Mercury in sediments and its bioaccumulation in fish in a contaminated fjord and in background freshwater lakes

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Thesis for the Master's Degree in Chemistry

60 Study points

Department of Chemistry Faculty of Mathematics and Natural Sciences

UNIVERSITY OF OSLO 2021

Mercury in sediments and its bioaccumulation in fish in a contaminated fjord and in background freshwater lakes

2021

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http://www.duo.uio.no

Printed at Reprosentralen, University of Oslo

Abstract

Mercury in soil is transported with DNOM to surface waters and enters the nutrient chain as methyl mercury, a neurotoxin. In this study concentration of Hg in two matrices, sediment and fish, from Gunneklevfjorden, as a locally polluted fjord was measured, and the results were compared with samples from two reference lakes. Flåte and Svanstulvatnet were chosen as the reference lakes since they are located in the vicinity of Gunneklevfjorden and they do not have any known local inputs of mercury. Concentration of Hg in both sediment and fish samples from Gunneklevfjorden was significantly ($p<0.05$) higher than Hg level in samples from two reference water bodies.

Since the transport, fate and impact of Hg in the environment is governed by the flux and fate of DNOM the spatial distribution of organic matter in sediment in Gunneklevfjorden was assessed. Highest amount of organic matter was found in sediments from southwest shore of the fjord. This may be due to that this is a mixing zone where DNOM containing water from Skienselva is mixed with salt water, thereby causing a flocculation and precipitation of DNOM. Lowest amount of organic matter was found in sediments from the shallow part of the fjord, which can be due to resuspension of the lighter organic sediments by the tidal water flushing back and forth.

Physicochemical properties of the water, such as pH and DNOM content, were studied to assess if they could have any explanatory value on the uptake of Hg and other heavy metals in biota. DNOM concentrations in water from Gunneklevfjorden and Flåte were lower than water from Svanstulvatnet. In a dystrophic lake in the boreal forest like Svanstulvatnet, there is usually a strong positive correlation between the concentration of DNOM and Hg in water because of transport of Hg by the DNOM from catchment area to the lake. Thus, higher levels of Hg were found in the sediments at Svanstulvatnet than at Flåte. Under slightly reducing condition sulphur reducing bacteria methylate Hg and produce bioavailable MeHg. So concentrations of Hg were thus also higher in fish from Svanstulvatnet compared to Flåte.

Anthropogenic activities have caused heavy metal contamination in the environment. These elements can enter the aqueous ecosystems and be taken up by fish. Levels of heavy metals in fish from three water bodies showed that fish from Gunneklevfjorden have higher concentration of Pb and As, which is likely due to the local anthropogenic source that has contaminated sediments. On the other hand, concentration of several borderline metals such as Mn and Zn were lower in fish from this fjord compared to two reference lakes. Higher pH in

Gunneklevfjorden could result in more hydrolysis of these elements, causing their precipitation and thereby lower bioavailability of them for fish.

Preface

Working on the master thesis has been a wonderful and often challenging experience. First, I would like to thank my supervisor Rolf. D. Vogt for his continued encouragement, endless patience and invaluable support during the course of two years at the environmental chemistry department.

I would also like to thank Hans Fredrik Veiteberg Braaten from NIVA for the discussions and comments and Marianne Olsen for providing samples. I am grateful to Jon Petter Omvendt for his support and making the submission process convenient.

My special thanks goes to Susanne Jøntvedt Jørgensen for her valuable help regarding lab work and performing analysis. The DOM analysis was carried out at the department of Biology, and I am therefore thankful for Berit Kaasa for her great help and hospitality.

I would like to acknowledge the whole Environmental Chemistry group for their academic and technical support and not the least the interesting discussions.

Finally, I want to thank my husband for his encouragement, support and always-positive attitude. A special thought is devoted to my parents for their never-ending support.

Abbreviations

As	Arsenic
Ba	Barium
Co	Cobalt
Cu	Copper
DMA	Direct Hg Analyzer
DNOM	Dissolved Natural Organic Matter
DOM	Dissolved Organic Matter
GPS	Geographical Position System
Hg	Mercury
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
MeHg	Methyl Mercury
Mn	Manganese
MP-AES	Microwave Plasma-Atomic Emission Spectroscopy
Ni	Nickel
NIVA	Norwegian Institute for Water Research
OM	Organic Matter
Pb	Lead
RSD	Relative Standard Deviation
SAR	Specific Absorbance Ratio
Se	Selenium
Sr	Strantium
sUVa	Specific UV Absorbance
sVISa	Specific Visible Absorbance
THg	Total Mercury
UV-VIS	Ultraviolet and visible light

Table of Contents

Abstract 5

Preface 7

Abbreviations 8

1 Introduction 12

1.1 Background 12

1.2 Aim of the study 13

2 Theory 14

2.1 Mercury 14

2.2 Accumulation of Hg in biota 16

2.3 Hg and DOM 17

2.3.1 Characterization of DOM using spectroscopic techniques 19

2.4 Effect of nutrients on Hg uptake 20

2.5 Other heavy metals in fish 21

2.6 Monitoring Hg in the environment 23

2.7 Previous studies on Gunneklevfjorden 23

2.8 Potential sources of Hg in Gunneklevfjorden 25

3 Materials and methods 27

3.1 Sampling sites 27

3.2 Fish, sediment and water sample collection 32

3.3 Physicochemical properties of the sediment samples 34

3.3.1 Determination of pH 34

3.3.2 Determination of hygroscopic humidity 34

3.3.3 Determination of organic matter content 35

3.3.4 Determination of sediment texture by feel 36

3.4 Physicochemical properties of water samples 37

3.4.1 pH 37

3.4.2 Spectrophotometry 37

3.4.3 Major anions 38

3.4.4 Major cations 39

3.5 Hg analysis of fish and sediment samples by Direct Hg Analyzer 40

3.6 Microwave digestion of fish samples for heavy metal analysis 43
3.7 Selected metal analysis of fish samples using ICP-MS 43
3.7.1 Calibration of the ICP-MS instrument 44
3.7.2 Analysis 44
3.8 Students t-test 46
4 Results and discussion 48
4.1 Physicochemical properties of sediment samples 48
4.2 Physicochemical properties of water samples 51
4.3 Water major anions and cations 54
4.3.1 Major anions 54
4.3.2 Major cations 55
4.4 Mercury 56
4.4.1 Total Hg (THg) in sediment samples 56
4.4.2 THg in water samples 58
4.4.3 THg in fish muscle samples 60
4.5 Selected metal concentration in fish samples 63
4 Conclusion 66
References 68
Appendix A Sample locations 73
A.1 GPS coordinates for sediment sample locations 73
A.2 GPS coordinates for water samples 73
Appendix B Freeze drying of samples 73
Appendix C Physicochemical properties of the sediment samples 74
C.1 pH 74
C.2 Loss on ignition 75
C.3 Clay estimates 76
C.4 Dry matter content of sediment samples 77
C.5 OM in sediment samples 78
C.6 Flow chart used for estimation of clay content 79
Appendix D Physicochemical properties of water samples 80
D.1 UV-Vis absorption of water samples 80
D.2 Major anions measurements by IC 80
D.2.1 General procedure 80
D.2.2 Instrument set up 81

D.2.3 Dinox standard 82
D.2.4 Calibration graphs 82
D.3 Major cations measurements by MP-AES 85
D.3.1. General procedure 85
D.3.2. Instrument set up 86
D.3.3 Calibration graphs 86
Appendix E THg measurements 88
E.1 Concentration of standard solutions and calibration graphs 88
E.2. General procedure to measure Hg by DMA-80 90
E.3. Instrument set up 91
E.3.1 Cleaning boats 91
E.3.2 Calibration graphs 91
E.3.3 Fish samples 92
E.3.4 Sediment samples 93
E.4 Measured THg 94
E.4.1 Fish samples 94
E.4.2 Sediment samples 97
E.5 Recovery of reference material in THg measurements 97
E.5.1 Fish samples 97
E.5.2 Sediment samples 99
E.6 Calculated RSD of doublets in THg measurement 99
E.7. Reference materials 100
E.8. Comparison of measured THg in wet fish samples 102
Appendix F Microwave digestion of fish samples 105
F.1. General procedure 105
F.2 Microwave digestion reagents 106
F.3 Cleaning of microwave vessels 106
F.4 Digested fish samples 107
Appendix G ICP-MS 108
G.1 Setup 108
G.2 Isotopes measured 109
G.3 Calibration curves 110
G.4 Concentrations measured of reference material 110

1 Introduction

1.1 Background

Mercury (Hg) is a metal which has been used by humans since thousands of years ago and been presents in many commercial products such as paints, lamps, batteries, medical devices and many others, leading to its release into the environment upon disposal (Horowitz et al. 2014). Almost two hundred years of anthropogenic activities has led to a large amount of Hg deposited in the soil, even in areas that do not have any local emission sources (Fitzgerald et al. 1998). In addition to its anthropogenic sources, which are both local and global sources, Hg has many natural sources such as volcanoes and weathering of minerals (Selin 2009). Since the elemental form of this element has a long life in the atmosphere (about one year), it can be transported over long distances, making it a global environmental challenge (Driscoll et al. 2013).

The concentration of Hg is especially high in boreal environment. This is both because the boreal biome receive long distance transported Hg from warmer regions in the world and since Hg is a type B or soft metal it binds strongly to organic matter which is abundant in the boreal domain. A layer of Hg in forest floor soils and lake sediments has thus been formed over the years.

A number of policies and regulations have been established in order to reduce Hg emissions. Minamata convention on Hg, which was signed by 93 countries in 2013, is an important Hg emission control (UNEP 2013). The aim of this agreement is to protect human health and the environment from the adverse effects of mercury. This international agreement emphasizes on phasing out products containing Hg and prohibition of establishing new Hg mines.

