td>1.32</td>
<td>48.7 ± 1.7</td>
<td>16.1 ± 2.5</td>
</tr>
<tr>
<td>Sand</td>
<td>0.063–0.125</td>
<td>2.05</td>
<td>41.8 ± 2.0</td>
<td>27.5 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>0.125–0.25</td>
<td>3.08</td>
<td>30.5 ± 1.1</td>
<td>25.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>0.25–0.5</td>
<td>6.04</td>
<td>25.5 ± 6.3</td>
<td>36.7 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>0.5–1</td>
<td>8.42</td>
<td>20.0 ± 3.2</td>
<td>35.1 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>1–2</td>
<td>11.11</td>
<td>23.9 ± 5.1</td>
<td>15.9 ± 3.2</td>
</tr>
<tr>
<td>Gravel/Stone</td>
<td>2–4</td>
<td>9.55</td>
<td>10.3 ± 0.8</td>
<td>4.1 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>4–6.3</td>
<td>14.65</td>
<td>7.9 ± 1.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6.3–12.5</td>
<td>15.51</td>
<td>6.2 ± 2.2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12.5–25</td>
<td>14.06</td>
<td>2.8 ± 1.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>&gt; 25</td>
<td>14.21</td>
<td>3.1 ± 3.0</td>
<td>-</td>
</tr>
</tbody>
</table>

This was partly due to the attachment of finer particles to coarser ones in the dry sieving, evidenced by the release of finer particles up to 0.5 mm when the coarser particles were subjected to wet sieving (Paper II). Hg concentrations in particle size fractions after wet sieving were not measured, but judging from the Hg concentrations in the leachates after wet sieving (Table 3), Hg removal by water was expected to be very low.

Successful physical separation (PS) applications have been repeatedly reported. Site demonstration of PS coupled with thermal desorption system for remediation of soils from Marktredwitz, Germany, achieved a Hg removal efficiency of 98% (BMBF, 1996). A pilot test on decontamination of Hg-polluted site in Czech Republic successfully decreased Hg in soils from originally 100–100,000 µg/g down to below 10 µg/g (NATO/CCMS, 2002). Moreover, an upgraded soil washing plant allowed a combined (thermal) treatment to reduce contaminated soils of Hg level from above 1000 mg/kg to below 10 mg/kg (Richter and Flachberger, 2010). However, PS is difficult or unfeasible when Hg is strongly bound to soil particles owing to high levels of insoluble humic substances or clay minerals of the contaminated soil (Paper II).

4.1.3 Soil washing assisted by chemical extraction

Soil washing in which chemicals are applied to facilitate contaminant removal is called chemical extraction and such method is often used in combination with PS when the contaminants are firmly bound to the soil matrix (Papers I and II). Hg solubility in distilled water was low in all particle size fractions, corresponding to 0.03–0.18% of total Hg in solid soil samples (Table 3), indicating a strong bonding of Hg to the soil. In order to evaluate the feasibility of applying chemical extraction to remediate this contaminated soil, Hg mobilisation was tested under the influences of
organic matter, pH, chlorides (Paper II) and leachates derived from various wastes (Xu, 2013).

4.1.3.1 Dissolved organic matter
Strong positive correlations between soluble Hg and dissolved organic carbon have been detected where Hg was released and co-transported with the dissolved natural organic matter (OM) when Hg was derived from wetlands and natural soils (Wallschläger et al., 1996; Åkerblom et al., 2008; Miller et al., 2011). However, increased dissolved OM in the water leachate did not enhance Hg dissolution as no correlation was observed between dissolved organic carbon and soluble Hg in the particle size fractions (Paper II). This could be because Hg contamination in the studied soils was from anthropogenic activities rather than natural sources. Meanwhile, the competition between Hg and other elements (Cd and Zn) which were significantly correlated with dissolved organic carbon (Paper II), for binding sites of dissolved OM could also have contributed to this (Lin and Chen, 1998; Turer and Maynard, 2003; Amir et al., 2005).

4.1.3.2 pH
It has been reported that pH could influence Hg mobility in soil (Barrow and Cox, 1992; Yin et al., 1996), and this was also observed in my study. All the size fractions reacted similarly to the pH changes in terms of Hg desorption, with the least Hg dissolution at pH 3 and the most Hg dissolution at pH 5 and pH 11 (Fig. 3). The behaviour of Hg dissolution under acidic to neutral pH was further confirmed by the results of HCl extraction, with the least Hg being mobilised around pH 3 and the most around pH 5 (Paper II).

![Figure 3](image)

*Figure 3.* The pH-dependent dissolution of Hg in soil particle size fractions using HNO₃ as the titration acid. Note the logarithmic concentration scale. Error bars represent standard deviation of means, n=3.

Low dissolution of Hg at pH 3 could be due to the co-precipitation of Hg with humic acid which is one of the major constituents of soil OM and is insoluble under strongly acidic conditions (Barrow and Cox, 1992;
Wallschläger et al., 1996; Yin et al., 1996). Besides this, adsorption of Hg to soil minerals e.g., goethite and hydrous MnO$_2$, could also have contributed in this pH range (Lockwood and Chen, 1973; Barrow and Cox, 1992). The apparent increase of Hg dissolution at pH 5 and pH 7 compared to at pH 3 could be due to the dissolution of humic acid. When pH increased from 7 to 9, Hg dissolution decreased, likely due to the increase of negative charges on the soil at elevated pH, which might attract and retain Hg$^{2+}$ ions (Semu et al., 1987). In addition, HgOH$^-$ resulted from Hg hydrolysis as pH elevated could have also contributed to increase Hg adsorption by soil constituents (Farrah and Pickering, 1978). An obvious increase of Hg dissolution was found from pH 9 to pH 11, probably due to decreasing concentrations of Hg$^{2+}$ and HgOH$^+$ with increasing pH, as hydrolysis of these charged species proceeded to the uncharged Hg(OH)$_2$ (Farrah and Pickering, 1978).

