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Heidi Pietilä

DEVELOPMENT OF ANALYTICAL METHODS FOR ULTRA-TRACE DETERMINATION OF TOTAL MERCURY AND METHYL MERCURY IN NATURAL WATER AND PEAT SOIL SAMPLES FOR ENVIRONMENTAL MONITORING

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU,
FACULTY OF SCIENCE,
DEPARTMENT OF PHYSICS AND CHEMISTRY;
FINNISH FOREST RESEARCH INSTITUTE

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Academic dissertation to be presented with the assent of the Doctoral Training Committee of Technology and Natural Sciences of the University of Oulu for public defence in the OP-Pohjola auditorium (L6), Linnanmaa, on 7 November 2014, at 12 noon

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Abstract

Mercury is a global pollutant that accumulates easily in forest soils, even in remote areas. Mercury accumulated in soils can be subsequently released into surface waters causing an increased eco-toxicological and human health risk. The most toxic form of mercury to humans and wildlife is methyl mercury (MeHg), which can be formed in the environment via methylation processes. In freshwaters, MeHg is readily accumulated in fish, which are the main source of human exposure to MeHg. The determination of both total mercury and MeHg concentrations in environmental samples, such as natural waters and soils, is important in environmental risk assessment. This study involved the development of analytical methods for the determination of ultra-trace total mercury and MeHg concentrations in humic-rich natural water and peat soil samples. Each developed method was carefully optimized and validated by using real natural water and peat soil samples, certified reference materials and/or reference methods. The cold vapor inductively coupled plasma mass spectrometry (CV-ICP-MS) method developed during this study was found to be a reliable method for the determination of total ultra-trace mercury concentrations in natural freshwaters. Purge and trap gas chromatography, coupled to an ICP-MS, was used in mercury speciation analysis. Together with species-specific isotope dilution this technique proved to be a reliable method in MeHg determinations. Prior to instrumental determination, MeHg was successfully isolated from humic-rich water and peat soil samples using N_2-assisted distillation. The analytical methods developed in this study were successfully applied to an investigation of the effects of forest harvesting practices on the mobilization of mercury in boreal forest catchments.

Keywords: CV-ICP-MS, environmental monitoring, mercury, methyl mercury, N_2-assisted distillation, natural waters, peat soils, purge and trap GC-ICP-MS, species-specific isotope dilution

Pietilä, Heidi, Elohopean analyysimenetelmien kehittäminen ympäristön seurantaa varten: pienten kokonaiselohopea- ja metyylielohopeapitoisuuksien määrittäminen luonnonvesistä ja turvemaista.

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Luonnontieteellinen tiedekunta, Fysiikan ja kemian laitos; Metsäntutkimuslaitos

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Tiivistelmä

Elohopeaa pääsee ilmakehään sekä luonnollisista lähteistä (mm. tulivuorenpurkaukset ja kiviaineksen rapautuminen), että ihmisen toiminnan kautta. Elohopean viipymäaika ilmakehässä on hyvin pitkä, minkä vuoksi se voi kulkeutua kauas päästölähteestä ennen päätymistään maaperään ja vesistöihin. Ympäristössä olevasta epäorgaanisesta elohopeasta voi muodostua erittäin myrkyllistä metyylielohopeaa, joka rikastuu helposti ravintoketjussa. Metyylielohopean muodostuminen on merkittävä osa elohopean biogeokemiallista kiertoa, minkä vuoksi metyylielohopean määrittäminen näytteen kokonaiselohopeapitoisuuden ohella antaa tärkeää tietoa elohopean käyttäytymisestä ympäristössä. Tutkimuksessa kehitettiin analyysimenetelmät, joilla määritettiin ultrapieniä kokonaiselohopea- ja metyylielohopeapitoisuuksia humuspitoisista luonnonvesistä ja turvemaanäytteistä. Tutkimuksessa käytetyt näytteet oli kerätty turvemaametsien valuma-alueilta Sotkamosta. Luonnonvesinäytteiden kokonaiselohopeapitoisuuksien määrityksessä käytettiin kylmähöyrymenetelmää (CV) yhdistettynä induktiiviplasma-massaspektrometriaan (ICP-MS). Vesi- ja turvenäytteiden metyylielohopeapitoisuuksien määrityksessä elohopeaspesiekset erotettiin kaasukromatografisesti (GC) ja määritettiin isotooppilaimennus-ICP-MS:lla. Ennen GC-ICP-MS -määritystä näytteet esikäsiteltiin typpiavusteisella tislausmenetelmällä ja esikonsentroitiin ’purge and trap’ -tekniikalla. CV-ICP-MS ja ’purge and trap’ GC-ICP-MS -menetelmät optimoitiin huolellisesti sekä laiteparametrien, että reagenssimäärien suhteen. Menetelmillä saatavien tulosten oikeellisuus varmistettiin vertailumateriaalien ja/tai vertailumenetelmien avulla. Kehitettyjä analyysimenetelmiä hyödynnettiin tutkimuksessa, jossa seurattiin metsähakkuiden mahdollisia vaikutuksia elohopean huuhtoutumiseen ja metyloitumiseen ojitetuilla turvemailla.

Asiasanat: elohopea, isotooppilaimennus, kaasukromatografi-ICP-MS, kylmähöyry ICP-MS, luonnonvesi, metyylielohopea, turve, typpiavusteinen tislaus, ympäristön seuranta

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Oulu, October 2014 Heidi Pietilä

Abbreviations

e.g.	exempli gratia
AFS	Atomic fluorescence spectrometry
BRL	Brooks Rand Labs
CV	Cold vapor
DOC	Dissolved organic carbon
EC	External calibration
EQS	Environmental quality standard
Et_2Hg	Diethyl mercury
EU	European Union
FIAS	Flow injection analysis system
FLPE	Fluorinated polyethylene
GC	Gas chromatography
HDPE	High-density polyethylene
HPLC	High-performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
ID	Isotope dilution
IDL	Instrumental detection limit
IS	Internal standardization
ISO	International Organization for Standardization
MDL	Method detection limit
MeEtHg	Methyl ethyl mercury
MeHg	Methyl mercury
Me_2Hg	Dimethyl mercury
OM	Organic matter
PTFE	Polytetrafluoroethylene
RF	Radio frequency
RIA	Randomized intervention analysis
RSD	Relative standard deviation
SD	Standard deviation
SOH	Stem-only harvesting
SRB	Sulfate reducing bacteria
SSID	Species-specific isotope dilution
THg	Total mercury
US-EPA	U.S. Environmental Protection Agency
UV	Ultraviolet

WTH	Whole-tree harvesting

List of original publications

This thesis is based on the following publications, which are referred to throughout the text by their Roman numerals:

I Pyhtilä H, Perämäki P, Piispanen J, Niemelä M, Suoranta T, Starr M, Nieminen T, Kantola M, Ukonmaanaho L (2012) Development and optimization of a method for detecting low mercury concentrations in humic-rich natural water samples using a CV-ICP-MS technique. Microchem. J. 103, 165–169

II Pyhtilä H, Niemelä M, Perämäki P, Piispanen J, Ukonmaanaho L (2013) The use of a dual mode sample introduction system for internal standardization in the determination of Hg at the ng L^{-1} level by cold vapor ICP-MS. Anal. Methods 5, 3082–3088

III Pietilä H, Perämäki P, Piispanen J, Majuri L, Starr M, Nieminen T, Kantola M, Ukonmaanaho L (2014) Determination of methyl mercury in humic-rich natural water samples using N_2-distillation with isotope dilution and on-line purge and trap GC-ICP-MS. Microchem. J. 112, 113–118

IV Pietilä H, Perämäki P, Piispanen J, Starr M, Nieminen T, Kantola M, Ukonmaanaho L. Determination of low methylmercury concentrations in peat soil samples by isotope dilution GC-ICP-MS using distillation and solvent extraction methods. (*Submitted manuscript*)

V Ukonmaanaho L, Starr M, Kantola M, Laurén A, Piispanen J, Pietilä H, Perämäki P, Merilä P, Fritze H, Tuomivirta T, Heikkinen J, Mäkinen J, Nieminen TM. Impacts of whole-tree harvesting vs. stem-only harvesting on the mobilization of Hg and MeHg in drained peatland forests. (*Manuscript*)

The present author is the principal contributor of papers I–IV and co-author of paper V. Most of the experimental analytical work related to papers I–IV was carried out by the author. In paper V, the present author was mainly responsible for performing the experimental work related to mercury analyses.

Contents

1 Introduction

Mercury (Hg) is a highly toxic element and known as a global pollutant. [1, 2] The toxicity, bioavailability and mobility of mercury in the environment are highly dependent on its chemical form. Due to their neurotoxic, lipophilic and bioaccumulative nature, organic mercury compounds, such as monomethyl mercury (CH_3Hg^+ = MeHg) and dimethyl mercury ($(CH_3)_2$ Hg = Me_2Hg), are the most toxic forms of mercury for humans and wildlife. [3, 4] Through atmospheric deposition and because of a strong affinity with organic matter, mercury accumulates easily in the environment. One third of Finland's land area is covered by peat and mire, which can act as sinks for atmospheric Hg deposition. [5–7] Hg retained in the soils can be subsequently released into surface waters, resulting in increased eco-toxicological and human health risks.

1.1 Mercury in the environment

In the environment, the major chemical species of mercury are elemental mercury (Hg^0), inorganic mercury (Hg^{2+}) and methyl mercury (MeHg). In natural waters and soils, the dominant form of mercury is Hg^{2+}, mainly existing as complexes. [8] Mercury (Hg^+, Hg^{2+}) is a soft Lewis acid which tends to associate with soft bases such as sulfur, less electronegative halides, and nitrogen. [9, 10] In soils and freshwaters, mercury is mainly bound to natural organic matter containing reduced sulfur groups (SH^-, S^{2-}). [11–14] Thus, soil organic matter plays an important role in the mobilization and transportation of mercury in ecosystems. [15–18]

In the environment, mercury can be converted into toxic MeHg through biogeochemical transformations. Mercury methylation rate is influenced by a number of environmental factors, such as pH, redox-potential, bacterial community, and organic matter content. [19] Although biological methylation due to sulfate- and iron-reducing bacteria are often considered the main source of MeHg in soils and freshwaters [20–22], abiotic methylation also occurs [23–25]. Biological methylation is favored by anaerobic conditions, which are easily formed in wetland areas, e.g. peat soils. [26–28] Due to methylation and demethylation processes taking place in the environment, MeHg concentrations are seldom related to the total mercury load.[29]

Another important transformation process affecting the biogeochemical cycle of mercury is the reduction of Hg^{2+} to Hg^0 e.g. due to direct photolysis and abiotic processes. [30–32] Through reduction, Hg^0 can be released from the soil into the atmosphere and deposited in surface waters, where it can be further methylated and

accumulated in the aquatic food chain. The biogeochemical cycle of mercury in the environment involves the circulation of mercury between air, water, sediment, soil and living organisms (Fig. 1).