Although the anthropogenic emissions of Hg has decreased due to the Minamata convention the large pool in the environment continues to leak Hg into the recipient water. Inorganic Hg can be converted to methyl mercury (MeHg) through bacterial processes (Ullrich et al. 2001). MeHg, which is a neurotoxin, bioaccumulates in aquatic food chains and affects human health through fish consumption (Mahaffey 2011). Even though the amount of MeHg in water might be low, the concentration in fish and other biota may be million times more. Fish from many Norwegian waters and watercourses have an elevated amount of Hg. This is mostly observed in predator fish species such as perch, which are at high trophic level of the freshwater food chain. A report (Braaten, Åkerblom, et al. 2017) shows that 20% of fish, from over one hundred lakes in Norway collected in the period from 1983 till 2015, exceeds the EU limit value for Hg

to protect human health which is 0.5 mg / kg (Commission 1995). Based on international concerns about the effect of Hg on health and environment the Minamata convention on Hg is an international treaty set up to protect human health and the environment from anthropogenic emissions and releases of Hg and Hg compounds (Kessler 2013).

Determination of levels of Hg in various environmental matrices such as water, sediment and biota can help us to find out more about the biogeochemical processes in the environment governing mobility, transport, fate and effect of Hg (Braaten, Lindholm, et al. 2020). This knowledge is a prerequisite for optimal abatement measures.

1.2 Aim of the study

In this study the levels of Hg in sediments and fish are measured in different types of waters which are located in the same region in order to estimate the effect of both long range atmospheric Hg and a local source of Hg. The freshwater lakes Flåte and Svanstulvatnet are chosen for estimation of loading from the long range transported atmospheric Hg, while Gunneklevfjorden is a brackish water body that has a local source of mercury. These measurements will aid in the selection of suitable abatement actions for the optimum effect of future planned remediation on the polluted seabed in Gunneklevfjorden.

In another part of this study, the amount of selected rare earth metals were measured in several fish samples from Gunneklevfjorden and two reference lakes.

The projects main objectives are:

- Compare Hg levels in fish and sediment from the locally polluted fjord with two reference lakes (Flåte and Svanstulvatnet).
- Map spatial distribution of Hg in sediments from Gunneklevfjorden.
- Map spatial distribution of organic matter in sediments from Gunneklevfjorden.
- Determine several rare earth metals levels in fish from Gunneklevfjorden and the two reference lakes.
- Assess if general water chemistry such as pH, DOM and nutrients can have any effects on Hg uptake and bioaccumulation of heavy metals in biota.

2 Theory

2.1 Mercury

Mercury (Hg) is a globally distributed environmental pollutant as it is capable of being long-range transported over long distances (Pacyna 2020). This is because of its conversion between different forms (gaseous and liquid) due to its low melting and boiling points. Hg is also a toxic contaminant due to its participation in biological cycles through its uptake into the food chain (Gworek et al. 2020).

Hg is naturally present in earth's biogeochemical system, but thousands of years of anthropogenic activities, such as mining and coal combustion, has caused increased amount of this element in atmospheric, terrestrial and aquatic systems (Mason et al. 2002). Hg is found only as a trace metal in igneous rocks, while higher levels are found in sedimentary bedrocks, especially in mercuryferous belt. This belt highlights where the Hg mines cinnabar (HgS) were located. In the mercuryferous belt the coal and limestone sedimentary deposits are enriched in mercury. Burning of these deposits for production of energy and cement emit high levels of Hg into the atmosphere. Volcanoes are a natural major source of Hg which annually release tones of Hg into the atmosphere (Siegel et al. 1984). The main anthropogenic sources of Hg includes mining, extraction and burning of fossil fuels, emissions from non-ferrous metal production and cement production and finally waste from Hg containing products (Assessment 2019) .
Mercury rapidly converts between Hg^{0}, Hg^{2+}, MeHg and particulate forms as it circulates at the earth's surface. This element accumulates in cold areas where there is a lot of organic matter that it can bind to, mainly in boreal ecosystems (Johnson et al. 2008). Therefore, the level of Hg in the environment increases when we move further north. The reason is that when Hg deposits as Hg^{2+} into the soil some is reduced to elemental Hg Hg^{0} which is semivolatile. Depending on the temperature, some of this elemental Hg evaporates and may be long-range transported by convective air movement. In the atmosphere the Hg^{0} oxidizes to Hg^{2+}, which dissolves in water and deposits readily as wet deposition. In soil it becomes reduced again to elemental Hg. Where there is a warm climate and little organic matter the Hg^{0} may again evaporate into the atmosphere (Graydon et al. 2012). This cycle continues until it reaches area region that is cold or rich in organic matter that Hg can strongly bind to. This global distillation

with multiple deposition and evaporation cycles is often referred to as the “grasshopper effect” (Wania et al. 1996).

Mercury contamination in boreal freshwater systems is common due to the long-range transport from warmer regions. Some of these boreal freshwater ecosystems are also affected by point sources of mercury. Devoid of any point and local sources, atmospheric Hg reaches freshwater ecosystems either by direct deposition to lake surfaces or via runoff from watersheds. Hg is mainly leached from the watershed as Hg^{2+} and MeHg associated to dissolved natural organic matter (DOM). Hg is deposited on the lake surface with both dry and wet depositions as Hg^{2+}. While Part of Hg^{2+} will be reduced to semivolatile Hg^{0}, which may evaporate and go back into the atmosphere, a small portion is converted to toxic MeHg if there is DOM available as energy source for the methylating bacteria (Figure 1.1). Environments with slightly reducing conditions, such as wetlands and lake sediments, are important compartments where methylation of Hg occurs. In the aquatic environment, MeHg is readily bioconcentrated into the primary producers. Once in the food web the Hg is bound to the muscular tissue that is consumed by the next trophic level and thus becomes further biomagnified up the food chain. (Selin 2009)

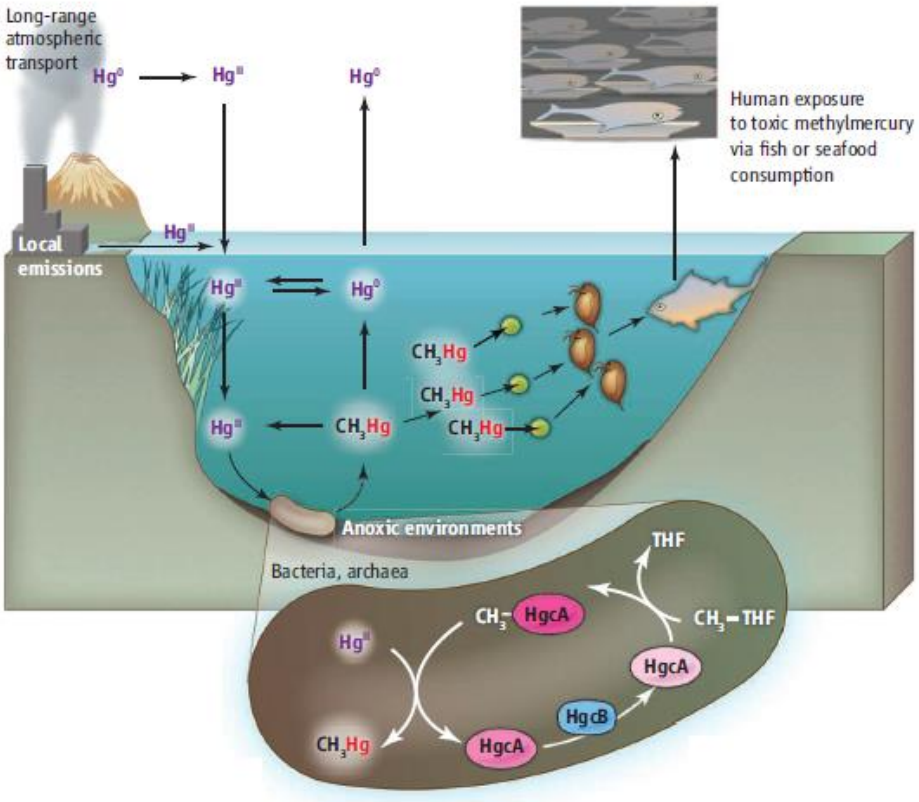

Figure 2.1. Geochemical cycle of Hg (Poulain et al. 2013).

2.2 Accumulation of Hg in biota

MeHg is a very important species of Hg as it is easily taken up into the aquatic food chain. Methylation process of Hg is mediated by sulfur- and iron reducing bacteria (Flemming et al. 2006). These bacteria need both organic matter and reducing conditions in order to produce MeHg (Compeau et al. 1985). Since MeHg bioaccumulates and biomagnifies in the food chain, the concentration of Hg in biota is strongly dependent on the trophic levels of the organisms in the food chain. The Hg also accumulates over the lifespan of the organism. We therefore expect to see higher Hg concentration in biota at higher trophic levels and in older individuals. This is why large predatory fish often have the highest Hg levels (Cabana et al. 1994). Also fish living in water bodies with different DOM levels, redox potential or pH will have different content of Hg due to the effect these governing factors have on the methylation of Hg (Chen et al. 2005). The Norwegian Food Safety Authority has thus issued dietary advice to avoid consuming large pike or perch over approx. 25 cm, trout over one kilo or char over one kilo. Pregnant, breastfeeding and young children under the age of five are warned against eating freshwater fish from self-catching (Matportalen.no).