4.1.3.3 Chloride
Chloride is regarded as one of the most mobile complexing agents for Hg (Kabata-Pendias, 2011) and is able to compete with OH$^-$ and even organic ligands for its association with Hg (Reimers and Krenkel, 1974; Gabriel and Williamson, 2004). However, a pH dependent solubility of Hg regardless of the concentrations of chloride was detected in this study (Paper II). Moreover, no obvious increase of Hg dissolution was observed at pH 3 and pH 5 when HCl was used as the titrant as compared to HNO$_3$ (Paper II). Similar findings were obtained by Yin et al. (1996), who found that the addition of Cl$^-$ at pH 3 had almost no effect on the desorption of Hg$^{(II)}$ in soils high in OM. A conceivable hypothesis is that the insoluble soil OM forms ternary complexes with Hg–Cl (Yin et al., 1996). Chloride content decreased in the leachate after HCl titration to pH 3 (data not shown), implying that chlorides were sorbed to the soil. Additionally, thermal desorption curves showed that Hg$^{(II)}$ was bound to OM as well as to chlorides (Paper II), indicating possible formation of ternary complexes of OM and Hg–Cl.

4.1.3.4 Various extractants
According to the results of pH-dependent dissolution, both weakly acidic and strongly alkaline pH could facilitate Hg mobilisation (Fig. 3). Therefore, acidic leachates generated from degradable wastes and alkaline leachates produced from the ashes were considered in the interest of pH adjustment. Distilled water, ethylenediaminetetraacetic acid (EDTA) and NaOH solutions were used as the references.

In acidic to neutral leachates, Hg dissolution seemed to follow the same pattern as in Figure 3, with the most Hg mobilised at pH 5 in the leachate from FW alone (Fig. 4). The peak of Hg dissolution was detected in 0.1 and 0.4 M NaOH leachates around pH 13, while the foot of that was detected in
ash leachates at pH around 12 (Fig. 4). Further work is needed to reveal the reasons for the sudden change in Hg dissolution within this narrow pH range.

All the extractants showed very low Hg desorption, with the largest fraction of mobilised Hg accounting for less than 1.5% of the total Hg. EDTA, commonly used to scavenge heavy metals through chelation, did not even exceed distilled water in Hg mobilisation (Fig. 4).

\[ \text{Figure 4. Hg dissolution in acidic and alkaline extractants, 0.01 M EDTA, NaOH solutions and distilled water (FW: food waste; PMS: paper mill sludge; DW: distilled water; MW-FA: municipal solid waste fly ash; CB-FA: coal burning fly ash; BF-FA: biofuel fly ash; PC-BA: phosphorus-containing bottom ash). Note the logarithmic concentration scale for mobilised Hg. Error bars represent standard deviation of means, n=3.} \]

Soil washing (SW) techniques incorporating both physical separation (PS) and chemical extraction (CE) have been successfully demonstrated over the past decades. A full-scale application of sediment washing technology applying both PS and CE for remediation of dredged materials (90% silt/clay) from the New York/New Jersey Harbour yielded a Hg removal of 92% (Enterprises BioGenesis, 1999). Later on, the same company performed a pilot-scale demonstration of decontamination of sediment from the lagoon of Venice Italy using similar strategy and obtained Hg removal rates of 75-93% (Enterprises BioGenesis, 2005).

However, my work shows that the SW strategy was hardly adequate for a satisfactory treatment of the Hg contaminated soil under scrutiny here. The studied soil exhibited very strong affinity for Hg. Commonly used and effective mobilising factors for Hg, e.g., dissolved organic matter or chloride, did not improve the Hg mobilisation; neither did the chelator
EDTA that has been widely used to scavenge metal contaminations (Paper I). Therefore, even with the assistance of CE, less than 1.5% of the total Hg could be mobilised (Fig. 3 and 4). Sierra et al. (2011) also showed difficulties in applying SW technique to separate Hg from soils affected by mining and metallurgical waste in Asturias, NW Spain. The presences of contaminants embedded in the soil and spoil heap aggregates were speculated to have caused such difficulties. Application of high resolution spectroscopic methods (e.g., X-ray spectroscopy) might facilitate the understanding of Hg bonding to soil organic matter/minerals thereby help evaluate the feasibility of SW (Kim et al., 2004).

4.2 Biological Hg\(\text{II}\) methylation in boreal soils and lake sediments

4.2.1 Representatives of Hg\(\text{II}\) methylating community in boreal soils and lake sediments

Boreal wetlands and forest soils are considered as sinks but can sometimes be significant sources of MeHg, contributing this contaminant to adjacent ecosystems (St. Louis et al., 1994; Grigal, 2003; Tjerngren, Meili, et al., 2012; Braaten and de Wit, 2016). In aquatic ecosystems, Hg\(\text{II}\) methylation can also be carried out by microorganisms at oxic-anoxic boundaries of sediments. In the boreal landscape, soils are generally enriched in organic matter (OM) with soil OM identified as a main vector of Hg and MeHg transport from catchments to surface waters in boreal areas (Grigal, 2002; Bravo, Sylvain Bouchet, et al., 2017). High MeHg levels in fish have caused considerable concern in many boreal regions over the past decades (Åkerblom et al., 2012; Gandhi et al., 2014). Since boreal soils and lake sediments are important sites for MeHg formation and subsequent export to the aquatic system, it is crucial to understand the role of the organisms for MeHg formation in these environments.