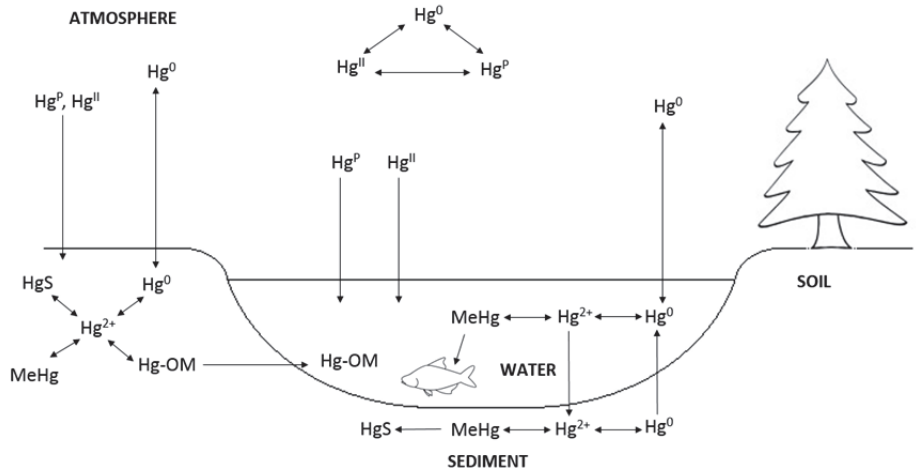

Fig. 1. Mercury cycling in the environment. (Hg^{II} = divalent reactive gaseous mercury, Hg^P = particle bound mercury, Hg-OM = mercury bound to organic matter, HgS = mercuric sulfide)

Mercury concentrations in uncontaminated freshwaters are at ultra-trace (ng/L) level and the proportion of MeHg is typically less than 10% of the total Hg concentration. [33] However, due to bioaccumulation, mercury concentrations in fish can be up to a million times higher than in ambient water and over 95% of accumulated mercury can be in the form of MeHg. [34] It has been shown that MeHg concentrations in fish respond rapidly to the Hg^{2+} added directly to the surface waters. [35, 36] Thus, a fall in mercury emissions and subsequent decrease in mercury depositions may have a significant effect on mercury contamination in fish.

1.2 Mercury sources and pollution control

Mercury is emitted into the atmosphere from both natural and anthropogenic sources. Globally, the main anthropogenic sources of mercury emissions are the burning of fossil fuels, primarily coal, and artisanal and small-scale gold mining. Other

anthropogenic sources include emissions from mercury-containing industrial processes and products, the incineration of waste, mining operations and metal production. [37] In terms of natural processes, mercury can be released into the atmosphere by volcanic and geological activity. Natural sources of mercury also include the re-emission of previously deposited mercury from top soil, vegetation and surface waters, and biomass burning due to wild fires and agricultural purposes. [38, 39]

In the atmosphere, the main mercury species are elemental mercury (Hg^0 ~95%), divalent reactive gaseous mercury (Hg^{II}, e.g. $Hg(OH)_2$ and $HgCl_2$) and particle bound mercury (Hg^P).[40, 41] Hg^{II} and Hg^P are more soluble in water compared to Hg^0 and are therefore most likely to be deposited on a local and regional scale. [42] Due to the long atmospheric residence time of Hg^0, it can be widely distributed and deposited in remote and pristine areas. [43] Deposited mercury is easily accumulated in soils; it has been estimated that over 90% of annual mercury deposition is retained within soils. [44] In freshwaters, mercury can originate from direct atmospheric deposition and runoff from soils. [45–47]

Inventories created for the estimation of global mercury emissions from both anthropogenic and natural sources have become increasingly important to controlling mercury pollution. [48] During recent decades, great efforts have been made to reduce mercury pollution by implementing laws and regulations on the uses and emissions of mercury. For example, mercury in electrical and electronic equipment has been restricted by the RoHS directive in Europe (RoHS directive 2002/95/EC). [49] In 2005, the European Union (EU) launched the mercury strategy, whose aim is to reduce mercury levels in the environment and to lower the exposure of humans and wildlife to mercury within the EU and globally. [50] The most recent global agreement on the prevention of mercury emissions and releases is the *Minamata Convention on Mercury*, named after the mercury poisoning tragedy which occurred in Minamata, Japan, in the 1950s. The Minamata Convention on Mercury was agreed at the fifth session of the Intergovernmental Negotiating Committee in Geneva, Switzerland, in January 2013 and was opened for signatures at the diplomatic conference held in Minamata in October 2013. By October 2014, this convention had been signed by 122 countries, including Finland. [51]

1.3 Why are mercury and methyl mercury in the environment monitored?

Large-scale mercury poisoning in Minamata, Japan, in the 1950s increased the attention drawn to mercury as an environmental and health concern. In Minamata, people were exposed to large amounts of MeHg by eating seafood contaminated with MeHg, which had been discharged into the water by a local acetaldehyde factory.[52] Another large-scale tragedy related to mercury occurred in Iraq in the 1970s, when people were exposed to high levels of MeHg by eating bread made from grain treated with an alkylmercury fungicide. [53]

MeHg is a neurotoxin that readily concentrates in the aquatic food chain, which is the dominant pathway for human MeHg exposure. [54] MeHg poisoning causes neurological damage and can have serious effects on fetal neurodevelopment. [55, 56] Thus, guidelines on fish consumption, especially for children and during pregnancy, have been proposed by several organizations such as the Finnish Food Safety Authority Evira.

Elevated mercury concentrations in fish have been observed despite the fact that mercury emissions and depositions have decreased in Finland and throughout Europe. [36, 57, 58] In Finland, mercury concentrations in predatory fish can exceed the EU's lower threshold limit of 0.5 mg/kg (EC 1881/2006). [59] One reason for this may be the disturbance of forest soil by forestry practices, e.g. harvesting, which increases the leaching of mercury into surface waters as a consequence of increased leaching of soil organic matter. [60–62] The EU's Water Framework Directive (2000/60/EG), which defines the requirements for the achievement of good ecological status among water bodies, classifies mercury as a priority hazardous substance whose presence in aquatic ecosystems must be monitored. [63] As set by the Priority Substances Directive (2008/105/EC), the environmental quality standard (EQS) threshold value for mercury in fish tissue is 0.02 mg/kg. [64]

The development of reliable analytical methods for mercury determination in environmental samples is an important part of environmental risk assessment. In addition to the total mercury, the determination of MeHg in freshwaters and soils is important to understanding the fate of mercury in ecosystems and estimating the aquatic food chain's potential exposure to mercury. Due to ultra-trace concentration levels of mercury in unpolluted environments (0.5–20 ng/L in freshwaters) [33, 65], sensitive analytical methods are required for the accurate determination and speciation of mercury.

1.4 Determination of ultra-trace amounts of mercury in environmental samples

Ultra-trace [66] mercury analysis requires a highly sensitive and element-specific detection technique, such as atomic fluorescence spectrometry (AFS) [67–70] or inductively coupled plasma mass spectrometry (ICP-MS) [71, 72]. Both AFS and ICP-MS are widely applied in mercury determination and speciation. AFS has also been adopted in standard methods of quantifying mercury in waters, published by the International Organization for Standardization (SFS-EN ISO 17852) and the U.S. Environmental Protection Agency (US-EPA). [73–76]

The advantages of ICP-MS in trace metal analysis include its multi-element analysis and isotope ratio measurement capabilities. In addition to external calibration and standard addition methods, these features enable the use of internal standardization (IS) and isotope dilution (ID) methods for accurate quantification. [77–79] IS with (a) carefully selected internal standard(s) is often used to correct signal instabilities and drift during ICP-MS measurements, e.g. those due to matrix effects and variations in the sample uptake rate. [80–82] Using the IS method, internal standard is added to both samples and calibration standards to enable accurate quantitative measurements. In the case of ID method, samples are spiked with known amounts of an isotopically enriched analyte. Since the method is based on isotope ratio measurements, no calibration standards are required in order to obtain quantitative analytical results. [83–85]

In addition to a highly sensitive analytical method, the selection of suitable sampling and sample pretreatment protocols are crucial in ultra-trace mercury analysis. [86]

1.4.1 Sampling and sample pre-treatment

The entire analytical chain, from sampling to instrumental determination, should be performed carefully in order to avoid contamination and analyte losses and, in the case of speciation analyses, in order to eliminate inter-species conversion. When ultra-trace mercury concentrations are determined, rigorous cleaning of sampling bottles and all laboratory ware is necessary in order to eliminate possible sources of contamination. [87] Analyte losses due to the volatilization and adsorption of mercury onto surfaces should be minimized, as should contamination from the surrounding atmosphere, by selecting the proper container materials.

The recommended sampling and storage materials for natural water samples in ultra-trace mercury analysis are Teflon (polytetrafluoroethylene, PTFE) and borosilicate glass. [88, 89] However, due to the high price of Teflon and impracticality of glass bottles in the field, alternative materials, such as fluorinated high-density polyethylene (FLPE), have been suggested. FLPE is much cheaper than Teflon and has lower permeability than an ordinary high-density polyethylene (HDPE). [90]

After sampling, the preservation of natural water samples is crucial to minimizing biological activity in the sample and reducing the volatility and adsorption effects of mercury.[91] Possible preservation methods in the case of water samples include acid addition and freezing. [92, 93] When total mercury concentrations are determined in natural freshwaters, acidic bromine chloride (BrCl) has proven to be an excellent reagent for oxidizing and stabilizing mercury. [88, 94–96] BrCl oxidation is particularly used when total mercury is determined using a cold vapor technique. [74] Water samples analyzed for MeHg are preserved e.g. with hydrochloric acid (HCl) or by freezing. [86] MeHg has been found to be extremely stable in water samples preserved with 0.4% (v/v) HCl and stored under cool and dark conditions. [89, 97] Use of 0.4% HCl is especially necessary when the distillation method is used to isolate MeHg from the freshwater sample matrix prior to instrumental determination. [75, 98]

When MeHg is determined in soil samples, the samples must be prevented from oxidizing immediately after sampling, in order to minimize potential mercury species transformations. After sampling, soil samples should be processed as soon as possible (preferably under inert gas) and stored in the freezer if they are not analyzed immediately. If samples are dried prior to analyses, freeze-drying should be used rather than oven drying in order to avoid possible losses of MeHg.