Fish absorbs Hg through the body surface, gills and primarily diet (Hall et al. 1997). Hg that is taken up from the water through the gills and skin is mostly Hg^{2+} since that is the most abundant form of Hg in water and sediments. Fish through diet absorb the organic MeHg. All forms of Hg bioaccumulates in fish (Barwick et al. 2003) while MeHg also biomagnifies the trophic chain (Kehrig et al. 2010). Organic Hg usually dominates in muscles of fish, therefore the majority of studies on levels of this element in freshwater fish are focusing on fish muscles (Polak-Juszczak 2018).

Recent studies have shown a decrease in the level of Hg in boreal food webs in recent decades (Braaten, Lindholm, et al. 2020). This is attributed to long term extensive environmental changes such as reduction in sulfate deposition, surface water browning (i.e. increased DOM) and climate warming(Braaten, Lindholm, et al. 2020). Reduced sulfate deposition decreases the activity of sulfate reducing bacteria, thus the amount of MeHg in surface waters decreases. Under less acidified and low ionic strength conditions the charge density of DOM increases which in turn increases its ion binding affinity and complexation with MeHg. This makes MeHg less bioavailable to aquatic food chain.

Although the concentration of Hg in fish and ecosystems has decreased in recent decades, it is still above the limit set for protection of human health. The safe concentration of Hg in fish to use by human is 0.5 ppm (Commission 1995) and in ecosystems is 0.2 ppm (Directive 2003).

2.3 Hg and DOM

Dissolved organic matter (DOM) generally exists in high concentrations in boreal aquatic systems. The DOM interacts strongly with Hg and affects its mobility, solubility, toxicity and speciation. Its interaction with Hg is by specific chemical bonding to the sulfhydryl functional groups and by general electrostatic attraction forming. This strong absorption results in increased mobility of Hg from soils and sediments into aquatic systems (Wallschläger et al. 1996). In addition, DOM provides the energy source for methylating bacteria in sulfate limited environments and stimulate microbial growth (Ravichandran 2004).

In addition, DOM has an important role in photochemical reduction and reoxidation of Hg species. The photolytic reduction of Hg^{2+} to Hg^{0} by sunlight can either enhance or diminish in the presence of DOM (Luo et al. 2020). Both the chemical structure of DOM and the light wavelength affects the role of DOM in photo reduction of mercury. When DOM has weak

binding sites (such as carboxyl group), it promotes Hg reduction by forming labile Hg-DOM complexes and acts as a photosensitizer. On the other hand, in eutrophic water sources that contain higher amounts of DOM, less penetration of UV light into the water happens that inhibits the formation of reduced mercury. Besides strong binding sites in DOM forms more stable complexes with Hg^{2+}, which makes it more difficult to be reduced.

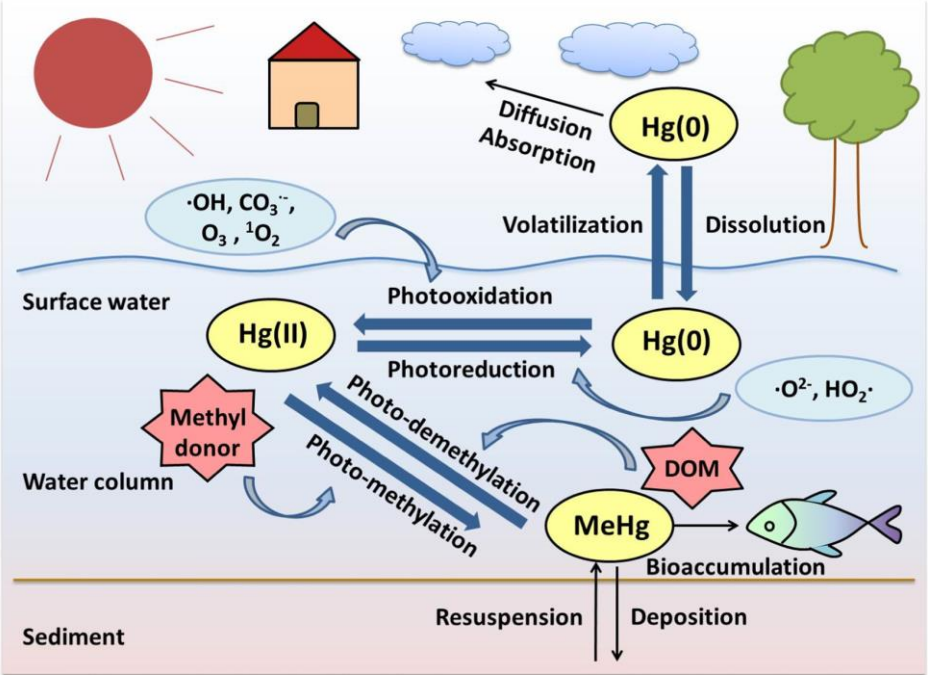

Figure 2.2. Photochemical transformations of Hg in natural environment.

The forest floors in Norway are rich in organic matter. In southern Norway the forest floor are pools of Hg due to accumulation of Hg through decades of long-range transportation by the grasshopper effect. Thus, they are considered as the sinks of mercury. In recent decades an increase in the levels and fluxes of DOM (i.e. Browning) in subarctic and boreal lakes has been observed (Braaten et al. 2014). In the past reduction in acid rain was one of the causes for the increased leaching of DOM into water. At present climate change and increased biomass is driving a continued increase Browning. Increase in DOM has also increased the flux of MeHg and Hg^{2+} from soil into the surface waters. In the lakes he Hg^{2+} can be methylated and form MeHg, while the MeHg can be photo-oxidized forming Hg^{2+}.

Differences in DOM quality is also suggested to affect MeHg bioavailability, for example humic fraction of estuarine wetland derived DOM is found to form MeHg complexes with low bioavailability (Schartup et al. 2015).

This is because DOM can both promote (Weber 1993) or inhibit (Barkay et al. 1997) the formation of toxic and bioaccumulative MeHg. MeHg can be produced in environments that are rich in DOM and have reducing or slightly reducing conditions. So high DNOM and low pe (reducing condition) in surface waters are considered to cause increased levels of Hg in invertebrates and fish (Chasar et al. 2009). However, DNOM can also have an antagonistic effect on MeHg production. DNOM is comprised of both low molecular weight compounds that can be easily used by methylating bacteria, and large refractory compounds. These large molecular weight compounds can bind Hg^{2+} and in this way, they can make it less available for methylation. Likewise, the more high molecular weight moieties of DOM can also bind the MeHg, rendering it less susceptible for bioconcentration since these macromolecules are too large to cross their cell membrane (Chakraborty et al. 2014). These more refractory moieties thereby serve to detoxifying the mercury. As a result, if the DOM present in water is generally of more low molecular weight quality the major process will be methylation, otherwise the detoxifying effect is the dominating mechanism (Braaten et al. 2018).

In addition, DOM mediate the reduction of Hg^{2+} to Hg^{0} specie. This would also reduce the bioavailability of Hg for methylation and subsequent biological uptake. The effect of DOM on Hg bioavailability has also something to do with the pH of the water column. At low pH, DOM is less negatively charged, and therefore less likely to complex mercury, making it more available to the methylating bacteria (Miskimmin et al. 1992) (Ravichandran 2004).

In summary, many factors affect the interaction of DOM and Hg that can either make Hg more or less bioavailable for aquatic food chain.

2.3.1 Characterization of DOM using spectroscopic techniques

DOM has a variety of functional groups, which give the molecule specific properties that are useful in characterization of DOM by spectroscopic techniques. The yellow-brown color of water containing DOM is due to conjugated double bonds in the structure of DOM. The absorbance of radiation by these chromophores can give us information about the structure of DOM.

Specific UV absorbance (sUVa), specific visible absorbance (sVISa) and specific absorbance ratio (SAR) are proxies to characterize the quality of DOM. These proxies are calculated based on UV and Vis absorptions of DOM containing water.

sUVa is the UV absorbency at 254 nm relative to the amount of dissolved organic carbon (DOC), measured in mg $L^{-}1$. This proxy is useful to describe the relative amount of aromatic moieties in DNOM (Weishaar et al. 2003). A high sUVa corresponds to more aromatic character, and thereby generally a higher molecular weight and higher hydrophobic moieties (Leenheer et al. 2003).

Specific visible absorbance (sVISa) is defined as the absorbance at 400 nm relative to the concentration of DOC. Absorption of radiation at higher wavelengths means that the molecules have longer chains of conjugated double bonds. sVISa thus generally reflects the relative amount of larger molecular size aromatic moieties in the DNOM.

Specific absorption ratio (SAR) is the UV absorbency at 254 nm relative to the visible absorbency at 400 nm. Thus, a higher SAR value corresponds to smaller size DOM, i.e. the absorbency is relatively more at lower wavelengths than at higher wavelengths reflecting shorter chains of conjugated double bonds.

2.4 Effect of nutrients on Hg uptake

Watras et al. (1995) reported maximum methylation rates in areas with maximum SO_4^{2-} reduction (Watras et al. 1995). Olsen et al. (2015) have also confirmed this theory. They found a strong negative correlation between MeHg and SO_4^{2-} concentrations together with a strong positive correlation between MeHg and S^{2-} which shows that sulfate reducing bacteria (SRB) are the main methylation reagents (Olsen 2016).

However, there are sometimes contradictory reports on the relationship between Hg uptake and total-N levels in water. Braaten et al. (2014) suggested that total-P concentrations have strong positive correlation with total-Hg in boreal lakes. This was confirmed by strong correlation between total-P and DOC and the subsequent positive link between total-Hg and DOC. They also mentioned that total-N concentration is positively correlated with concentration of MeHg. This may be due to that methylation is stimulated by N availability in boreal lakes since total-

N is an indicator of N availability. However, a negative correlation between nitrate concentration and MeHg production was found in water and sediments from a lake in North America (Todorova et al. 2009). The authors speculated that nitrate-reducing microorganisms will compete with SRB as electron acceptors in high nitrate concentrations and this will suppress the formation of MeHg. Also in another study done on marine environment, a negative correlation between nutrient loading especially N and the availability of Hg was found. (Driscoll et al. 2012).