Diverse microbial communities have been identified as Hg\(\text{II}\) methylators within these ecosystems. Schaefer et al. (2014) investigated Hg\(\text{II}\) methylating communities from a temperate swamp in southern Sweden and a tropical swamp in southern Florida, USA and found that potential methylators affiliated with Deltaproteobacteria, Chloroflexi and Methanomicrobia were common in both wetlands. Experimental assays of Kronberg et al. (2016) implicated methanogens and sulphate reducing bacteria as key Hg\(\text{II}\) methylators in boreal forest soils. In the present work, taxonomic classification of \textit{hgcA} sequences from the forest hotspots, four wetlands and lake sediments revealed methylating communities consisting mainly of representatives from \textit{Proteobacteria}, \textit{Firmicutes} and
Euryarchaeota, all of which are previously identified Hg(II) methylating groups (Gilmour et al., 2013, Papers III, IV and V).

4.2.1.1 Iron reducing bacteria

Deltaproteobacteria has been considered the predominant Hg(II) methylating class in anaerobic soils (Bae et al., 2014; Schaefer et al., 2014; Y.-R. Liu et al., 2014) and its central role among the Hg(II) methylators was also confirmed in the current study. In the 34 hotspots from boreal forests, in boreal wetlands and in the lake sediments, respectively 85.4%, 71% and 98.1% of all the hgcA sequences clustered with Deltaproteobacteria (Papers III, IV and V). This is in agreement with earlier work suggesting that most currently confirmed Hg(II) methylating bacteria are affiliated with the Deltaproteobacteria (Ranchou-Peyruse et al., 2009; Yu et al., 2012).

Geobacteraceae is the most represented family within the Deltaproteobacteria, contributing over 30% of all hgcA reads in both boreal soils (Paper III and IV) and 27% of those in the fresh water sediments (Paper V). Iron reducing bacteria have previously been shown to be important for Hg(II) methylation in some environments (Fleming et al., 2006; Kerin et al., 2006; Yu et al., 2012; Bravo et al., 2018). Most Geobacter species belonging to Geobacteraceae tested so far are particularly efficient at MeHg formation in the laboratory (Kerin et al., 2006). In addition, every Geobacter species for which a genome is available (except G. lovleyi) contains an hgcAB orthologue, suggesting the ability to methylate Hg(II) is common among the Geobacteraceae (Fleming et al., 2006; Kerin et al., 2006; Schaefer and Morel, 2009). The importance of Geobacteraceae in Hg(II) methylation was further backed up by earlier evidences of hgcA distribution in wetlands and paddy soils (Bae et al., 2014; Schaefer et al., 2014; Y.-R. Liu et al., 2014). Our results combined with previous findings highlight the role of Geobacteraceae as Hg(II) methylators in the landscape.

4.2.1.2 Firmicutes

Firmicutes are newly confirmed representatives of Hg(II) methylating communities and have expanded the niches and metabolisms associated with MeHg production (Gilmour et al., 2013; Bae et al., 2014; Y.-R. Liu et al., 2014). Methylation rates vary among the Firmicutes, and a few species have been found to have those equalling some Deltaproteobacteria under similar incubation conditions (Gilmour et al., 2013; Bae et al., 2014; Y. R. Liu, Yu, et al., 2014).

Ruminococcaceae under Firmicutes were relatively abundant in soils from Örebro forest region (9.12% of the total hgcA reads) and was a major family across all the wetlands (averagely 12.05 % of the total hgcA reads) with a particularly high abundance in KSN (18 % of the total hgcA reads) (Papers III and IV). In forest soils, they could have played a role in shaping
the composition of Hg\(^{II}\) methylating communities, as a negative correlation was seen between \textit{Ruminococcaceae} and C/S, a primary geochemical factor shaping Hg\(^{II}\) methylating communities in the hotspots (Fig. 5 and Paper III). Low C/N along with low C/S, denoting high quality OM enriched in N and S, seemed to increase the presence \textit{Ruminococcaceae} (Paper III). The relationship between organic S and Hg\(^{II}\) methylation has already been extensively studied (Ullrich \textit{et al.}, 2001; Skyllberg \textit{et al.}, 2002; Drott \textit{et al.}, 2007; Tjerngren, Karlsson, \textit{et al.}, 2012; Jonsson \textit{et al.}, 2014). Organic S and particularly thiols can facilitate bacterial uptake of Hg\(^{II}\) in low molecular mass Hg\(^{II}\)–thiol complexes, yet, not much research has been devoted to the possible relationship between organic S and Hg\(^{II}\) methylating \textit{Ruminococcaceae}.

![Image](image.png)

\textit{Figure 5}. Non-metric multidimensional scaling (nMDS) of potential Hg\(^{II}\) methylating families (based on \textit{hgcA}) in 34 hotspots overlaid with families (black) and geochemical factors (brown) that were moderately (R > 0.5) correlated with the biotic ordination positions

\textit{Ruminococcaceae} were also detected among Hg\(^{II}\) methylators in the sediments impacted by wastewater releases, accounting for 0.02 % of all the \textit{hgcA} reads (Paper V). In addition, it has been speculated that strains of \textit{Ruminococcaceae} could even be the main Hg\(^{II}\) methylators in the sediments of Nam Co Lake in Tibet Plateau (Ma \textit{et al.}, 2017). Considering the abundance of this group in boreal soils and other landscapes, further work is needed to shed light on the metabolic or physiological pathways of Hg\(^{II}\) methylating \textit{Ruminococcaceae}.
4.2.1.3 Sulphate reducing bacteria (SRB)
SRB were the first organisms identified as primary mediators of Hg\(^{(II)}\) methylation in aquatic systems and have also been highlighted as the dominant Hg\(^{(II)}\) methylators in a range of other environments (Gilmour et al., 1992; King et al., 2000; Ullrich et al., 2001; Yu et al., 2010; Achá et al., 2012).