1.4.2 Determination of total mercury using CV-ICP-MS

Liquid nebulization using a pneumatic nebulizer/spray chamber is the most frequently used method for sample introduction in ICP-MS. However, this system includes several drawbacks, such as low analyte transport efficiency (1–5%) and matrix and memory effects. [99] Due to these drawbacks, the detection limits achieved with conventional liquid nebulization are insufficient for determining ultra-trace mercury concentrations in natural freshwaters.

Gaseous sample introduction using a cold vapor (CV) system coupled to an ICP-MS is a highly sensitive technique for ultra-trace mercury analyses. [100–103] The

use of CV in sample introduction enables the separation of mercury from the sample matrix, resulting in reduced spectral interferences and high analyte transport efficiency, which subsequently lowers the detection limits. CV is based on the reduction of mercury to elemental mercury Hg^0 (mercury vapor, termed as "cold vapor") which is then transported to the detector. The reduction of mercury in an aqueous solution is usually performed based on chemical reduction, using stannous chloride ($SnCl_2$) [74] or sodium borohydride ($NaBH_4$). [104] However, an electrolytic [105, 106] and photo-induced [107, 108] reduction methods have also been used.

Determination of total Hg concentrations in freshwater samples with CV-ICP-MS requires an oxidation step to release all Hg compounds from the sample matrix and to convert Hg species, such as organomercury compounds, into divalent "reducible" inorganic mercury (Hg^{2+}). BrCl is the oxidizing agent most commonly used for this purpose and is also recommended as part of SFS-EN ISO 17852 and US-EPA 1631 standard methods. [73, 74, 94] When natural water samples containing high amounts of organic matter are analyzed, the oxidation step requires particular care since an insufficient amount of BrCl added can result in low mercury recoveries.[95, 96] On the other hand, too high BrCl concentration in samples may cause high blank levels due to mercury impurities in the reagents, resulting in elevated detection limits. The efficiency of BrCl oxidation can be improved using an elevated temperature or ultraviolet (UV) photo-oxidation. [96, 109] BrCl oxidation has also been applied in online mode, using a flow injection analysis system (FIAS). [67, 110, 111]

As an alternative to chemical oxidation, a "reagent-free method" using UV irradiation to decompose organic matter and nano-gold collectors to pre-concentrate mercury species has been successfully applied to the determination of ultra-trace mercury concentrations in natural water samples. [112, 113] Although this method has significant advantages, such as the avoidance of harmful chemicals, it may be inadequate for freshwater samples containing high amounts of organic matter.

1.4.3 Determination of methyl mercury using GC-ICP-MS

The most commonly used instrumental technique in mercury speciation analysis is based on chromatographic separation, such as gas chromatography (GC) [114–116] or high-performance liquid chromatography (HPLC) [117–119], coupled to an element specific detector. However, in the case of HPLC-ICP-MS methods, the detection limits obtained are usually higher than when using GC-ICP-MS methods. [120] GC-ICP-MS coupling was first reported in the mid-80's, since when it has been increasingly applied to trace metal speciation. [121, 122] GC is connected to an ICP-

MS with a transfer-line which can be at the ambient temperature, partially heated or fully heated depending on the detected species. [123]

Prior to GC separation, ionic mercury species in an aqueous sample are converted into volatile derivatives. This is most commonly performed using an aqueous phase ethylation with sodium tetraethylborate ($NaBEt_4$). [124] However, propylation and phenylation have also been applied. [125, 126] When ultra-trace mercury speciation is performed, a pre-concentration technique, most commonly purge and trap, is used combined with an aqueous phase derivatization step. In the case of the purge and trap technique, mercury derivatives are purged from the aqueous sample solution with an inert gas and trapped in an adsorbent material (e.g. Tenax). [127] Volatile mercury species are released from the trap by heating and are introduced to the GC column for subsequent separation. Separated mercury species are detected using ICP-MS and identified based on their retention times.

When ICP-MS is used in speciation analyses, a species-specific isotope dilution (SSID) method can be applied to the quantification and correction of incomplete analyte recoveries. [128–130] SSID also enables the investigation and correction of possible species transformations during the analytical procedure. [131, 132] Furthermore, SSID can be used to study environmental methylation processes e.g. to understand which factors in the ecosystem control the transformation of inorganic mercury species into the more toxic MeHg. [133, 134]

1.4.4 Isolation of methyl mercury from environmental samples

The determination of MeHg in soil and sediment samples using a purge and trap GC-ICP-MS requires the isolation of MeHg from the sample matrix prior to instrumental determination. Isolation of MeHg is also required in the case of organic-rich freshwaters, since the MeHg complexed by organic matter may not be reactive in the derivatization phase leading to incomplete recovery. In addition, high amounts of other matrix constituents, such as sulfide and chloride, may interfere with the derivatization step, subsequently decreasing the method's sensitivity. [135, 136] If the water sample matrix does not excessively interfere with the derivatization step, the SSID method may be used to correct incomplete recoveries during the analysis. [128]

The most widely used methods of isolating MeHg from both water and soil/sediment samples are nitrogen (N_2) -assisted distillation and acid treatment with solvent extraction followed by back-extraction into water. [75, 98, 137–140] In the case of solid samples, acid treatment with microwave and sonic extraction techniques have also been used for the isolation of MeHg. [141–144] Although, many studies

have been performed on the isolation of MeHg from environmental solid samples, but few of them deal with soil samples.

One of the major concerns in MeHg analysis is the methylation of inorganic mercury during isolation [145–149], derivatization [150, 151] and/or GC separation steps [152] leading to erroneous results. N_2-assisted distillation was previously the most commonly used method of MeHg isolation due to significant advantages such as high recoveries and the capability to eliminate interferences during the derivatization step. However, it was shown that N_2-distillation is more prone to artificial MeHg formation compared to other isolation methods. [148] Methylation particularly occurs when organic-rich environmental samples containing high amounts of inorganic mercury, such as sediments, are distilled. [145, 146] Solvent extraction method using acidic KBr/$CuSO_4$ and methylene chloride has proven to be less prone to artificial MeHg formation compared to N_2-distillation. [145, 153, 154]

It should be noted that the sediment reference materials used in many studies contain much higher amounts of inorganic mercury than those found in natural unpolluted freshwaters and soils. The possible error associated with artifact formation depends on the sample type and amount of inorganic mercury present in the samples. [155] N_2-distillation can therefore be considered an isolation method if the potential methylation of inorganic Hg does not affect the accuracy of the results. N_2-assisted distillation is also used for water samples in the case of the US-EPA standard method 1630. [75] The investigation of possible methylation during the analytical procedure is possible using the SSID-ICP-MS method, in which the sample is spiked with an isotopically-enriched inorganic mercury in order to reveal the possible formation of MeHg artifact. [129, 148, 150, 156]

2 Aims of the research

The main objective of this research was to develop reliable analytical methods for the determination of ultra-trace total mercury (THg) and methyl mercury (MeHg) concentrations in natural water and peat soil samples. The precision and accuracy of the analytical results were improved by applying internal standardization and isotope dilution techniques as part of the developed method. Using the methods developed in this study, important information can be obtained on the mercury biogeochemical cycle and accumulation in the environment. The key aims of this research were:

1. To develop and optimize a cold vapor ICP-MS method for the determination of ultra-trace THg concentrations in humic-rich natural water samples. [I]
2. To study the potential of a dual mode sample introduction system with internal standardization, in order to improve the accuracy and precision of results in the determination of ultra-trace THg concentrations by cold vapor ICP-MS. [II]
3. To develop a reliable method for the determination of ultra-trace MeHg concentrations in humic-rich natural water samples, using N_2-assisted distillation with isotope dilution and purge and trap preconcentration GC-ICP-MS. [III]
4. To compare distillation and solvent extraction methods for the determination of MeHg concentrations in peat soil samples. [IV]
5. To apply the developed methods to the investigation of the possible consequences of forest harvesting practices on mercury mobilization in boreal forest catchments. [V]

3 Materials and methods

3.1 Sample types, sampling and sample pre-treatment

The natural water and peat soil samples analyzed in this study were collected from eight drained peatland forest catchments located in Sotkamo, eastern Finland (Fig. 2). Six of the catchments were clear-cut either using conventional stem-only harvesting or whole-tree harvesting (tree tops, branches and stumps also removed). Two of the catchments were left unharvested to serve as control plots. More information on the sampling sites is given in the original paper V.

Natural water samples were collected from drainage ditches in each area, and in the case of whole-tree harvesting sites samples were also taken from the standing water pools formed after stump lifting. Samples for THg and MeHg analyses were collected in carefully cleaned 250 mL fluorinated polyethylene (FLPE) bottles, in accordance with the ultraclean sampling protocols. [157] MeHg samples were preserved in the field by adding 1 mL of concentrated HCl to the FLPE bottles prior to field sampling. Collected samples were transported to the Trace Element Laboratory (University of Oulu) and were handled under clean room conditions. All samples were stored at 4 °C in the dark prior to analyses. Field blanks (ultrapure water) were included in each sampling batch and treated similarly to the samples throughout the analytical procedures.

Natural water samples analyzed for dissolved organic carbon (DOC) were collected in 500 mL polyethylene bottles and transported to the laboratory of the Finnish Forest Research Institute (Vantaa unit). Samples were filtered using a glass filter (pore size < 1μm) prior to analyses.

Peat soil samples were taken from three sampling points in each catchment area. Samples were collected in double plastic zip-lock bags and the excess of air was removed before sealing the bags. In the lab, samples were handled in a glove-box filled with N_2. Samples for THg analyses were stored under cool (4 °C) conditions, while samples for MeHg analyses were kept in a freezer and thawed just prior to analysis. A more detailed description of the sampling and pre-treatment of peat soil samples is given in the original papers IV and V.