A study focusing on methylation of Hg in boreal wetlands, showed that an intermediate levels of nutrients, C/N ratios in soil and nitrate in stream waters gives the highest MeHg production rates (Tjerngren et al. 2012).

Another nutrient that affects the bioavailabity of Hg in aquatic environment is selenium (Turner et al. 1983). It has been shown that eliminating selenium from aquatic environment leads to increased accumulation of Hg in fish. A possible explanation is formation of very low soluble HgSe ($K_{sp} = 10^{-58}$) by microbial community under anoxic conditions which easily precipitates and removes Hg from the water (Yang et al. 2008).

All in all the effect of nutrients on Hg uptake is not very well studies and more research needs to be done on this subject.

2.5 Other heavy metals in fish

The term heavy metal is usually used as synonym to toxic metals with density above 8 g/ml, which is a criticized term. In other words, not all heavy metals are particularly toxic, for example, Bi is a non-toxic heavy metal as well as Fe that is an essential element for living organisms. Besides there are lighter elements with density lower than 8 g/ml, which are toxic such as beryllium.

A better way to define and classify metals is by using their covalent index, which reflects their reactivity. In this way metals are classified as type A, B and borderline metals. Type B metals tend to form more stable complexes than type A, because they form covalent bonds with highly polarizable, big and low charged donor ligands. Therefore, they form stable complexes with organic carbon, sulfides, organo-sulfide, ligands containing nitrogen and functional groups on humus that act as a ligand (VanLoon et al. 2011). The heavy metals measured in this study that

belong to this group are Pb (II) and Hg. Type A or alkali metals usually bind ligands containing oxygen typically hydroxide, carbonate, sulfate and phosphate. Type A metals measured in this study are Sr and Ba.

Borderline metals in this classification show characteristics that are intermediated to type A and B. They can form complexes with all types of donor ligands (VanLoon et al. 2011). Borderline metal cations measured in this study are Cd^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+} and As^{2+}.

Heavy metals are naturally occurring elements, nevertheless years of anthropogenic activities has caused a wide distribution of heavy metal contamination in the environment. These elements can enter the marine ecosystems, some of them bioconcentrate into the food web and biomagnify in aquatic food chain. Consuming fish with high content of heavy metals by human has detrimental effects on digestive, cardiovascular and central nervous systems (Crespo-López et al. 2007).

Although some heavy metals such as Fe, Mn, Co, Zn and Ni are essential elements for organisms and participate in oxidation-reduction reactions in their body, the excess amounts of them can cause serious problems such as damage in tissues (Tchounwou et al. 2012). On the other hand, metals such as Cd, Pb and Hg have no biological functions and are toxic even in trace amounts (Inoue 2013).

Among heavy metals lead (Pb) is one of the highly toxic and bioaccumulative metals since it can easily bind to sulfur and oxygen atoms in proteins and forms stable complexes (Verstraeten et al. 2008). Lead can accumulate in various tissues of fish such as kidney, liver, spleen, intestine, and gills (Kim et al. 2015).

Cadmium is also very toxic to all living organisms even at very low concentrations. Cadmium accumulates in kidney, liver, and gills of freshwater fish due to binding to molecules called metallothioneins that are present in these organs (Chowdhury et al. 2004). Consumption of fish containing high levels of cadmium causes cancer, birth defects, and genetic problems in human (Levit 2010).

2.6 Monitoring Hg in the environment

Monitoring Hg in the environment can help us to achieve a good understanding of the biogeochemical processes in the watershed that govern the mobility, transport, fate and bioavailability of this toxic element. There are clearly higher concentrations of Hg in lakes which have both local Hg sources and long range transported atmospheric Hg compared to those which only have long range atmospheric deposition as their Hg source (Braaten, Åkerblom, et al. 2019). The Hg concentration has decreased between 1965 and 2015 in Norwegian lakes only exposed to long-range transported pollution and in lakes also receiving local Hg pollution. This is mainly due to policies that restrict the use of Hg in business or industry which resulted in emission reductions (Braaten, Gundersen, et al. 2019). However, also other environmental factors control the flux of Hg into surface waters. These explanatory factors can be uncovered by understanding the hydrological and biogeochemical processes that govern the mobility and transport of Hg in the environment. As an example increased amount and intensity of precipitation leads to increased transport of DOM directly from the forest floor to the surface water bodies. Since Hg in the environment is mainly transported by being bound to DOM this results in an increase in Hg concentration in water (Shanley et al. 2012). So measuring Hg in environmental compartments, such as water, sediment and biota, over different time intervals can aid us to have a better understanding of the biogeochemical processes and their explanatory parameters that govern the concentration of Hg in the environment. Consequently, we can have an overview of the efficiency of the abatement actions implemented with the aim of reduction in local Hg pollution.

2.7 Previous studies on Gunneklevfjorden

Gunneklevfjorden is a small (0.7 km^2), shallow (max. depth 11 m), and brackish fjord located in southern Norway. It has been estimated that sediments in this fjord contain 20-30 tons of mercury, which mainly originates from emissions from Norsk Hydro's chlorine plant in Herøya between 1947 and 1987. (Braaten, Johnson, et al. 2019).

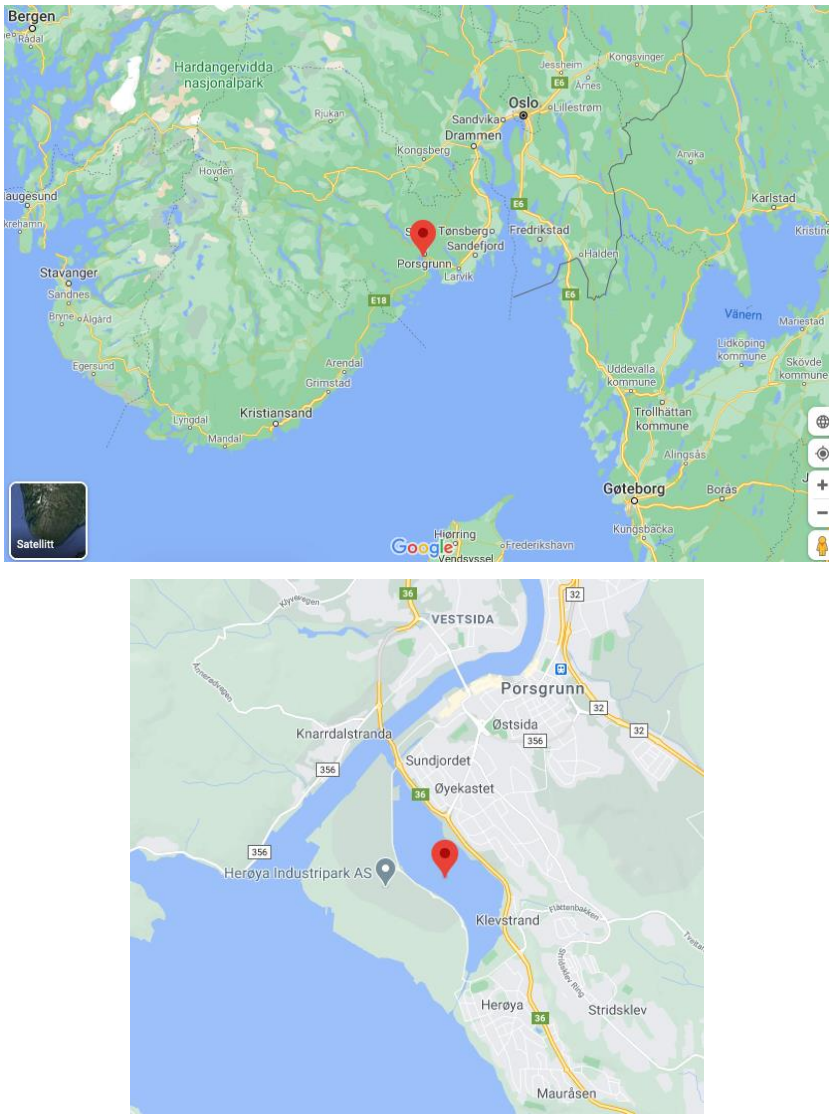

Figure 2.3. A map showing location of Gunneklevfjorden (taken from google map).

Investigations on Hg content in fish from Gunneklevfjorden were first done in 1989 (Berge et al. 1989) and showed high concentrations of mercury. Hg measurements on a significant collection of fish was carried out in 2013, with the main emphasis on perch (Olsen et al. 2015). They found that for perch that were between 22 and 29 cm long there was a significant decrease in the concentrations of Hg between 1989 (0.55 - 1.34 mg / kg) and 2013 (0.16 - 0.68 mg / kg).

In another study on Hg content in fish in 2016 it was showed that there were significantly higher levels of Hg in populations of perch in Gunneklevfjorden (0.56 ± 0.32 mg / kg) compared to two reference lakes, Flåte (0.18 ± 0.06 mg / kg) and Svanstulvatnet (0.25 ± 0.17 mg / kg) (Braaten, Olsen, et al. 2017). The reasons that these two lakes were chosen as reference lakes are as follows:

- These two freshwater lakes are located in the vicinity (<50 km) of Gunneklevfjorden.
- Previous data exist on measurements of Hg in fish (perch) in these lakes
- They do not have known local inputs of Hg, so the main source of Hg to these lakes is assumed to be long-transported atmospheric Hg
- They have different nutrient conditions, pH and concentrations of DOM, so the effect of these two factors on Hg levels in fish can also be investigated.