However, identified SRB that use sulphate as the terminal electron acceptors only accounted for a minor portion of all Hg\(^{(II)}\) methylators in both forest soil hotspots and the four wetlands (Paper III and IV). This could have resulted from low availability of sulphate for SRB in these boreal landscape elements (Papers III and IV). Low sulphate concentrations coupled with sporadic detection of SRB-like hgcA sequences was also reported previously (Schaefer et al., 2014). For the lake sediments, the role of SRB could have been outcompeted by iron reducing bacteria due to the Fe-rich but low-sulphate anoxic aquatic settings (Paper V).

Nevertheless, SRB are also capable of syntrophic fermentation of simple organic acids in the absence of sulphate as the terminal electron acceptors (McInerney et al., 2008; Plugge et al., 2011). Negative correlations were found between the abundances of syntrophs and sulphate concentrations across four wetlands (Paper IV). The physiological traits of Hg\(^{(II)}\) methylating SRB are complex and varied (Bae et al., 2014). The importance of syntrophs could also be backed up by the sediment study that pointed out the role of syntrophs in the lake ecosystem rich in organic matter and Fe (Paper V).

A previous study carried out in the boreal region suggested that SRB played an important role in MeHg formation in boreal forest soils (Kronberg et al., 2016). Additionally it has been demonstrated that even when SRB belong to the ‘rare biosphere’ of peatlands, they contribute significantly to respiration (Pester et al., 2010). Therefore, we cannot rule out the possibility that SRB also contribute significantly to Hg\(^{(II)}\) methylation in the boreal regions. Their highly diverse metabolisms suggest that at least some of the hgcA sequences annotated as unclassified Deltaproteobacteria in both forest hotspots and wetlands (Papers III and IV) might be unknown Hg\(^{(II)}\) methylating SRB or Hg\(^{(II)}\) methylating sulphate-reducing syntrophs.

4.2.1.4 Methanogens
Methanogens were early on suspected to be responsible for Hg\(^{(II)}\) methylation (Wood, 1975), but not until recently were they verified as significant mediators of Hg\(^{(II)}\) methylation in the environment (Hamelin et al., 2011; Gilmour et al., 2013; Yu et al., 2013; Schaefer et al., 2014). Their possible active role in this process was suggested in the hotspots from the forest soils even if they overall featured rather low relative abundances in the composition of the Hg\(^{(II)}\) methylating microbial communities (Paper III).
In the studied wetlands especially in SKM, 2.05-6.80% of the total hgcA reads clustered with the methanogens, indicating that they may serve an important role in Hg transformations within boreal wetland systems (Paper IV). In addition, methanogens reached their highest abundance in the deepest sediment layers, where the main electron acceptors FeIII and sulphate were depleted (Paper V).

A syntrophic relationship between methanogens and SRB has been observed when sulphate is limiting, and SRB were found to be able to methylate Hg(II) in co-culture with H2-utilising methanogens (Bryant et al., 1977; McInerney et al., 2008). Moderately positive correlations between syntrophs and methanogens were observed across all the studied wetlands, in particular in the two northern wetlands where sulphate was generally low (Paper IV). This might explain the highest relative abundances of methanogens in SKM where syntrophs were also at their highest relative abundances (Paper IV). This also supports the finding in lake sediment study, for the methanogens could have maintained the activity of syntrophic Hg(II) methylators, especially in deeper sediment layers where FeIII and SO4^2- are depleted (Paper V).

4.2.2 Factors affecting the distribution of microbial communities

Understanding how the Hg(II) methylating microbial communities respond to environmental factors and how they interact with each other as well as with non-Hg(II) methylating bacterial communities is of major interest and relevance for research in Hg(II) methylation (Bae et al., 2015).

4.2.2.1 Effect of geochemistry on Hg(II) methylating communities

MeHg levels

For some time, %MeHg has been proposed as a useful proxy for Hg(II) methylation efficiency (Skyllberg et al., 2007; Drott et al., 2008), and high %MeHg has occasionally also been found to be positively linked to the estimated abundance of Hg(II) methylators (Remy et al., 2006; Eklöf et al., 2018).

In the forest soils, families of Syntrophobacteraceae, Methanosarcinaceae, Methanoregulaceae, Desulfobulbaceae, Syntrophaceae, Desulfo bacteraceae and Dehalococcoidaceae, which potentially host Hg(II) methylators (Gilmour et al., 2013; Podar et al., 2015), were found relevant to the bacterial community composition at sites with high %MeHg according to 16S data (Paper III). However, neither %MeHg nor MeHg concentrations were significantly correlated to any dimensions of the Hg(II) methylating community composition at the 34 hotspots (Paper III). This could possibly be due to the fact that all the samples where hgcA could be successfully amplified featured quite high %MeHg (Paper III).
In wetland soils, there was no significant correlation between either %MeHg or MeHg concentrations and the distribution of Hg\textsuperscript{(II)} methylating community (Paper IV). Generally, this can be interpreted as the composition of the Hg\textsuperscript{(II)} methylating communities is not decisive for net MeHg production or accumulation. This could be due to the known fact that methylation potential differs within various Hg\textsuperscript{(II)} methylating families, where the ability to methylate Hg\textsuperscript{(II)} can even be strain-dependent rather than being manifested at species, genus or family level (King \textit{et al.}, 2000; Ekstrom \textit{et al.}, 2003; Ranchou-Peyruse \textit{et al.}, 2009). Moreover, %MeHg in soils is the net result of methylation and demethylation where the activity of MeHg demethylating bacteria might also affect the final %MeHg (Tjerngren, Karlsson, \textit{et al.}, 2012). The ability for the same microbial groups to also demethylate will make their net effects on MeHg levels uncertain, and this must be taken into account. Lu \textit{et al.} (2016) demonstrated that an iron reducing bacterium was capable of simultaneously producing and degrading MeHg under anoxic conditions. Accordingly, the abundance of such Hg\textsuperscript{(II)} methylators would not always result in high net MeHg production. In the future, a better understanding of demethylation by the Hg\textsuperscript{(II)} methylating families, which in some way is equally important in determining net MeHg formation, is required.