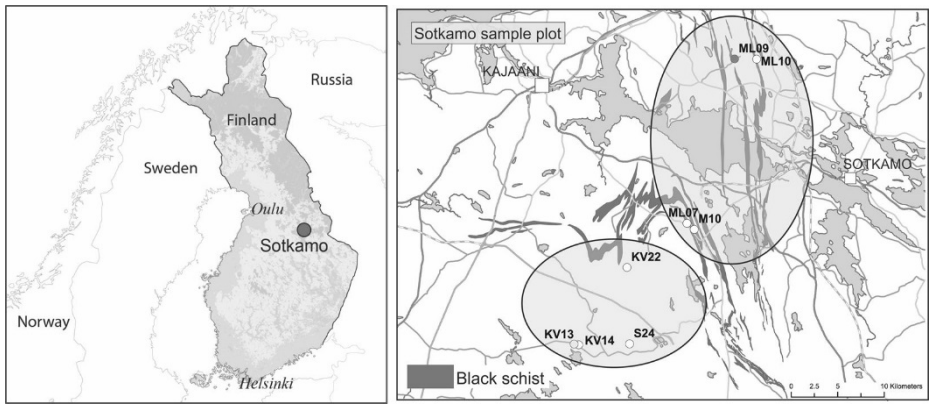

Fig. 2. Location of sampling sites in Sotkamo, Finland.

3.2 Sample preparation

3.2.1 Reagents and standards

All chemicals used in this study were of at least analytical grade, and reagents and standard solutions were prepared using ultrapure water (Millipore Gradient water purification system, Millipore Corp.). Working standard solutions of Hg^{2+} and CH_3Hg^+ with a natural isotopic composition were prepared daily through the appropriate dilution of $HgCl_2$ and CH_3HgCl stock solutions (1000 mg/L). Isotopically-enriched spike solutions ($^{201}Hg^{2+}$ and $CH_3{}^{201}Hg^+$) were prepared from stock solutions (10 µg/g) purchased from Applied Isotope Technologies (Sunnyvale, CA, USA).

3.2.2 Pretreatment of water samples prior to THg analyses

Mercury can be present in several chemical forms in natural water samples (organic and inorganic) and can also be associated with organic matter. Mercury compounds present in natural water samples were released and converted into inorganic mercury (Hg^{2+}) using an acidic BrCl solution. The samples used in this study were not filtered prior to the analyses. Due to the high concentrations of DOC present in the samples, the amount of BrCl was carefully optimized. 3 mM was found to be a sufficient BrCl concentration for natural water samples containing DOC > 70 mg/L. [I] An optimized

amount of BrCl reagent was added directly to the sampling bottles as soon as possible after field sampling. The BrCl was allowed to react at least overnight and any excess reagent was eliminated using hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) just prior to the analyses.

3.2.3 Isolation of MeHg from natural water and peat soil samples using N_2-assisted distillation

An N_2-assisted distillation method was used to separate MeHg in natural water and peat soil samples from the sample matrix. For this purpose, an in-house made distillation system, which enabled the simultaneous distillation of 20 samples, was used.

Distillation of natural water samples was performed by distilling a 50 g aliquot of the unfiltered sample at 145 ºC under an N_2 gas flow. The distillation was stopped when 70–90% of the distillate had been collected, which corresponded to a distillation time of 2–3 hours. Distilled samples were stored in the fridge until the analyses, which were usually performed on the next day.

When MeHg was isolated from peat soil samples, 2–4 g of wet peat soil was mixed with 20 mL of ultrapure water. Aliquots of H_2SO_4 and KCl reagents were added to the sample suspensions just before distillation and samples were transferred to the distillation vessels with ultrapure water (the final volume of sample was 60–80 mL). Samples were distilled until 20–30 mL of the distillate was collected within 1–3 hours. Collected distillates were diluted to around 50 mL with ultrapure water and stored in a fridge until analyses.

Distillation of organic-rich environmental samples can cause the formation of MeHg artifact from the inorganic mercury present in the sample. Thus, the potential methylation of inorganic mercury during the distillation of natural water and peat soil samples was studied using an enriched stable isotope tracer of inorganic mercury to reveal the possible formation of MeHg artifact (further discussed in section 3.4).

A more detailed description of the distillation system and the methods used are given in papers III and IV.

3.2.4 Isolation of MeHg from peat soil samples using solvent extraction

When using the solvent extraction method, a 5–10 g wet peat soil sample was weighted into a 50 mL centrifuge tube and leached with 20 mL of a

$CuSO_4/KBr/H_2SO_4$ solution by shaking the samples for 2 hours in a rotary shaker. After that, 10 mL of CH_2Cl_2 was added and the samples were shaken for an hour to extract MeHg into the organic phase. Samples were centrifuged for 30 min at 3000 rpm and a subsample of the organic layer was transferred into a clean centrifuge tube. 45 mL of ultrapure water was added and the organic solvent was removed by purging the sample with argon gas, leaving the extracted MeHg in the aqueous phase.

3.2.5 Digestion of peat soil samples for total mercury analysis

Prior to the THg analysis, wet peat soil samples were dried in an oven at 35 °C and homogenized by grinding. The samples were then digested in a microwave oven (MDS-2000, CEM Corporation) using a 500 mg sample mass and mixture of HNO_3 (5 mL) and H_2O_2 (3 mL). [158] These digested samples were diluted in ultrapure water and analyzed as soon as possible.

3.3 Instrumentation

Determination of THg in water samples was performed using a Thermo Elemental X7 quadrupole ICP-MS equipped with a CETAC HGX-200 cold vapor sample introduction system and CETAC ASX-500 autosampler. In CV system, mercury (Hg^{2+}) was reduced to volatile Hg^0 by a $SnCl_2$ solution and mercury vapor was transported into the ICP by an argon carrier gas. Data was collected by monitoring ^{200}Hg and ^{202}Hg isotopes at a dwell time of 50 ms per isotope. When internal standardization was used in Hg cold vapor analyses, ICP-MS was equipped with a unique dual mode X series sample introduction system, which enabled the simultaneous introduction of gaseous and liquid samples.

During speciation analysis, the ICP-MS instrument was equipped with a Tekmar LSC-2000 Purge and Trap concentrator and a HP 5890 gas chromatography. GC was connected to ICP-MS with a simple non-heated in-house-made interface (Fig. 3). Ionic mercury species in an aqueous sample solution were converted into volatile derivatives by ethylation and preconcentrated on a Tenax adsorbent. The maximum sample volume that could be introduced into the glass sparger of the purge and trap instrument was 20 mL. Ethylated Hg species (MeEtHg, Et_2Hg) were separated in a capillary GC column and identified according to their retention times (Fig. 4). GC-ICP-MS parameters were optimized in order to obtain a narrow and good shaped peak profile for ethylated MeHg, to enable accurate and precise isotope ratio measurements based on the peak area (further discussed in section 3.4). More detailed information

on the instrumental conditions and optimized measurement parameters used with each instrumental configuration are given in papers I–IV.

Total mercury concentrations in peat soil samples were determined using a cold vapor atomic fluorescence spectrometry (CV-AFS, Merlin PSA). DOC concentrations in water samples were determined according to the SFS-EN 1484:1997 standard, using a Shimadzu TOC-$V_{CPH/CPN}$ analyzer. [159]

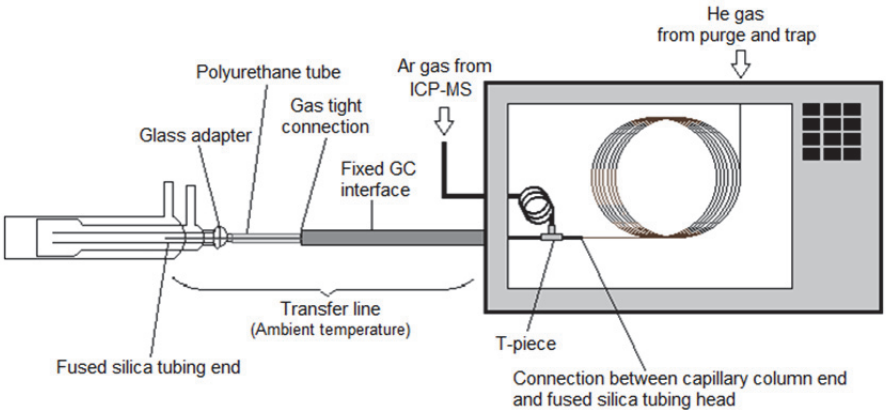

Fig. 3. The GC-ICP-MS coupling. [III]

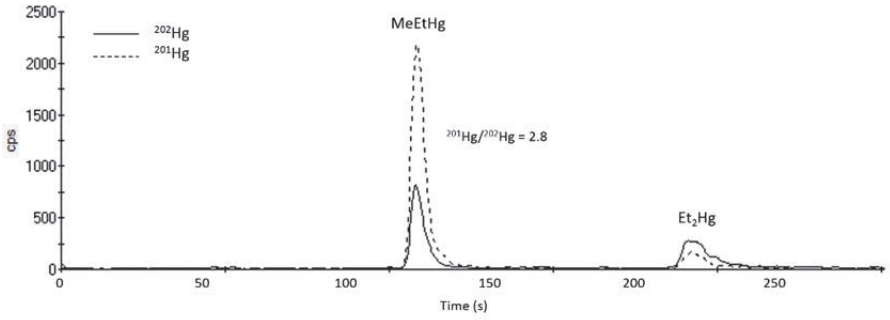

Fig. 4. A typical chromatogram for ethylated Hg species obtained with a purge and trap GC-ICP-MS after the distillation step. The MeHg concentration in a natural water sample spiked with $CH_3{}^{201}Hg^+$ was 0.82 ng/L.

3.4 Calibration methods

In most cases, quantitative results for THg with CV-ICP-MS were obtained by external calibration. Other calibration methods used with the CV-ICP-MS were based on isotope dilution (ID) and internal standardization. In the case of the ID method, a 10 g aliquot of BrCl-oxidized water sample was accurately weighted into a 15 mL polypropylene centrifuge tube and spiked with a known amount of $^{201}Hg^{2+}$ solution. After a few hours of equilibration time, the sample was measured using CV-ICP-MS to detect the $^{201}Hg/^{202}Hg$ ratio. When internal standardization was applied, aqueous internal standards (^{195}Pt, ^{205}Tl and ^{209}Bi) were used for drift correction in Hg cold vapor analysis with the dual mode sample introduction system.