The higher levels of Hg in fish samples from Gunneklevfjorden compared to Flåte and Svanstulvatnet is due to higher Hg content of surface sediments in Gunneklevfjorden compared to two other lakes. The amount of Hg in surface sediment samples (0-5 cm) from Flåte (0.13 mg / kg) and Svanstulvatnet (0.06 mg / kg) reported in 2016 (Braaten, Olsen, et al. 2017) is in agreement with the Hg content in sediments from southern Norway that has long-range transported atmospheric Hg as their only Hg source (Rognerud et al. 2001). This shows that these two lakes are convenient choices of reference lakes in this study. The conclusion is while Flåte and Svanstulvatnet have long-range transported Hg as their main source of mercury, Gunneklevfjorden has both atmospheric and local Hg pollution.

2.8 Potential sources of Hg in Gunneklevfjorden

Gunneklevfjorden has been one of the major recipients for waste from different industries located in Herøya industrial park in Telemark, one of the biggest industrial parks in Norway. According to Hg mass balance investigation, the main source of Hg in Gunneklevfjorden are deduced to be (Olsen et al. 2015):

- Atmospheric input
- Storm water/run off from urban area
- Storm water from Herøya
- Cooling water from Yara
- Inflow from Skienselva and Frierfjorden
- Internal flux from sediment to water

Other potential sources that were not estimated in the mass balance model were groundwater flows from Herøya and airborne dust from Eramet Norway's production on Herøya of refined

manganese alloys. The mass balance model concludes that re-suspended sediment contributes significantly to the transport of Hg out of the fjord.

3 Materials and methods

3.1 Sampling sites

Norwegian Institute for Water Research (NIVA) has studied Gunneklevfjorden since 1970's and it is known as a fjord that is heavely polluted through several local pollution sources on Herøya (Skei 1978).

Figure 3.1. Gunneklevfjorden has been a recipient of waste from Herøya industrial park (photo from www.heroya-industripark.no).

Figure 3.2. Gunneklevfjorden located in vicinity of Herøya industrial park.

Samples were collected from Gunneklevfjorden as the main study point, as well as Flåte and Svanstulvatnet as two reference lakes. These two lakes located in the vicinity of Guuneklevfjorden have been previously studied and have no local inputs of Hg which makes them appropriate candidates as reference lakes.

Figure 3.3. Flåte as one of the reference lakes.

Figure 3.4. Svanstulvatnet as the second reference lakes (photo from www.runesturer.com).

The locations of the three studied water bodies as well as watershed for the two reference lakes are shown below.

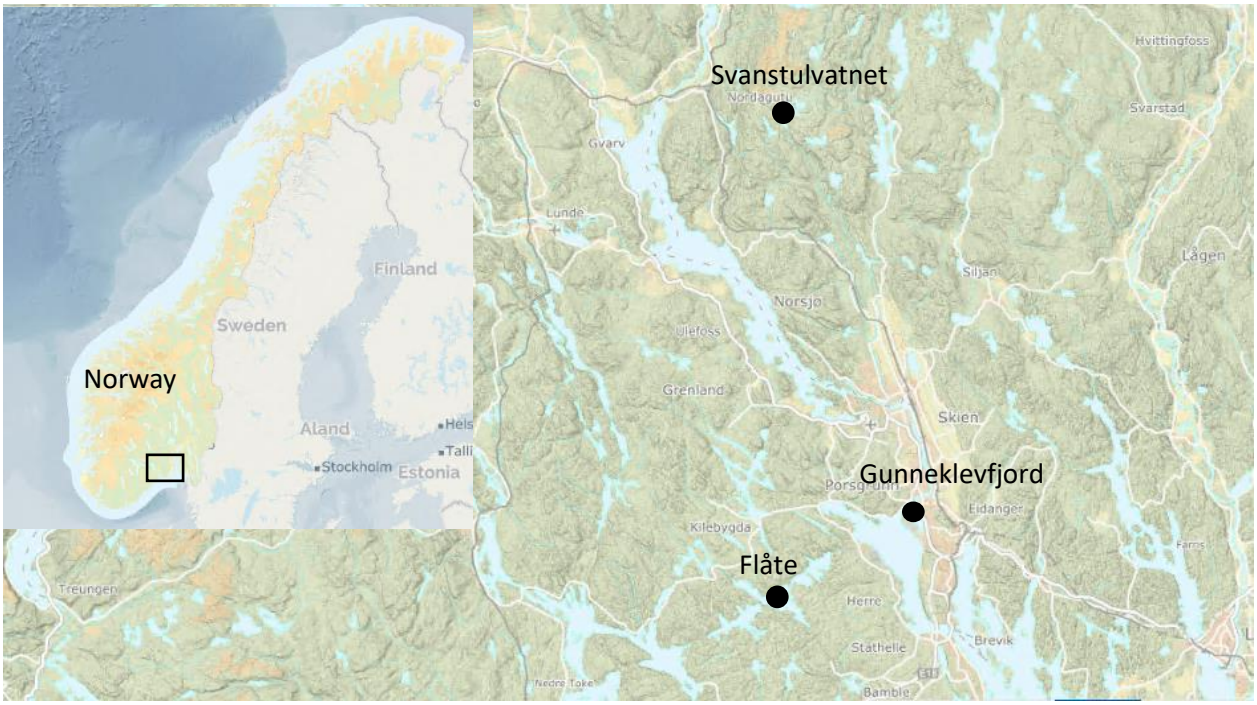

Figure 3.5. Location of the three studied lakes, Gunneklevfjorden, Flåte and Svanstulvatnet (source:kartverket.no).

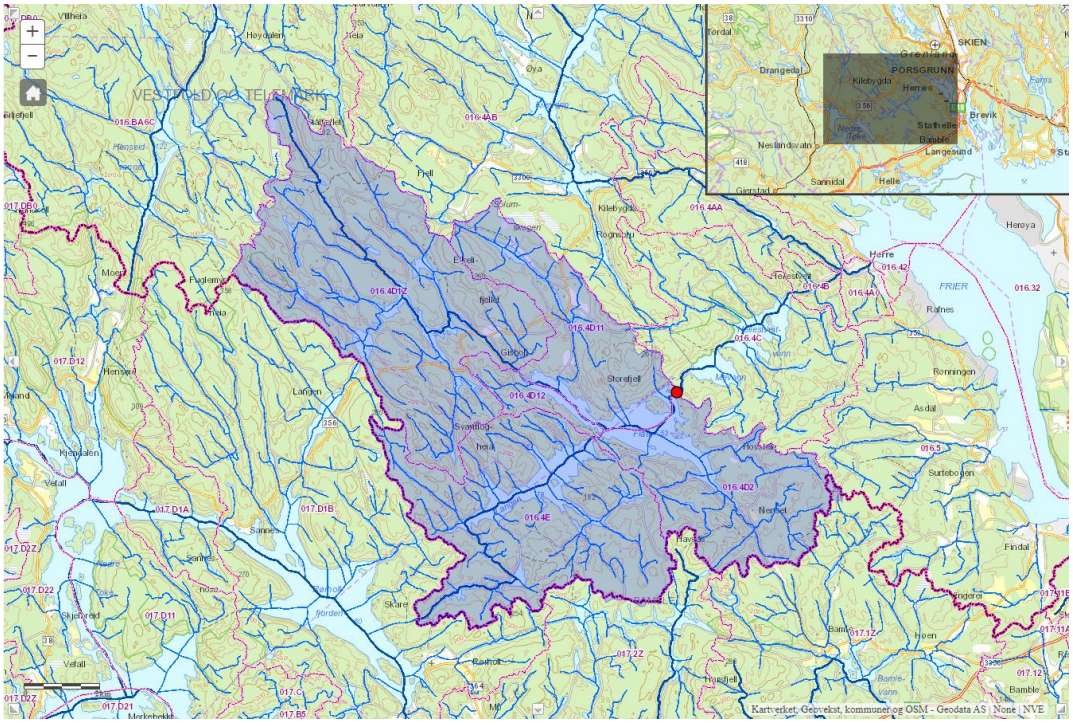

Figure 3.6. Flåte watershed (taken from http://nevina.nve.no).

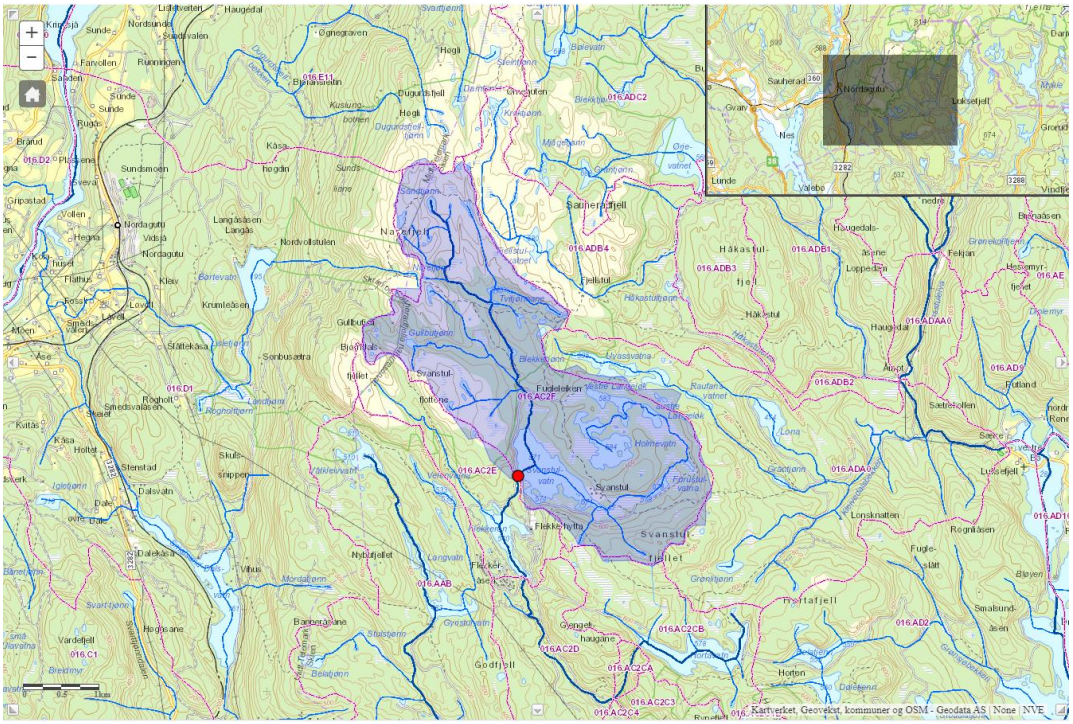

Figure 3.7. Svanstulvatnet watershed (taken from http://nevina.nve.no).