\textit{Nutrient status}

Across all four wetlands, the changes in total Hg\textsuperscript{(II)} methylating communities were mainly related to PO\textsubscript{4}\textsuperscript{3-} concentrations (Paper IV). Strong correlation between this typically limiting nutrient and the Hg\textsuperscript{(II)} methylating communities was observed (Fig. 6). In particular, high productivity coupled to high phosphorus availability seems to promote the growth of \textit{Syntrophorhabdaceae} and \textit{Methanoregulaceae} among all the potential Hg\textsuperscript{(II)} methylators (Paper IV). Nutrient status in soils denoted by C\%, N\% and S\% was also the contributing factor that affects the shaping of Hg\textsuperscript{(II)} methylating communities and also the entire bacterial communities in boreal forests (Fig. 5 and 7).
Figure 6. NMDS of Hg$^{(II)}$ methylating communities (based on hgcA) in all wetland soil samples overlaid with families moderately (R > 0.5) correlated with the biotic ordination positions. Bubble sizes indicate the PO$_4^{3-}$ concentrations in the porewater.

Figure 7. NMDS of bacterial community composition in all forest soils (based on 16S rRNA) overlaid with families (black) and geochemical factors (brown) moderately (R > 0.5) correlated with the biotic ordination (%MeHg: MeHg/THg).
Numerous studies have investigated the impact of nutrients, in particular P on soil microbial community structure. For instance, assemblages of SRB, synthrophs and methanogens, have been shown to be dependent on the position along nutrient gradients (Wright and Reddy, 2001; Chauhan et al., 2004; Bae et al., 2015). In addition, nutrient levels were found to be strong regulators of bacterial growth and activity, including MeHg production (Bigham et al., 2017; Bravo, Sylvain Bouchet, et al., 2017). Liem et al. (2016) investigated ecosystem effects of enhanced nutrient N and P loadings and showed that increased nutrient loads caused increased phytoplankton biomass productivity with stimulated microbial activity in the sediments. However, the exact mechanisms of how different Hg(II) methylating populations respond to the availability of various nutrients remain unclear and merit further study.

Water content
Water content is likely to be another important factor in wetland soils, as the distribution of Hg(II) methylating communities within individual wetlands SKM, LDN and GTN seemed to be governed by water content (Paper IV). An increase in moisture could facilitate MeHg formation through stimulation of microbial methylators by enhanced inputs of organic material from decaying vegetation and soil, and also by the creation of anaerobic conditions and microzones. This might also explain the positive correlation observed between the entire soil microbial community and water content in boreal forest soils (Fig. 7 and Paper III). It has been previously noticed that Hg(II) methylators are stimulated by enhanced water saturation of organic soils, a feature that would provide readily available electron donors for formation and buildup of MeHg (Kronberg et al., 2016). Eklöf et al., (2018) also found that the highest %MeHg was coupled with water-filled cavities and water-logged soils, conditions which might favour methylating microorganisms and thereby enhance MeHg formation.

However, in wetland KSN where water content was generally very high (Paper IV), other environmental factor such as depth seemed to be more important in shaping the local Hg(II) methylating communities (Paper IV). This could be linked to the redox condition and enhanced availability of fresh organic matter and nutrients that would boost microbial activity (Fierer et al., 2003; Kramer and Gleixner, 2008; Lorenz and Lal, 2005).

Bioavailable Hg(II)
The availability of Hg(II) for uptake by methylating organisms has been found to limit the methylation process (Schaefer et al., 2011). This might have been important in wetland KSN and LDN, where soil Hg seemed to influence the total Hg(II) methylating community (Paper IV).
Hg\(^{\text{II}}\) availability could certainly affect the behaviour of Hg\(^{\text{II}}\) methylators and MeHg formation (Schaefer et al., 2011) and a recent mesocosm study of estuarine sediments showed that increased nutrients only led to increased MeHg when Hg\(^{\text{II}}\) was in a highly bioavailable state (Liem-Nguyen et al., 2016). This might also explain why GTN presented the lowest %MeHg in both porewater and soil among the four wetlands (Paper IV). GTN featured the highest amount of total dissolved thiols but the lowest amount of low molecular mass thiols that is likely to be bioavailable (Paper IV). This could have limited the availability of porewater Hg\(^{\text{II}}\) to the methylators at GTN.

**FeIII**

FeIII acting as a direct electron acceptor for iron reducing bacteria has been found to significantly influence Hg cycling and Hg\(^{\text{II}}\) methylation (Fleming et al., 2006; Si et al., 2015).

FeIII was found to contribute to the shaping of Hg\(^{\text{II}}\) methylating communities in wetlands SKM and GTN (Paper IV). This observation was in line with the results from the studies of lake sediments, denoting that ferruginous conditions enhance MeHg formation (Bravo et al., 2015, Paper V). Meanwhile, the dominant Hg\(^{\text{II}}\) methylating family Geobacteraceae was also identified as an influential family for the distributions of Hg\(^{\text{II}}\) methylating community in SKM and GTN (Paper IV). However, no apparent correlations were found between Geobacteraceae and FeIII in these wetlands (Paper IV). Further studies for a better understanding of this particular group are warranted to reveal if geochemistry features other than Fe affect the distribution of this abundant Hg\(^{\text{II}}\) methylating family.