Quantitative MeHg results in water and soil samples were obtained using a species-specific isotope dilution (SSID) technique which enables the correction of incomplete analyte recoveries during analysis. Using the SSID method, an accurately weighted amount of an isotopically enriched methylmercury solution ($CH_3{}^{201}Hg^+$) was added to a weighted amount of sample and allowed to equilibrate for about 20 hours prior to the isolation step. Following the isolation of MeHg, transient isotope signals were determined with the purge and trap GC-ICP-MS and $^{201}Hg/^{202}Hg$ ratios based on the peak areas were calculated using ThermoElement PlasmaLab software (Fig. 4).

When ID and SSID methods were used, the quantitative results were calculated using an isotope dilution equation:

$$c_s = c_{sp} \frac{m_{sp}}{m_s} \frac{M_s}{M_{sp}} \frac{A_{sp}}{A_s} \left(\frac{R_{sp} - R_m}{R_m - R_s} \right)$$

where R_m is the mass-biased $^{201}Hg/^{202}Hg$ ratio in the isotope-diluted sample, which is the only variable that needs to be measured. [160] In this equation, c_s and c_{sp} are the concentrations of Hg/MeHg in the sample and spike, m_s and m_{sp} are the masses of the sample and spike, M_s and M_{sp} are the atomic weights of Hg in the sample and spike, A_s and A_{sp} are the abundances of ^{202}Hg in the sample and spike, and R_s and R_{sp} are the $^{201}Hg/^{202}Hg$ ratios in the sample and spike.

When the potential methylation of inorganic Hg was studied, the sample was spiked with $^{201}Hg^{2+}$. The measured $^{201}Hg/^{202}Hg$ ratio of MeHg peak was compared to the natural ratio (0.44) in order to reveal the formation of MeHg artifact. [161]

3.5 Quality control

The reliability of the methods developed in this study was verified by performing replicate analyses of field samples, analyzing certified reference materials and using reference methods. Groundwater reference material, ERM-CA615, was used to estimate the accuracy of the CV-ICP-MS method. The certified Hg concentration with expanded uncertainty at a 95% confidence level in ERM-CA615 was 37 ± 4 ng/L.

Sediment reference material, ERM-CC580, was used to evaluate the accuracy of the solvent extraction and distillation methods in the determination of MeHg in peat soil samples. The certified MeHg and THg concentrations in ERM-CC580 with expanded uncertainty at a 95% confidence level were 75 ± 4 µg/kg and 132 ± 3 mg/kg, respectively. The amount of ERM-CC580 used in the MeHg analysis was 250 mg.

When the methods for the determination of THg and MeHg in natural water samples were developed, the reliability of the methods was studied by analyzing parallel samples with the reference methods in Brooks Rand Labs (BRL, Seattle, U.S.). The reference methods used were based on the US-EPA standards 1630 and 1631. [74, 75] Parallel samples were collected in separate bottles during field sampling and shipped to the BRL for analyses.

4 Results and discussion

4.1 Determination of THg in natural water samples [I]

CV-ICP-MS method was developed and optimized for the determination of ultra-trace THg concentrations in unfiltered humic-rich natural water samples. Prior to analyzing field samples, the most important instrumental parameters of the CV-ICP-MS system were carefully optimized using statistical experimental design software (Modde 7.0, Umetrics AB). The instrumental parameters assumed to have the most significant effects on the Hg response were the sample and reagent ($SnCl_2$) uptake rates, the carrier and additional gas flow rates of the CV system and the plasma radio frequency (RF) power. Optimization was performed using a 10 ng/L Hg standard solution and monitoring of ^{200}Hg and ^{202}Hg count rates.

A screening experiment with a full two-level factorial design (2^4) showed that the RF power and both gas flow rates were the most significant variables affecting the Hg response. Since the sum of both gas flow rates had a greater effect than the individual gas flow rates, the sum of both gas flow rates was used as a variable in further studies. More precise optimization of the plasma RF power and the gas flow rate (carrier + additional gas) was performed using a response surface methodology (Fig. 5). Optimal conditions for mercury determination were obtained based on an RF power of 1250 W and a gas flow rate of 0.86 L/min.

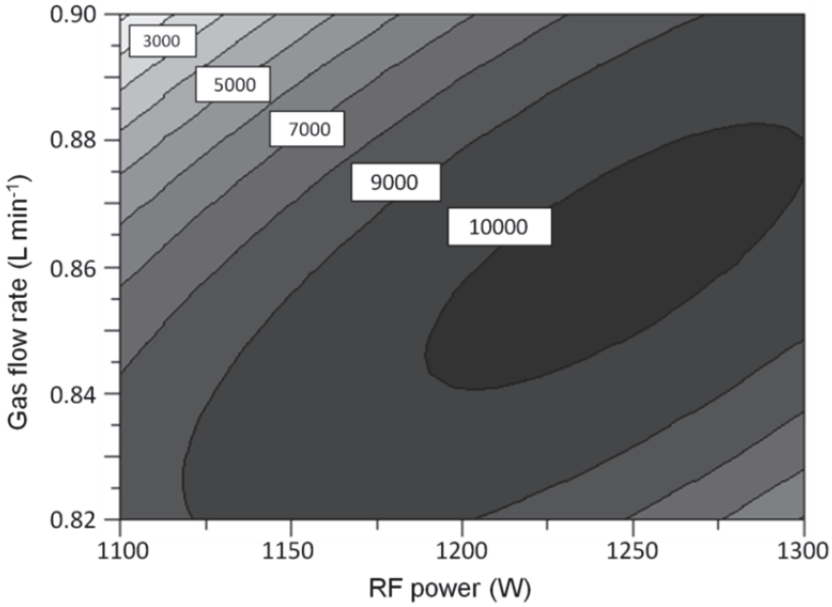

Fig. 5. Response contour plot for ^{200}Hg (10 ng/L). The model was fitted on the basis of the central composite (CCC) design. [I]

After the careful optimization of the instrumental parameters, the method was validated for the most common performance parameters, such as instrumental detection limit (IDL), method detection limit (MDL), precision and accuracy. IDL and MDL were calculated based on their correspondence to three times the standard deviation (3σ) of replicate blanks. The IDL obtained by analyzing the reagent blank solution (n = 11) on the same day was found to be 0.2 ng/L when using external calibration. A slightly lower IDL (0.09 ng/L) was achieved if the ID method was applied. The MDL was determined by analyzing an artificial peat water sample containing THg < 2 ng/L and DOC 15 mg/L. An artificial peat water sample was prepared to be similar to the water samples collected from peatland forest catchments and could be used to determine a more realistic detection limit than that obtained using a reagent blank. MDL determined using the artificial peat water sample as a blank was found to be 0.7 ng/L (n = 10). The detection limits obtained using the developed CV-ICP-MS method are similar to those reported in the US-EPA method 1631.

The accuracy and precision of the method were evaluated by analyzing ERM-CA615 reference material and performing replicate measurements of Hg standard

solution (10 ng/L). Replicate analyses of ERM-CA615 on different days gave a mean value of 39.5 ± 0.7 ng/L (n = 6), which is in a good agreement with the certified value (37 ± 4 ng/L). The precision (relative standard deviation, RSD) obtained for the replicate measurements of Hg standard solution was 2.6% (n = 6).

Finally, THg concentrations in 36 field samples were determined using the optimized CV-ICP-MS method and the results were compared to those obtained with the reference method. THg concentrations in these field samples varied from 3.3 to 43.1 ng/L (obtained with the CV-ICP-MS method) and DOC concentrations from 16 to 134 mg/L. THg concentrations obtained using the CV-ICP-MS method and the reference method (CV-AFS) were generally in good agreement with each other (Fig. 6). When the results for two samples (18.1; 3.7 and 9.4; 4.5 ng/L) were rejected as outliers, no statistically significant difference (p = 0.05) was observed between the results obtained using the two methods.

Based on the results obtained in this study, the developed and optimized CV-ICP-MS was shown to be a sensitive and reliable method for the determination of ultra-trace THg concentrations in unfiltered humic-rich natural water samples.

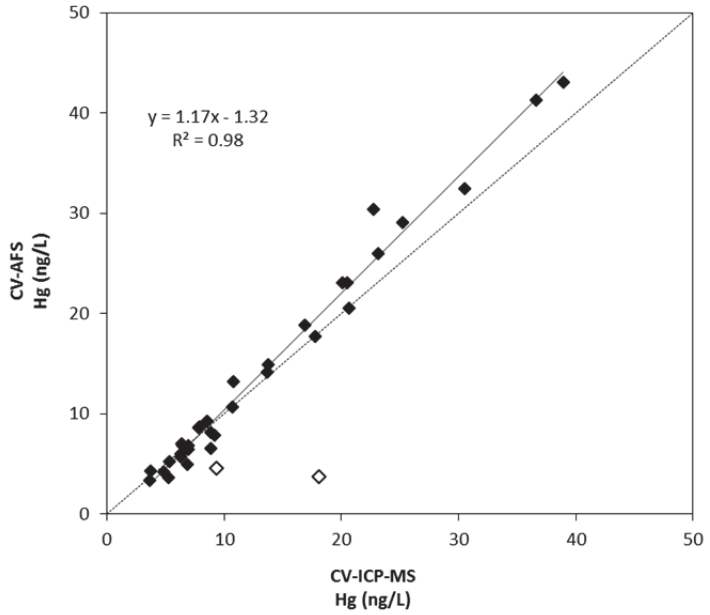

Fig. 6. The relationship between the THg results obtained using the developed CV-ICP-MS and the reference CV-AFS methods. [I] ◇ = result was excluded in the regression analysis as an outlier.

4.1.1 Internal standardization [II]

Although ICP-MS is a powerful detection technique, it is known to be susceptible to instrumental drift, which may affect its analytical performance. Signal instability can usually be corrected by using a carefully selected internal standard(s). However, with the CV-ICP-MS method it is not possible to use internal standardization in the common way since basically only mercury is vaporized by $SnCl_2$ and transported to the plasma. Thus, a dual mode sample introduction system for the simultaneous introduction of liquid sample and Hg vapor was studied in order to apply aqueous internal standards to drift correction in Hg cold vapor analysis.