Gunneklevfjorden, Svanstulvatnet and Flåte are located in Porsgrunn, Skien and Bamble respectively. Selected characteristics of the three water bodies such as lake and catchment area, altitude, lake volum and medium lake depth are summarized in Table 3.1. Not all specifications are available or applicable for the three sites.

Table 3.1. Characteristics of the three studied sites.

Specification	unit	Gunneklevfjorden	Svanstulvatnet, Linddalselva	Flåte, Herreelva
NVE ID[1]	-	-	6467	110
Lake area	km^2	0.76	0.53	
Catchment area	km^2	-	10.52	97.99
Altitude	m a.s.l.	0	568	53
Lake volume	10^6 m^3	3.5	2.2	-
Medium lake depth	m	4.6	5.1	-
Runoff	10^6 m^3 yr^{-1}	-	10.5	53.2

– denotes not available or applicable.

[1] Norwegian Water Resources and Energy Directorate's water body ID

The physical properties of the watershed such as its geology affects the watershed water quality such as the quality and quantity of the anions and cations in water. The watershed bedrock in case of Flåte consists of Granite, Båndgneis and Granittic gneiss which all have very low weathering rate (Figure 3.8).

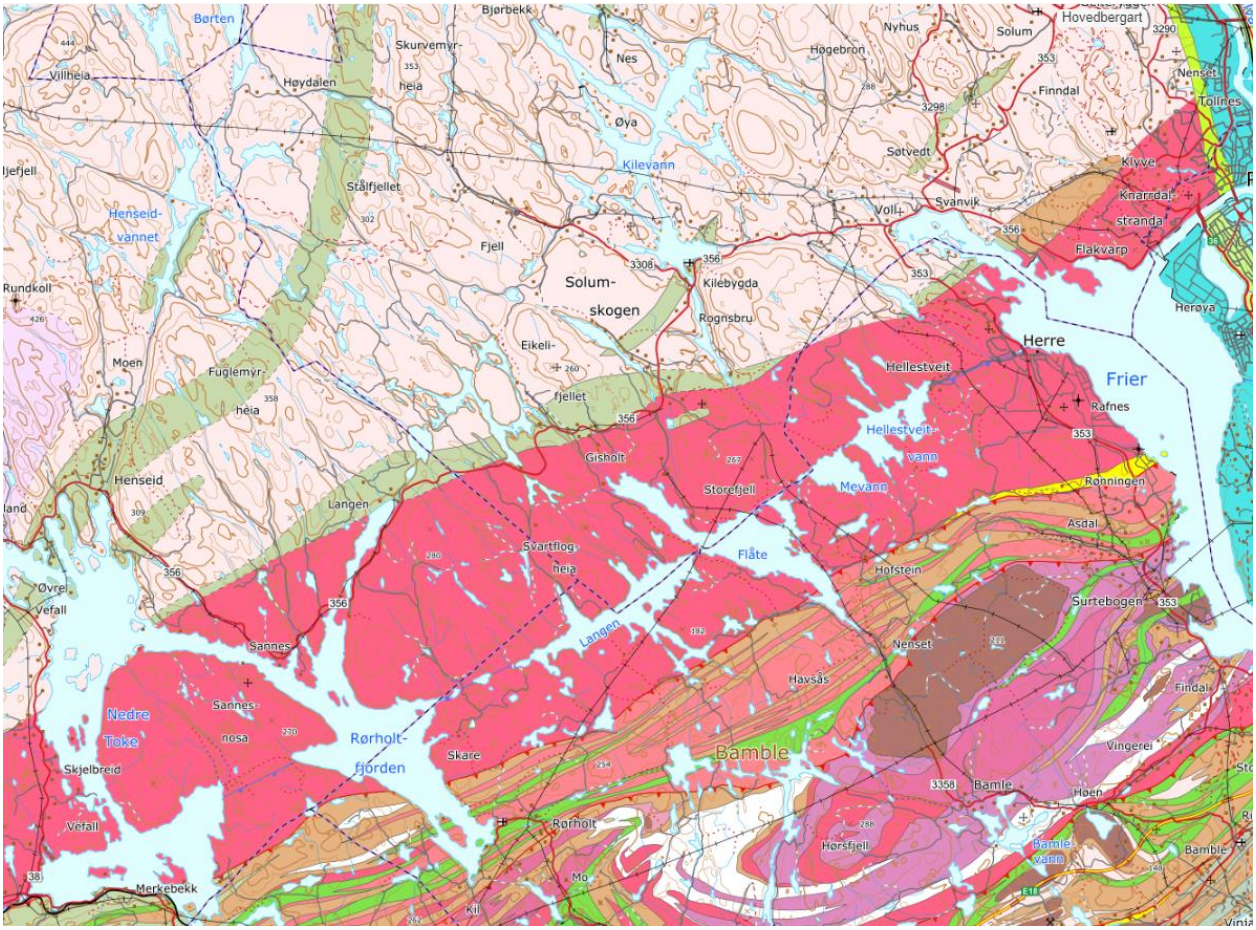

*Figure 3.8. Flåte watershed bedrock. Light red, green and light pink show Granite, Båndgneis and Granittic neiss respectively (*https://geo.ngu.no/kart/berggrunn_mobil/).

In case of Svanstulvatnet the watershed bedrock mostly consists of granite and larvikite which both are resistant to weathering and erode very slowly. Therefore, we expect to observe low concentrations of cation in water from this lake (Figure 3.9).

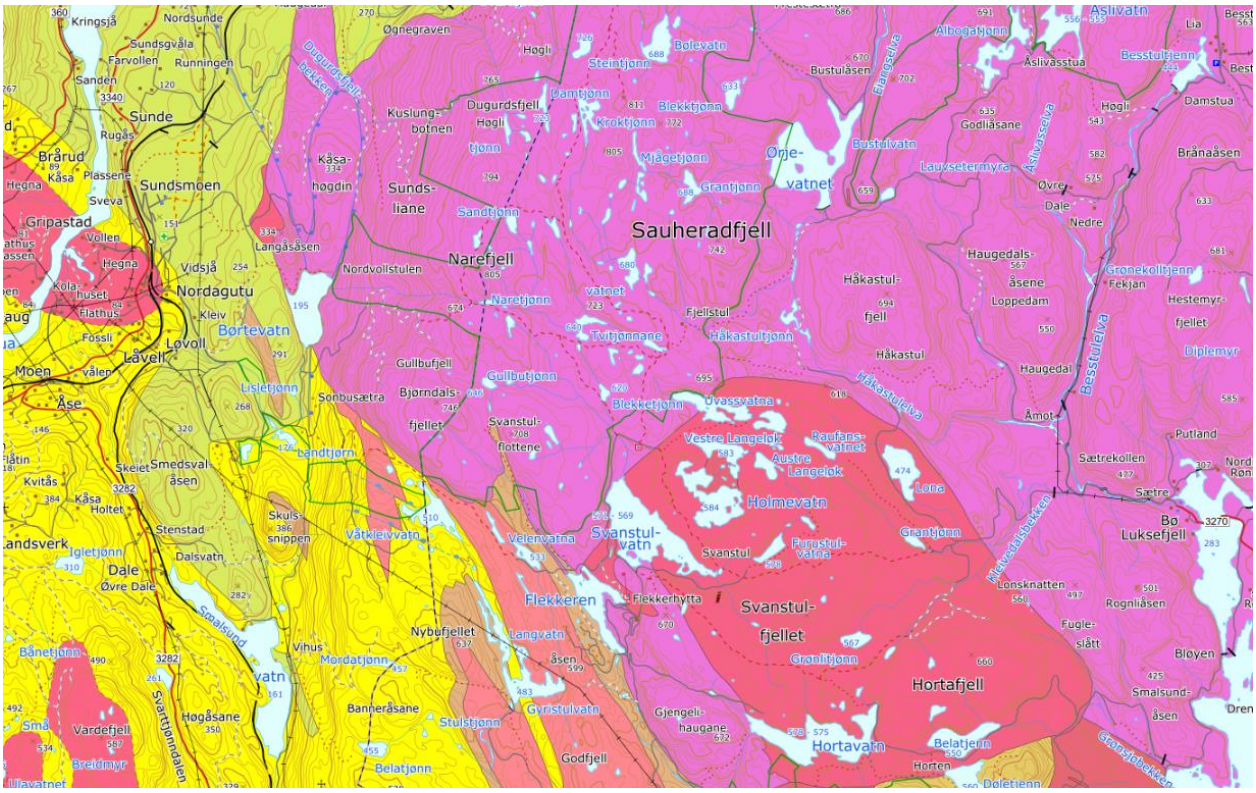

*Figure 3.9. Svanstulvatnet watershed bedrock. Pink and light red show Larvikite and Granite respectively (*https://geo.ngu.no/kart/berggrunn_mobil/).