Instead, the distributions of Methanoregulaceae and Ruminococcaceae were highly affected by porewater FeIII in SKM and KSN (Table 4). The growth of Methanoregulaceae might be suppressed under aerobic conditions reflected by the presence of FeIII, which in turn could have favoured the occurrences of Ruminococcaceae.

Table 4. Correlations between geochemical factors and relative abundances of Methanoregulaceae and Ruminococcaceae at sites in SKM and KSN (Significant correlations R≥0.7, moderate correlations 0.5≤R<0.7)

<table>
<thead>
<tr>
<th></th>
<th>FeIII</th>
<th>FeII/III</th>
<th>PO(_4^-)</th>
<th>NH(_4^+)</th>
<th>Methanoregulaceae</th>
<th>Ruminococcaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeIII</td>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeII/III</td>
<td>-0.9</td>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO(_4^-)</td>
<td>-0.6</td>
<td>0.7</td>
<td>1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH(_4^+)</td>
<td>-0.6</td>
<td>0.7</td>
<td>1.</td>
<td>1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanoregulaceae</td>
<td>-0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>1.</td>
<td></td>
</tr>
<tr>
<td>Ruminococcaceae</td>
<td>0.9</td>
<td>-0.8</td>
<td>-0.6</td>
<td>-0.6</td>
<td>-0.7</td>
<td></td>
</tr>
</tbody>
</table>
4.2.2.2 Interactions between microbial communities

*Non-Hg(II) methylating bacterial communities and the actual Hg(II) methylators*

%MeHg has previously been used as a proxy for methylation efficiency (Skyllberg *et al.*, 2007; Drott *et al.*, 2008), and high %MeHg has in a few cases been shown to correlate positively with the abundance of Hg(II) methylators (Remy *et al.*, 2006; Eklöf *et al.*, 2018). In the studied forest soils, sites with high %MeHg hosted characteristic bacterial communities (Fig. 7). Not only families known to contain Hg(II) methylators were found relevant at sites with high %MeHg, but also several non-Hg(II) methylating families were positively correlated to %MeHg (Paper III).

*Anaerolineaceae, Spirochaetaceae and Holophagaceae* are known to generate acetate (Hunger *et al.*, 2015), while *Fibrobacterales* have been suggested to have an important role in cellulose hydrolysis in soils (Ransom-Jones *et al.*, 2012). Therefore, these families that are associated with high %MeHg, could possibly promote MeHg production by degradation of long chain organic matter compounds in the process of providing appropriate substrates (e.g. acetate) for the Hg(II) methylators (Liang *et al.*, 2015; Juottonen *et al.*, 2017). Non-Hg(II) methylating members of *Desulfobulbaceae*, known to oxidise organic substrates incompletely to acetate (Kuever, 2014) might also have provided the necessary substrate to Hg(II) methylators (Paper III). In particular, *Methanothrix* has been observed to establish syntrophic interactions with *Anaerolineaceae* (Liang *et al.*, 2015) or *Geobacteraceae* (Holmes *et al.*, 2017) in methanogenic degradation of long chain carbon compounds. As *Geobacteraceae* are major contributors to the Hg(II) methylating microbial community in the forest soils (Paper III), the correlation found between *Methanothrix* and %MeHg could be the result of the interaction between the non-Hg(II) methylating *Methanothrix* and the Hg(II) methylating *Geobacteraceae*.

This suggests an important role of the supporting and interacting non-Hg(II) methylating bacterial communities in sustaining the activity of Hg(II) methylating microorganisms and thereby boosting MeHg formation in boreal forest soils. More in depth studies with metagenome-level sequencing and metabolic pathway reconstruction will be a logical next step to gain a better understanding of how Hg(II) methylating bacterial and non-methylating species interact in soils.

*Ruminococcaceae and Methanoregulaceae*

There seemed to be an antagonism or a competition between *Methanoregulaceae* and *Ruminococcaceae*. This was especially apparent when looking at the northern wetlands SKM and KSN (Table 4). Strong positive correlations were found between *Methanoregulaceae* and FeII/III.
and NH$_4^+$ both of which could indicate the redox status (Table 4). This might have contributed to the low abundance of *Methanoregulaceae*, a clade of methanogen whose growth and activity is likely to be inhibited in the presence of even low levels of dissolved oxygen. Furthermore, less productive sites also seem to host lower numbers of *Methanoregulaceae*, indicated by the positive correlations between this group and PO$_4^{3-}$ across all four wetlands (Fig. 6) and especially in northern wetlands (Table 4).

Depending on the availability of both electron donors and electron acceptors, the interaction between SRB and methanogens could be either competitive when sufficient sulphate is available to serve as a terminal electron acceptor for SRB; or cooperative via a syntrophic relationship when sulphate is limited (Bryant *et al.*, 1977; McInerney *et al.*, 2008). In wetlands SKM and KSN, due to the generally low sulphate, both syntrophic SRB and methanogens could have outcompeted fermentors (*Ruminococcaceae*) under more anaerobic condition with sufficient P levels (Paper IV). Further research is needed for a better understanding of the physiology and metabolism of *Ruminococcaceae* in order to elucidate the competitive mechanism between Hg$^{(II)}$ methylating fermentors and methanogens.
5. Conclusions

Soil washing has historically been one of the most widely used techniques for remediation of Hg contamination in soils with primary sand/gravel content. The studied soil was coarse-grained and the total Hg concentration decreased with increasing particle sizes. However, soil washing by physical separation was proven insufficient to treat this soil Hg contamination, as even the least contaminated fraction exceeded the acceptable level for Hg in soils.