Thallium (^{205}Tl) and bismuth (^{209}Bi) were used as internal standards for Hg, since the masses of these isotopes are very close to the mass of the most abundant Hg isotope (^{202}Hg). However, the first ionization potentials of Tl and Bi (6.11 and 7.29, respectively) are quite low compared to that of Hg (10.44 eV). Thus, platinum was also selected as an internal standard, since its first ionization potential (9.00 eV) and the mass of the most abundant isotope (^{195}Pt) are quite close to those of Hg.

The suitability of the selected internal standards (Pt, Tl and Bi) for drift correction in the cold vapor determination of mercury was studied by changing the plasma conditions to induce changes in the Hg intensity. Usually, the most important ICP-MS parameters affecting the analyte response are the plasma RF power, the nebulizer gas flow rate, and the sampling depth (distance from the sampling orifice to the end of the load coil). [99] However, with the dual mode sample introduction system, the nebulizer and cold vapor gas flow rates were dependent on each other and were therefore kept constant. Thus, the variables studied were the plasma RF power, auxiliary gas flow rate and sampling depth. The results showed that changes in the levels of these variables had a similar effect on both Hg and internal standard signals, indicating that ^{195}Pt, ^{205}Tl or ^{209}Bi can be considered a single internal standard for the correction of Hg signal instabilities during CV-ICP-MS measurements. The effect of plasma RF power on the ^{202}Hg, ^{195}Pt, ^{205}Tl and ^{209}Bi intensities is shown in Fig. 7.

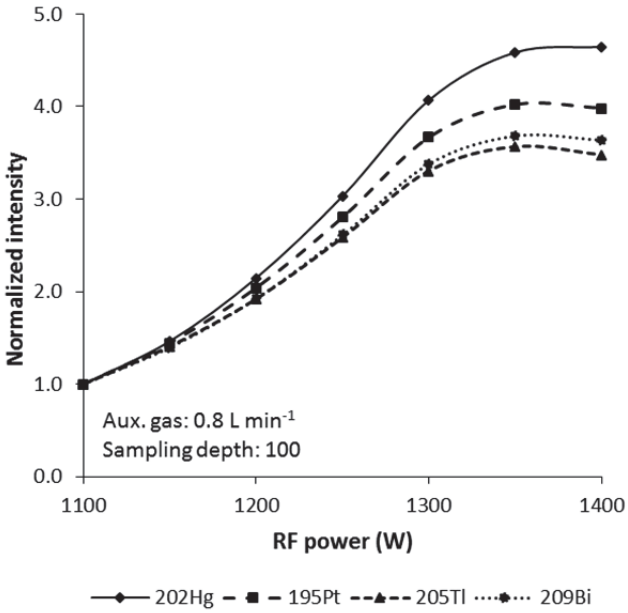

Fig. 7. The effect of plasma RF power on ^{202}Hg, ^{195}Pt, ^{205}Tl and ^{209}Bi signal intensities (normalized to the first measurement at 1100 W).

The use of internal standardization in the determination of ultra-trace THg concentrations in natural water samples was studied by analyzing 6 field samples. THg concentrations obtained with the internal standardization (IS) were compared to those obtained with the external calibration (EC) and isotope dilution (ID) methods (Table 2). The results obtained using the IS method agreed with the ID results (considered as a reference method), particularly when ^{205}Tl was used as an internal standard. However, the standard deviations of replicate measurements (n = 5) obtained with a single internal standard correction were somewhat higher than those obtained with EC and ID methods. Thus, no improvement in short-term precision was achieved when internal standardization was used. In addition, IDLs (3σ, n = 11) obtained using an internal standard correction (^{195}Pt, ^{205}Tl or ^{209}Bi) were slightly higher (0.4, 0.5 and 0.4 ng/L, respectively) than those obtained using EC and ID methods (0.2 and 0.09 ng/L, respectively). These observations are most probably due to decreased mercury sensitivity and the instability of liquid sample introduction when using the dual mode system.

Table 2. Results of THg concentrations in natural water samples (ng/L ± sd, *n* = 5)[a] obtained using a single internal standard correction, external calibration and isotope dilution method.

Sample	Internal standardization[b]			External calibration[c]	Isotope dilution[c]
	^{195}Pt	^{205}Tl	^{209}Bi		
Sample 1	2.4 ± 0.41	2.4 ± 0.37	2.4 ± 0.35	3.0 ± 0.24	2.4 ± 0.08
Sample 2	4.6 ± 0.40	4.2 ± 0.44	4.3 ± 0.21	5.1 ± 0.13	4.2 ± 0.17
Sample 3	4.1 ± 0.24	3.7 ± 0.24	4.1 ± 0.23	5.4 ± 0.13	3.7 ± 0.07
Sample 4	10.4 ± 0.43	9.6 ± 0.33	10.1 ± 0.27	9.6 ± 0.29	9.3 ± 0.14
Sample 5	2.0 ± 0.38	1.3 ± 0.38	2.0 ± 0.40	1.9 ± 0.11	1.3 ± 0.07
Sample 6	2.3 ± 0.24	1.8 ± 0.23	2.2 ± 0.18	2.0 ± 0.08	1.9 ± 0.04

[a] *n* = replicate measurements

[b] Measurements were performed using a dual mode sample introduction

[c] Measurements were performed using a conventional CV-ICP-MS

To study the effect of internal standardization on the long-term precision of CV-ICP-MS determination using dual mode sample introduction, a mercury standard solution (10 ng/L) was measured 5 times during a two-hour analysis period. In the case of external calibration, the RSD value of 5 measurements was 11% whereas for internal standardization using ^{195}Pt, ^{205}Tl or ^{209}Bi for correction, the RSD values were < 5%. Thus, some improvement in long-term precision was observed when the single internal standard correction was used.

By using internal standardization in mercury cold vapor analysis, the accuracy and long-term precision of measurements were slightly improved. However, since no major advantages were observed over conventional CV-ICP-MS with external calibration, the dual mode system was not applied as a routine method for the determination of THg in natural water samples.

4.2 Determination of MeHg in natural water samples [III]

A method for the determination of ultra-trace MeHg concentrations in humic-rich non-filtered natural water samples was developed next. MeHg was isolated from the water sample by N_2-assisted distillation and determined using purge and trap SSID-GC-ICP-MS. The potential methylation of inorganic Hg during the distillation of humic-rich natural water samples was studied based on three field samples containing different amounts of DOC (20, 35 and 50 mg/L). No significant formation of MeHg artifact was observed when the field samples were distilled (Fig. 8, measured $^{201}Hg/^{202}Hg$ ratio of 0.45 corresponds to the theoretical isotope ratio of 0.44),

indicating that the N_2-assisted distillation can be used to separate MeHg from the interfering sample matrix prior to instrumental determination.

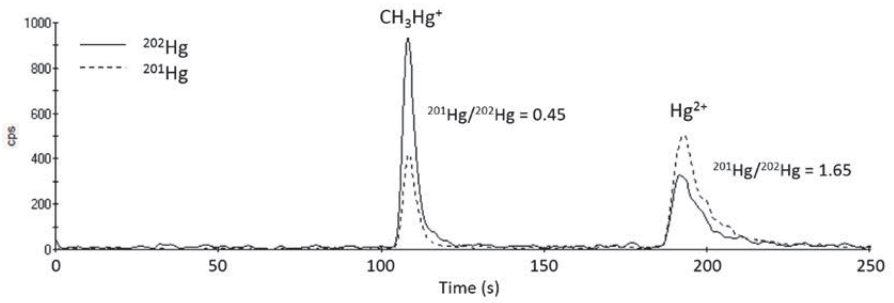

Fig. 8. Chromatogram of a natural water sample (DOC 35 mg/L) spiked with 10 ng/L of $^{201}Hg^{2+}$ prior to the distillation step.

The validity of the developed method for the determination of MeHg in natural water samples was studied by determining the MDL, precision and accuracy. The MDL was obtained by analyzing reagent blank on different days (n = 6) and was found to be 0.05 ng/L (3σ) for the 20 mL sample volume used in the instrumental determination. The MDL obtained is similar to those reported in the US-EPA method 1630.

The precision of the method was evaluated by analyzing six field samples containing different concentrations of MeHg (0.4 – 3.9 ng/L) with three replicates on the same day. The RSD values were found to be better than 10% for each sample, showing good repeatability of the method. The precision of the method was estimated by analyzing the same sample with two replicates on four different days. One way-analysis of variance showed that the between days variance (n = 4) did not differ significantly from the 'within day' variance (n = 2), indicating good reproducibility of the method.

The accuracy of the method was verified by using the developed SSID-GC-ICP-MS method to analyze natural water samples and by comparing the results with those obtained with a reference method (GC-CV-AFS).[75] The MeHg concentrations in the 26 field samples varied from 0.05 to 1.0 ng/L (obtained using the SSID-GC-ICP-MS method). The results obtained with the two methods were generally in good agreement with each other (Fig. 9), with no statistically significant difference being observed (n = 26, p = 0.05).

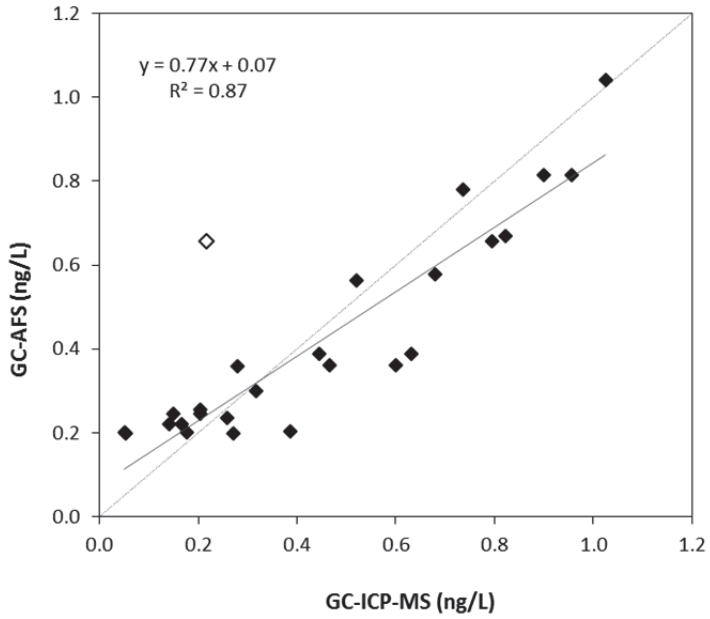

Fig. 9. The relationship between the MeHg results obtained for natural water samples with SSID-GC-ICP-MS and GC-CV-AFS methods. ◇ = result was excluded from the regression analysis as an outlier.