3.2 Fish, sediment and water sample collection

Perch fish and sediment samples were collected from Gunneklevfjorden, Flåte and Svanstulvatnet. Sediment samples from Flåte and Gunneklevfjorden were collected in 2018 and sediments from Svanstulvetnet were collected in 2019. Fish samples from all three water bodies are from fall 2019 . The perch (Perca fluviatilis) was targeted because it is one of the fish species that often exceeds Norwegian threshold values for Hg content in freshwater fish. The reason for this is that this type of fish is located at the highest trophic levels in Norwegian lakes. The collection of fish was carried out by net fishing. Nets of different sizes were used to catch as wide age range of fish as possible. The fish muscle samples were freeze-dried and used for analysis. The freeze drying procedure is described in Appendix B.

Water samples are collected from Gunneklevfjorden and Flåte in April 2021. GPS coordinates for sediment and water sample locations are given in Appendix A. Locations of Gunneklevfjorden sediment samples are shown in Figure 3.10. Figure 3.11 shows the location of collected water samples in gunneklevfjrden and Flåte.

Figure 3.10. Samples G-1-4, G-5-6, G-7-8 and G-9-10 are collected from locations 12, 19, 23 and 2, that are referred to as Mixing zone, Deep mid-fjord, Deep fjord-shore and Shallow eater, respectively. Area shown with dark blue has depth more than 5 meters. (https://www.norgeskart.no).

Figure 3.11. Gunneklevfjorden and Flåte water sample locations.

3.3 Physicochemical properties of the sediment samples

3.3.1 Determination of pH

pH determination of sediment samples was carried out with type 1 water as the suspension agent in the laboratory. pH of the mixture was measured by Thermo Scientific OrionTM DualStar TM pH/ISE Dual Channel Benchtop Meter. The procedure was according to ISO10390-Determination of pH in a soil/water ratio of 1:5 (ISO 1994).

3.3.2 Determination of hygroscopic humidity

Sea and lake sediments are hygroscopic and can absorb water which will affect their weight. In order to have higher accuracy in sediment organic matter measurements, we should correct the weight of the samples based on their moisture content.

Determination of hygroscopic humidity was done according to ISO 11465 – Determination of dry matter and water content on a mass basis – Gravimetric method (ISO 1993).

The relative amount of water in air dried sediment samples wes determined gravimetrically based on loss of weigh after 32 hours heating at 110° C. The following equation was used to calculate hygroscopic humidity. These values were used to correct the measured organic matter content in air dried sediments.

$$\%\ Dry\ matter = \frac{m3 - m1}{m2} \times 100$$

where

m1 = weight of crucible

m2 = weight of sediment before drying

m3 = weight of crucible and sediment after drying

Equation 3.1. Determination of dry matter in sediment samples.

3.3.3 Determination of organic matter content

Organic matter content of sediments affects their ability to bind heavy metals such as mercury. On the other hand they can provide energy for methylating bacteria which accelerates the production of MeHg in aquatic environments.

Determination of organic matter was done by measuring loss on ignition after heating 3 grams of each sediment sample in 6 hours at 600 ° C . The following equation was used to calculate loss on ignition (Krogstad 1992).

$$\%\ Loss\ On\ Ignition = \frac{m3 - m4}{m3 - m1} \times 100$$

where

m1 = weight of crucible

m2 = weight of sediment before drying

m3 = weight of crucible and sediment after drying

m4 = weight of crucible and sediment after combustion

Equation 3.2. Determination of LOI in sediment samples.

The water bound to clay fraction of the sediment does not evaporate with heating at 110 ° C, as a result we need to correct the calculated OM for the clay content (Ekström 1926). The corrected OM values are presented in Appendix C.5.

3.3.4 Determination of sediment texture by feel

A simplified method was used to determine the soil texture. Based on this method the three building blocks of soil, sand, silt and clay feel very different and give different properties to soil (Ritchey et al. 2015). As a result, soil texture can be found by coherency and feel of the soil. After soil type determination, the clay content of soil can be estimated according to the following triangle (Figure 3.12).

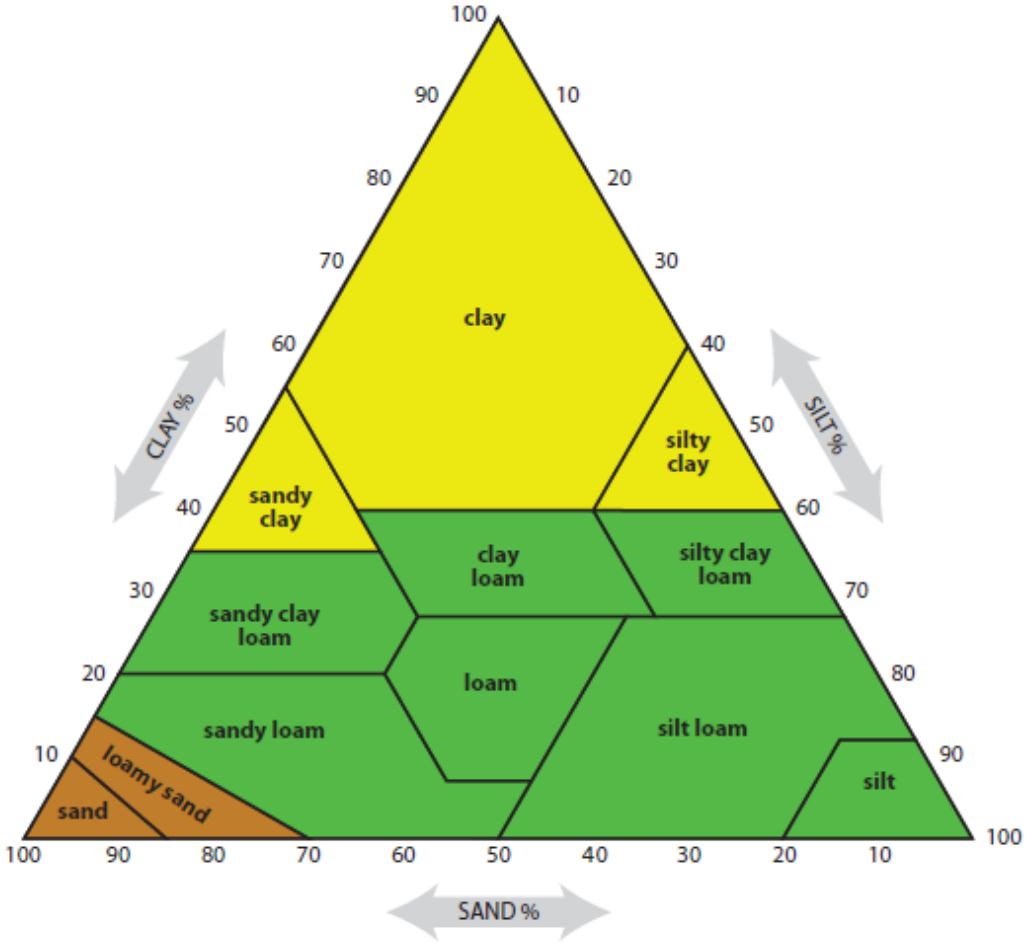

Figure 3.12. Soil texture triangle used for estimating the clay content of sediment samples (Ritchey et al. 2015).

We used the same method for sediment samples texture. A more detailed procedure to determine the soil or sediment texture has been mentioned in the appendix C.6.

3.4 Physicochemical properties of water samples

3.4.1 pH

The pH of the water samples were measured using a Thermo Scientific OrionTM DualStar TM pH/ISE Dual Channel Benchtop Meter with an OrionTM ROSS UltraTM pH electrode. This electrode is a combination electrode that combines the glass electrode and the reference electrode. pH is measured by the potential differences between the two electrodes. Prior to pH measurements, the pH meter was first calibrated with buffer solutions with pH 7.00 and 4.01.

3.4.2 Spectrophotometry

Absorbance spectra of water samples were measured using a Shimadzu UV-1800 UV-VIS spectrophotometer. This was done at wavelengths 200-800 nm with 1 cm quartz cuvettes. A cuvette containing Type 1 water was used as reference during the scan of the samples.

The resulting absorbance values at 254 and 400 nm, as well as DOC concentrations were used to calculate specific UV absorbance (SUVA), specific visible absorbance (SVISA) and specific absorption ratio (SAR). These values give us information about the structure and aromaticity of DNOM material. The following equations were used to calculate these spectrophotometric DNOM quality proxies.

$$SUVA = \frac{Abs\ (254\ nm)}{DOC} \times 100$$

$$SVISA = \frac{Abs\ (400\ nm)}{DOC} \times 100$$

$$SAR = \frac{Abs\ (254\ nm)}{Abs\ (400\ nm)} \times 100$$

Equations 3.3, 3.4 and 3.5. Determination of SUVA, SVISA and SAR.

A high SUVA corresponds to more aromatic character which shows higher molecular weight and higher hydrophobicity of DNOM.

3.4.3 Major anions

The concentration of major anions, fluoride (F^-), chloride (Cl^-), sulfate (SO_4^{2-}) and nitrate (NO_3^-) in the water samples were determined by ion chromatography, using a Thermo Fisher Scientific Dionex Integrion HPICTM instrument with a DionexTM AS-DV autosampler. For separation of the ions, a DionexTMAG18 guard column and AS18 separation column were used. The instrument was operated using the Thermo Fisher Scientific software Chromeleon 7.