Due to the strong affinity between soil particles and Hg contaminants, soil washing at pH from 3 to 11 could only remove up to 0.3% of the total soil Hg even if Hg mobilisation was enhanced at pH 5 and pH 11. Elevated concentrations of chloride, a competent complexing agent for Hg, also did not improve Hg mobilisation. Various extractants including acidic leachates from food waste, alkaline leachates from bottom ash, as well as a strongest chelating agent EDTA, were applied to test the potential of soil washing by chemical extraction. The Hg extraction efficiency was hardly satisfying, with less than 1.5% of the total Hg removed. All this highlights the extremely firm bond between Hg contamination and the soil particles, questioning the feasibility of soil washing to treat Hg pollution in this studied soil.

The newly developed strategy of combining high-throughput hgcA amplicon sequencing with molecular barcoding revealed diverse clades of Hg(II) methylating bacteria and archaea in soils from boreal forests, wetlands and impacted lake sediments, including Proteobacteria, Firmicutes and Euryarchaeota. This study confirms a predominant role of Deltaproteobacteria, in particular Geobacteraceae, as Hg(II) methylators in boreal soils and lake sediments. Firmicutes, in particular Ruminococcaceae, were also abundant members of Hg(II) methylating microbial community in the boreal landscape. In addition, a role of syntrophic SRB interacting with methanogens may be important in boreal soils.

In forest soils, certain clades of non-Hg(II) methylating bacterial community (e.g. Anaerolineaceae, Holophagaceae and Spirochaetaceae) were positively correlated with MeHg production and this could be explained by their inferred role in processing complex OM and thereby providing low OM compounds as a substrate to the organisms actually carrying out the Hg(II) methylation. By revealing the linkages between Hg(II)
methylators and non- Hg\(^{\text{II}}\) methylators, our results highlight the role of the entire complex soil bacterial community. The nutrient status appears to play a significant role in shaping the Hg\(^{\text{II}}\) methylating community composition across the four wetlands and in shaping the entire soil bacterial community in all the forest soils. Meanwhile, the water content plays a major role in controlling the distribution of Hg\(^{\text{II}}\) methylators within individual wetlands and that of the entire microbial community across all forest regions. Furthermore, anaerobic and productive conditions seemed to favour *Methanoregulaceae*, which might in turn hamper the growth of *Ruminococcaceae* in boreal wetlands. For the sediments, our results suggest that geochemical conditions dominate net MeHg formation. Our studies shed light on the diversity and distribution of populations active in Hg\(^{\text{II}}\) methylation in forest soils, wetlands and lake sediments in the landscape.
6. Outlook

The thesis highlights the complex transformation and speciation of Hg and the importance of geochemical conditions of the soil matrix for selecting appropriate remediation options. For instance, quantitative studied relying on high resolution spectroscopic methods (e.g., X-ray spectroscopy) might facilitate the understanding of Hg adsorption/desorption on soil organic matter/minerals. Site-specific investigations of Hg contamination could provide important information about Hg(II) and MeHg concentrations, Hg(II) availability, microbial activity and bioaccumulation, etc. At certain sites with specific biogeochemical characteristics, even more detailed and comprehensive assessments are required to facilitate remedial decision-making.

My results suggests that there is a need for more in depth studies on the interactions between various Hg(II) methylating groups in contributing to the overall MeHg production. There is also a need for more targeted studies of linkages between Hg(II) methylators and non- Hg(II) methylators. Special attention should be paid to the physiology as well as the metabolism of different Hg(II) methylating microbes, in order to understand how they respond to various levels of limiting nutrients and organic matter of different composition and availability. The design of primers for the hgcA amplification also requires further optimisation to minimise possibly oversights and for certain Hg(II) methylating clades.

Additionally, combing the quantitative methods, e.g., rate measurements of MeHg, and DNA based methods revealing the presence of Hg(II) methylating organisms could be a promising approach for verifying the activity of potential Hg(II) methylators. Further experiment could also be designed to test if the prediction of MeHg formation based on the presence of Hg(II) methylating community is valid. Apart from soils and sediments, Hg(II) methylation in the periphyton which could be an important source of food and nursing sites for fish, could also have significant implications for biota. Even the water column that has been recently suggested to play an important role in Hg(II) methylation in certain aquatic environments should be more investigated. These are critical points to gain a better understanding of Hg(II) methylating community in the landscape.
Kvicksilver (Hg) är en mycket skadlig tungmetall som förekommer naturligt i de flesta naturmiljöer, men då normalt i mycket låga halter. Under årtionden har vi använt oss av kvicksilver för olika processer och ändamål, och detta har orsakat betydande kvicksilverförorening av luft, vatten, mark och frilevande djur i olika områden.

Alla former av kvicksilver är skadliga för levande organismer, men organiska kvicksilverföreningar, såsom metylkvicksilver (MeHg) är särskilt farliga. Detta ämne fungerar som ett neurotoxin och ackumuleras och ansamlas också i levande organismer och näringsvävar i olika naturmiljöer.

Kvicksilver betraktas som en global förorening eftersom substanserna kan transporteras långväga i atmosfären samtidigt som de dröjer kvar och ackumuleras i naturen: Väl där kan kvicksilver orsaka allvarligt skada på människor, djur och ekosystemet i stort.

Under senare tid har en rad framgångsrika åtgärder genomförts för att reducera användande och spridning av kvicksilver. Exempel är bland annat insatser för ökad medvetenhet hos allmänheten och globala samarbetsavtal för att hantera problemet som etablerades redan 1990. Trots detta kvarstår de hälsor och miljöproblem som kan kopplas till ökade kvicksilverhaltar i främst naturvatten och marksystem, miljöer som även möjliggör de biologiska processer som omvandlar oorganiskt kvicksilver till mer problematiska organiska former (MeHg). Kvicksilverproblemet kvarstår som en av de stora miljöutmaningarna, nu och sannolikt också i framtiden.