4.3 Determination of methyl mercury in peat soil samples [IV]

N_2-assisted distillation and the acidic $KBr/CuSO_4$ solvent extraction methods were applied to isolate MeHg from wet peat soil samples. Acidic solvent extraction has been recommended as an isolation method for MeHg by many authors and was therefore considered as a reference method for N_2-assisted distillation. Compared to solvent extraction, the distillation method used in this study was less time-consuming and not as labor-intensive, and did not require the use of a toxic organic solvent.

Although, compared to solvent extraction, N_2-assisted distillation is known to be more prone to artifact formation of MeHg, no significant methylation of inorganic Hg was observed when natural peat soil samples were distilled. When the MeHg concentrations – obtained using purge and trap SSID-GC-ICP-MS after the application of the two isolation methods – were compared, no significant difference was observed (p = 0.05) between the results (Table 3). Furthermore, the standard

deviation values for the replicate determinations were satisfactory, even though wet peat soil samples were used in the analyses.

Table 3. MeHg and THg concentrations (dry weight)[a] in peat soil samples. MeHg concentrations were obtained using purge and trap GC-ICP-MS after distillation and solvent extraction methods.

Sample	MeHg (µg/kg)		THg (µg/kg)
	Distillation	Solvent extraction	
Peat 1	0.8	0.9	69
Peat 2	3.4	3.6	94
Peat 3	2.2 ± 0.4 (n = 3)[b]	2.4 ± 0.2 (n = 2)	100
Peat 4	3.2 ± 0.3 (n = 3)	3.0 ± 0.3 (n = 3)	63
Peat 5	18.1 ± 3.3 (n = 3)	14.5 ± 1.6 (n = 3)	198
Peat 6	13.6 ± 1.9 (n = 3)	14.5 ± 1.6 (n = 3)	134
Peat 7	5.7 ± 1.3 (n = 3)	4.8 ± 0.7 (n = 3)	49

[a] Dry matter content varied from 15 to 20% of the total matter content.

[b] Standard deviation and number of replicates.

Compared to the solvent extraction method, fewer and smaller amounts of reagents are needed with the distillation method. A typical blank value obtained by N_2-assisted distillation was therefore approximately ten times lower than that obtained using solvent extraction. The MDL of the distillation with the purge and trap SSID-GC-ICP-MS method was found to be 0.02 µg/kg (n = 4) for a 20 mL sample volume used in instrumental determination. MeHg concentrations in wet peat soil samples analyzed during this study were usually >0.1 µg/kg.

The sediment reference material (ERM-CC580) was used to evaluate the accuracy of both isolation methods, since no proper certified reference material is available for low-level MeHg in soil. The MeHg concentration in ERM-CC580 obtained by solvent extraction was 76 ± 2 µg/kg (n = 3), which was very close to the certified value (75 ± 4 µg/kg). However, when N_2-assisted distillation was used as an isolation method, the MeHg concentration obtained was significantly higher (117 ± 9 µg/kg, n = 3) than the certified value. The most probable reason for the overestimation of MeHg concentration is the methylation of inorganic Hg present in the ERM-CC580 during the distillation step. It should be noted that the THg concentration in ERM-CC580 is extremely high, around 500 to 3000 times higher than that typically found in unpolluted peat soils. Furthermore, the ratio of MeHg/THg in ERM-CC580 is very low. Thus, although only a minor amount of

inorganic Hg (~0.03%) was methylated during the distillation step, the measured MeHg concentration was significantly biased.

Since some degree of artifact MeHg was formed during the distillation of the ERM-CC580, the potential methylation of inorganic Hg during the distillation of peat soil sample was studied by spiking the peat soil with increasing amounts of Hg^{2+}. The linear increase in the measured MeHg concentration as a function of THg concentration (ambient + added Hg^{2+}) after distillation was observed, revealing that methylation of inorganic Hg occurred during the distillation of a peat soil sample. (Fig. 10) The average methylation yield in the Hg^{2+} spiked peat soil sample was 0.08%, which was fairly similar to that of ERM-CC580. However, the proportion of MeHg of the THg concentration in natural unpolluted peat soil samples is typically over 1% and the observed methylation yield should not therefore affect the reliability of the MeHg results.

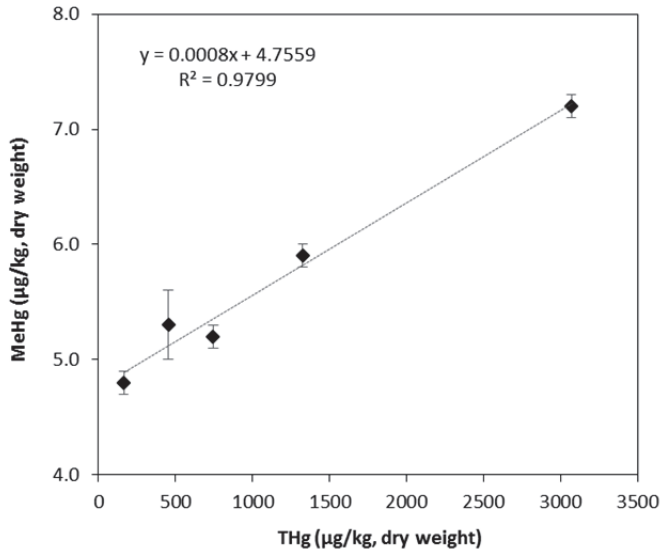

Fig. 10. MeHg concentrations (*n* = 2) for peat soil samples as a function of THg (ambient + added Hg^{2+}), obtained after distillation and SSID-GC-ICP-MS.

MeHg concentrations determined in 26 peat soil samples with SSID-GC-ICP-MS after distillation varied from 0.8 to 18 µg/kg (dry weight). THg concentrations in these samples varied from 35 to 235 µg/kg. Based on measured THg concentrations and the proportion of MeHg of the THg concentration found in the

analyzed samples (1.2–12%), distillation using SSID-GC-ICP-MS can be applied to the determination of trace MeHg concentrations in unpolluted peat soil samples.

4.4 Application of methods developed for investigating the mobilization of mercury in boreal forest catchments [I, IV, V]

The methods developed in the previous studies [I–IV] were applied to monitoring THg and MeHg concentrations in natural water and peat soil samples, in order to investigate the potential effects of forest harvesting practices on the leaching and mobilization of mercury in boreal forest ecosystems. [162] According to the current Finnish energy policy, whole-tree harvesting (WTH), which includes the harvesting of logging residues (tree tops, branches and sometimes stumps) should be favored in addition to the traditional stem-only harvesting (SOH) method, in order to meet bioenergy production targets. [163, 164] In Finland, 4.9 Mha of peatland forests have been drained for forestry purposes and much of the related harvesting will be performed within 10 to 30 years, providing a major source of bioenergy.

The real impacts of WTH on the mobilization of mercury and other heavy metals in drained peatland forests are largely unknown. The disturbance associated with WTH can perhaps be expected to be greater than in the case of SOH, resulting in increased leaching of mercury and other heavy metals into surface waters. An investigation has therefore been performed on the potential consequences of both SOH and WTH on mercury mobilization in drained peatland forest catchments underlain by black schist or felsic bedrock. Because mercury and other heavy metal concentrations may be higher in areas underlain by black schist, note was taken of the bedrock type when interpreting the results. [165]

In total, 41 THg and 37 MeHg ditch water samples were taken per catchment during April 2008–October 2012. Peat soil samples were collected twice in 2012 and once in 2013. More information on sampling sites and collected samples is given in paper V. Natural water samples were analyzed for MeHg and THg in BRL (Seattle, U.S.), using methods based on the US-EPA 1630 and 1631 standards. However, since 2011 THg concentrations in water samples have been determined using the CV-ICP-MS method developed in this study.[I] All results for MeHg in the water samples presented in paper V were obtained in BRL, because the SSID-GC-ICP-MS method developed was not routinely used for natural water samples until 2013. MeHg concentrations in peat soil samples were determined using the developed SSID-GC-ICP-MS, either based on solvent extraction (samples collected in 2012) or N_2-assisted

distillation (samples collected in 2013). The methods developed in previous studies [I–IV] can be applied to freshwater and soil samples of all kinds and have also been used in other environmental monitoring and risk assessment-related study.

THg concentrations in ditch water samples collected during the years 2008–2012 varied from 1.5 to 53 ng/L and MeHg concentrations from 0.05 to 23 ng/L. THg concentrations in peat soil samples varied from 35 to 242 µg/kg (dry weight), and MeHg concentrations from 0.5 to 19 µg/kg (dry weight). These observed concentrations were on a similar level to those reported in the other boreal catchment studies. [26, 65, 166, 167] THg and MeHg concentrations in ditch water and peat soil samples were generally higher at the sites underlain by black schist bedrock compared to those underlain by felsic bedrock. This observation is in accordance with the fact that the bedrock type has an effect on soil, stream and lake water quality. The positive correlation (r = 0.56, p < 0.05) between THg and DOC concentrations in water samples indicated that THg is mainly associated with organic matter in the studied freshwater systems. MeHg concentrations in ditch water were typically higher at the end of the summer and the abundance of sulfate reducing bacteria (SRB) was usually highest at sites with high MeHg concentrations, suggesting mercury methylation by SRB.

In general, annual THg and MeHg concentrations in ditch water were increased during the first year of harvesting (Fig. 11). However, clear increases in annual MeHg concentrations were seen at WTH sites only. THg concentrations continued to increase at both WTH and SOH sites throughout the second and third year after harvesting, and decreased after that. A randomized intervention analysis (RIA) was used to determine whether the difference in the mean values between the control and harvested plots was significant. [168] Although the annual average THg and MeHg concentrations in the ditch water increased one or two years after harvesting, the RIA analysis revealed that there was no significant difference in the THg or MeHg concentrations as a response to the forest harvest treatment (SOH or WTH), with the exception of sites KV22 and ML10. During the calibration year, 2008, THg and MeHg concentrations were determined only four times, which may have reduced the power of RIA analyses and subsequently caused low significances.

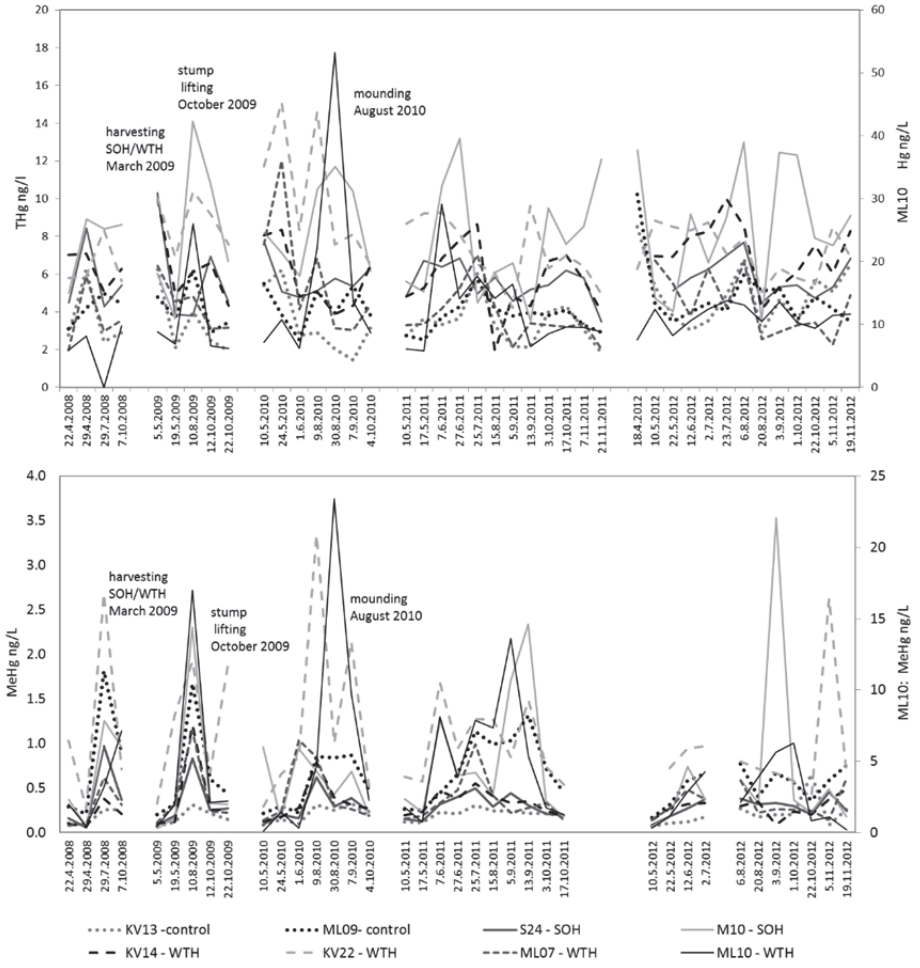

Fig. 11. THg and MeHg concentrations in ditch water during the years 2008–2012. [V]

Since an increase was evident in the annual THg and MeHg concentrations in ditch water samples after both harvesting methods, simple 'treatment effect ratios' were calculated in addition to the RIA analyses. These treatment effect ratios were determined by dividing THg and MeHg concentrations from the treated sites by the respective THg and MeHg concentrations from the control sites. It was assumed that, in the absence of a treatment effect, the treatment/control ratios would remain the same before and after harvesting. Based on the simple treatment

effect ratio calculation, THg and MeHg concentrations in ditch water increased regardless of the harvesting method used (WTH/SOH).

By monitoring THg and MeHg concentrations in ditch water samples during a 5-year period, the potential effects of forest harvesting practices on mercury mobilization could be evaluated. Although the RIA analysis gave only a weak indication of the harvest-induced mobilization of mercury, the 'treatment effect ratio' suggested that forest harvesting had a stronger effect on the THg and MeHg leaching from soil into recipient ditches. This also provided an indication that the effect of forest harvesting on mercury leaching was more pronounced at the sites underlain by black schist bedrock. When the effects of WTH and SOH on mercury leaching were compared, no clear difference could be discerned between the two harvesting methods, since the difference was almost significant only in the case of MeHg. However, higher MeHg concentrations at WTH sites than in the case of SOH constitute a slight indication that soil disturbance may create conditions which favor methylation. A more extensive and detailed discussion of the results related to this environmental monitoring study are given in paper V.

5 Conclusions

A CV-ICP-MS method was developed for the determination of ultra-trace THg concentrations in unfiltered humic-rich natural water samples. The instrumental parameters were carefully optimized and the amount of BrCl, used as an oxidizing agent prior to cold vapor analyses, was adjusted to be sufficient for water samples containing high amounts of DOC (> 70 mg/L). The IDL and MDL obtained with an optimized CV-ICP-MS based on an external calibration were found to be 0.2 ng/L and 0.7 ng/L, respectively. The CV-ICP-MS method showed good accuracy and precision and was successfully applied to determining ultra-trace THg concentrations in humic-rich natural water samples.

A dual mode sample introduction system was applied with the CV-ICP-MS method, in order to study the potential of internal standardization (^{195}Pt, ^{205}Tl and ^{209}Bi in aqueous solution) for drift correction in ^{202}Hg cold vapor measurements. When real natural water samples were analyzed, THg concentrations obtained based on a single internal standard correction were in agreement with those obtained using the isotope dilution method suggesting good accuracy. In addition, long-term precision was slightly improved when internal standardization was used for drift correction in CV-ICP-MS measurements. However, no improvement was observed in short-term precision or in IDLs when the results were compared to those obtained using the external calibration method. Since no major advantages were achieved over the external calibration method, the internal standardization based on dual mode sample introduction in CV-ICP-MS was not used as a routine method for ultra-trace THg determination.

Purge and trap GC-ICP-MS with SSID quantification was developed for the determination of ultra-trace MeHg concentrations in humic-rich natural water and wet peat soil samples. N_2-distillation was successfully applied to isolating MeHg from the water and soil matrix prior to instrumental determination, and no significant methylation of inorganic Hg was observed during the distillation step. The distillation method developed in this study is fast, easy to perform and has a high sample throughput capacity. In addition, since no toxic solvents are required the method is safe and environmentally friendly. When N_2-distillation with SSID-GC-ICP-MS was used for the analysis of water samples, an MDL of 0.05 ng/L was obtained and the method showed good accuracy and precision when analyzing real humic-rich natural water samples.

In the case of peat soil samples, the reliability of N_2-assisted distillation was evaluated by using solvent extraction as a reference method. When sediment

reference material containing a high amount of inorganic mercury was analyzed using both methods, the MeHg concentration obtained with solvent extraction was in good agreement with the certified value, whereas the result obtained using distillation was significantly higher. Overestimation of the MeHg concentration was caused by the methylation of inorganic mercury during the distillation step, which is an acknowledged problem associated with the N_2-assisted distillation method. Methylation of inorganic mercury was also observed when the peat soil sample was spiked with increasing amounts of inorganic Hg. However, the methylation yields observed for the certified reference material and spiked peat soil sample were very low (< 0.1%). Hence, in practice the slight artificial methylation of inorganic mercury has no effect on the reliability of the final MeHg result when unpolluted peat soils, such those used in this study, are analyzed. This was confirmed by the strong correlation between the results obtained for peat soil samples with N_2-assisted distillation and solvent extraction methods. In addition, the precision of replicate determinations was good even though wet peat soil samples were used in the analyses. Thus, N_2-assisted distillation with SSID-GC-ICP-MS can be considered a convenient method when MeHg is determined in unpolluted peat soil samples.

The analytical methods developed in this study were applied to the research project, whose aim was to investigate the consequences of forest harvesting practices (WTH and SOH) on the mobilization of mercury in boreal forest catchments. THg and MeHg concentrations were determined in water and peat soil samples collected from eight peatland forest catchments located in Sotkamo, Finland. The determined concentrations were on a similar level to those reported in other boreal catchment studies carried out in Finland, Sweden and Norway. Based on the results obtained during the 5-year monitoring period, a weak indication could be seen of the harvest-induced mobilization of mercury. In addition, the higher MeHg concentrations observed at WTH sites than SOH sites may be related to increased methylation caused by greater disturbance of the forest floor.

During this research work, sensitive and reliable analytical methods were developed for the determination of ultra-trace THg and MeHg concentrations in natural water and soil samples. These methods were successfully applied to determining THg and MeHg concentrations in real humic-rich natural water and peat soil samples. The methods developed in this study can be applied to natural freshwater and soils of all kinds and can also be used to study mercury methylation processes in order to achieve a better understanding of the biogeochemical cycle of mercury in the environment.

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I Pyhtilä H, Perämäki P, Piispanen J, Niemelä M, Suoranta T, Starr M, Nieminen T, Kantola M, Ukonmaanaho L (2012) Development and optimization of a method for detecting low mercury concentrations in humic-rich natural water samples using a CV-ICP-MS technique. Microchem. J. 103, 165–169

II Pyhtilä H, Niemelä M, Perämäki P, Piispanen J, Ukonmaanaho L (2013) The use of a dual mode sample introduction system for internal standardization in the determination of Hg at the ng L^{-1} level by cold vapor ICP-MS. Anal. Methods 5, 3082–3088

III Pietilä H, Perämäki P, Piispanen J, Majuri L, Starr M, Nieminen T, Kantola M, Ukonmaanaho L (2014) Determination of methyl mercury in humic-rich natural water samples using N_2-distillation with isotope dilution and on-line purge and trap GC-ICP-MS. Microchem. J. 112, 113–118

IV Pietilä H, Perämäki P, Piispanen J, Starr M, Nieminen T, Kantola M, Ukonmaanaho L. Determination of low methylmercury concentrations in peat soil samples by isotope dilution GC-ICP-MS using distillation and solvent extraction methods. *(Submitted manuscript)*

V Ukonmaanaho L, Starr M, Kantola M, Laurén A, Piispanen J, Pietilä H, Perämäki P, Merilä P, Fritze H, Tuomivirta T, Heikkinen J, Mäkinen J, Nieminen TM. Impacts of whole-tree harvesting vs. stem-only harvesting on the mobilization of Hg and MeHg in drained peatland forests. *(Manuscript)*

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