Av ovan nämnda skäl är det av stor betydelse att minimera och motverka förekomsten och utbredningen av kvicksilver i förorenade och särskilt känsliga miljöer. Ett symptom på problemets omfattning är till exempel att sötvattensfisk i den boreala klimatzonen vanligtvis uppvisar kvicksilverhaltar som överstiger de gränsvärden för skadlig exponering som satts upp av världshälsoorganisationen (WHO). Boreala skogar och våtmarker verkar kunna fungera som såväl källor som sänkor för det mer skadliga metylkvicksilveret, vilket sedan transporteras med vatten till nedströms ytvattens-recipienter. Väl där ansamlas metylkvicksilveret i organismer och kan transporteras och koncentreras i näringsväven vilket i förlängningen kan leda till exponering hos människor. För att bättre förstå detta problem och bakomliggande mekanismer och processer behöver vi
studera var och varför oorganiskt kvicksilver omvandlas till de mer skadliga organiska formerna.

I denna avhandling studeras såväl processer och strategier för att behandla kvicksilverkontaminerad mark och de biologiska processer som ligger till grund för kvicksilvermetylering i olika landskapselement (skogsmark, våtmarker, sjösediment). Inledningsvis granskas och summeras de naturliga och antropogena källor som har bidragit till de förhöjda kvicksilverhalter som vi nu ser i vår naturmiljö. I denna översikt beskrivs också några av de vanligaste metoderna för att behandlas kvicksilverförörenad jord.

Potentialen hos några lovande behandlingstekniker studerades därefter experimentellt. I dessa studier behandlades jord som förorenats av kvicksilver genom industriella klor-alkaliprocesser, avfallshantering och sjöfartspåverkan behandlades med olika tvättprocesser. Genomförbarheten testades för olika metoder: (1) fysikalisk sållning under torra eller våta förhållanden, (2) kemisk extraktion med olika lakningslösningar och varierande pH. Mina resultat visade på att kvicksilvret är starkt bundet till jordpartiklarna och att stabiliseringstekniker snarare än kemiska extraktionstekniker bör väljas för behandling av denna typ av förorenad jord.

I avhandlingens andra del studerades de mikrobiella samhällen som ligger bakom metyleringen av kvicksilver i vårt landskap. I tre kopplade studier belystes även de miljöfaktorer som synarbligen hade stor inverkan på dessa mikroorganismers samhällsstruktur och hur detta sedan kunde kopplas till förekomst av MeHg i skogsmark, våtmarker och sjösediment. Genom att använda ny DNA-baserad teknik identifierades en rad olika grupper av kvicksilvermetylerande mikroorganismer där deltaproteobakterier som sannolikt använder endera sulfat eller trevärt järn för att driva sin metabolism dominerade. I skogsmark fanns det inget tydligt samband mellan artsammansättningen hos dessa metylerare och förekomsten av MeHg, utan metyleringen verkade snarare kunna härledas till artsammansättningen hos andra bakterier som inte själva kan utföra kvicksilvermetylering. Detta indikerar att dessa grupper kan driva på kvicksilvermetyleringen genom en “stödfunktion” där de genom sin egen metabolism förser kvicksilvermetylerande grupper med olika näringsämnen och substrat.

I våtmarker verkar systemets näringsstatus och vattenhållande förmåga styra förekomsten av kvicksilvermetylerande grupper. Många olika typer av metylerande grupper kunde påvisas, med dominerande inflytande från järnreducerande och sulfatreducerande bakterier. Även metanogener och grampositive fermenterade förekom och den relativa betydelsen av de olika grupperna kunde kopplas till vattenhalt och andra miljöfaktorer som skilde sig åt mellan de fyra våtmarker som ingick i studien.

Betydelsen av järnoxiderande bakterier i kvicksilvermetyleringen var ett genomgående resultat i såväl skogsmark som våtmarker och detta skiljer sig
åt från den gängse uppfattningen att kvicksilvermetyleringen huvudsakligen utförs av sulfatreducerande bakterier. För att ytterligare belysa detta genomfördes en riktad studie av kvicksilvermetylering i sjösediment som påverkas av utsläpp från ett avloppreningsverk där fosfor avlägsnas med järnutfällning. Även i dessa sediment som kännetecknas av förhöjda halter av trevärt järn och biotillgängligt organiskt material ingick järnoxiderande bakterier, men även sulfatreducerare, metanogener och syntrofer i det kvicksilvermetylerande samhället. Dessa tre studier bidrar till betydligt ökad kännedom om kvicksilvermetylerande mikroorganismer och hur dessa populationer påverkas av olika miljöförhållanden i olika delar av vårt landskap.
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9. References


Bravo, A.G., Bouchet, S., Guédron, S., Amouroux, D., Dominik, J., and Zopfi, J.


Gionfriddo, C.M., Tate, M.T., Wick, R.R., Schultz, M.B., Zemla, A., Thelen, M.P.,
et al. (2016) Microbial mercury methylation in Antarctic sea ice. *Nat. Microbiol.* 1:


Jonsson, S., Skyllberg, U., Nilsson, M.B., Lundberg, E., Andersson, A., and Björn,
Liem-Nguyen, V., Bouchet, S., and Björn, E. (2015) Determination of sub-nanomolar levels of low molecular mass thiols in natural waters by liquid chromatography tandem mass spectrometry after derivatization with p-


Robles, I., Lakatos, J., Scharek, P., Planck, Z., Hernández, G., Solís, S., and and


plants through phytoremediation. *Int. J. Chem. Eng.*


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