The samples are injected into an eluent stream with bicarbonate which is pumped through the column, where the ions are separated based on their charge, radius and interaction with the ion exchange sites on the separation column. The stationary phase in the column is positively charged and is interact with the negatively charged anions. Stronger interaction results in a longer retention time. After moving through the column, the eluent and sample move through a suppressor (Figure 3.13). In the suppressor, all cations are exchanged with an equivalent amount of H^+. This converts the analytes into the form of their dissociated strong acids, while the bicarbonate in the eluent is fully protonated to carbonic acid. The high specific conductivity of the proton and no conductivity of the carbonic acid enhances sample detection and decreases background noise, respectively. The anions are identified based on their retention time and the chromatogram peak areas are compared to the peaks produced by the standard solution. The concentration of each anion is calculated based on its peak area in the chromatogram. Calibrations curves for each ion were created by preparing calibration solutions from a Dionex Seven Anion Standard solution.

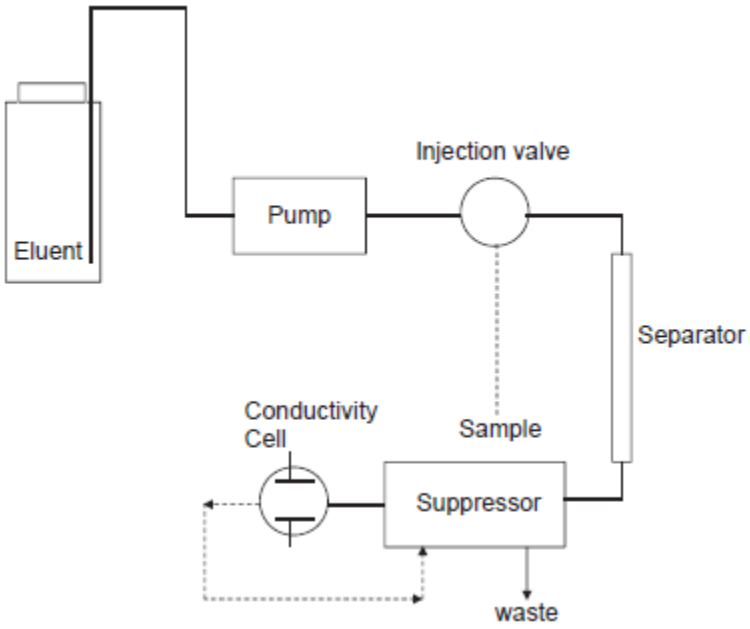

Figure 3.13. The instrument set up for a standard ion chromatograph (Srinivasan 2017).

When running the analysis of the water samples first standard solutions with 0, 0.5, 1, 5 and 10 mg/L concentration of each anion were prepared from Thermo Fisher Scientific Dionex Seven Anion standard solution and inserted at the beginning of the sequence. The samples were analyzed after the standard solutions. At the end of the sequence a solution with a known concentration of anions was added to test the accuracy and precision of the selected method. For chloride (Cl^-) and sulfate (SO_4^{2-}) measurements water sample from Gunneklevfjorden was diluted 100 times and reanalyzed.

3.4.4 Major cations

Agilent Microwave Plasma Atomic Emission Spectroscopy (MP-AES) was used to analyze the major cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) concentrations in water samples. The basic principle in MP-AES is that when an atom of a specific element is excited by an external energy source (i.e., microwave plasma (MP)), it emits radiation and forms an atomic emission spectrum (AES) while it returns to the ground state. In MP-AES the source for the elemental emission spectrum is thus the MP. Inside a MP-AES instrument, microwave energy is used to form a plasma from nitrogen gas. An aerosol from a liquid sample is created using a nebulizer and a spray chamber. This aerosol is then introduced into the center of the hot N_2 plasma. The sample aerosol dries, decomposes and is then atomized. The atoms in the sample are excited and emit light at

wavelengths characteristic for each element as they return to lower energy states. Emission of radiation from the plasma is directed into a fast scanning monochromator. The selected wavelength range is imaged onto the detector (Agilant 2016).

Prior to analysis, standards with increasing cation concentrations were prepared in order to create a calibration curve. For each element calibration solutions were prepared from certified standard solutions which contain 1000 ppm of the corresponding element.

For K^+ and Mg^{2+} standard solutions with 0 to 25 mg/L concentration, for Ca^{2+} standard solutions with 0 to 20 mg/L concentration and for Na^+ standard solutions with 0 to 500 µg/L concentration were prepared.

Calibration curves in addition to instrument settings are presented in Appendix D.3. all samples and standard solutions contained 2% nitric acid. After creating a calibration curve, a blank sample was analysed in order to make sre we have a clean system. The system was rinsed between every sample by placing the tube in Type I water and pumping water through the system for approximately 30 seconds A solution with known concentration of cations was added at the end of the sequence to ensure the accuracy and precision of the method.

3.5 Hg analysis of fish and sediment samples by Direct Hg Analyzer

THg content in fish and sediment samples were measured using a Direct Hg Analyzer (Milestone DMA-80, Sorisole, Italy). This analytical instrument decomposes the sample thermally under oxygen flow. Hg and other combustion products are then transported to a catalyst section by oxygen flow where Hg is reduced to elemental Hg^0 and trapped in a gold amalgamator. The trapped Hg is then subsequently thermally released into the measuring cells which are positioned along the optimal path of a fixed wavelength (253 nm) atomic absorption spectrophotometer (Figure 3.14).

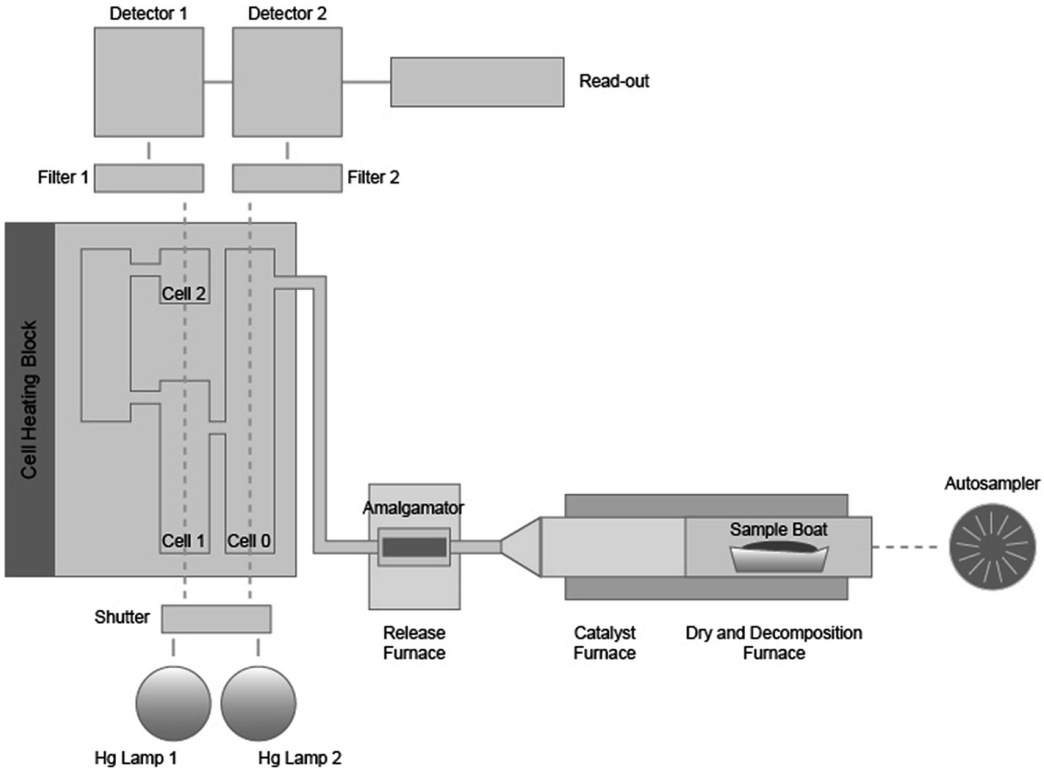

Figure 3.14. Schematic drawing of the DMA components (Windmöller et al. 2017).

Prior to the fish samples analysis, standard Hg solutions were prepared and analyzed to generate calibration curves. DMA has two separate cells to measure the Hg (one cell for low concentration samples and one for high concentration ones). Two calibration curves, for both low and high concentration standards, were thus prepared.

Standard solutions were prepared from certified Hg standard solution (Merck, 1000 ±3 µg/mL in 2.5% HNO_3). Both standard and blank solutions were prepared using 2% nitric acid solutions in order to maintain the solubility of Hg ion.

75 samples of freeze-dried fish muscle from Flåte, Svanstulvatnet and Gunneklevfjord were analyzed (25 samples from each lake). No further sample pre-treatment was done. A small portion of dry fish muscle (5-50 mg) was weighed in sample boats and analyzed directly. The output result of DMA-80 is the total amount of Hg found in the sample boat.

In order to ensure analytical quality in regards to both accuracy and precision the following analytical measures were taken:

- Calibration curves were prepared before analysis of fish samples.
- 2-3 blank samples were run at the beginning of each sequence to eliminate any Hg carryover in the instrument.
- Three Standard Reference Material (SRM) samples were analyzed following the blank samples.
- After every 10th sample the SRM sample was analyzed.
- 10% of samples were analyzed as duplicates.

The standard reference material that was used was DORM-4 fish protein (PROONRCDORM-4, VWR). The Relative Standard Deviation (RSD) of the duplicates was always less than 10% and the recovery of the reference material was between 90 and 110%, otherwise the analysis was repeated.

Since we expected very high concentrations of Hg in several sediment samples from Gunneklevfjorden which could be more than the maximum concentration measurable by DMA-80, we mixed the sediment samples with silica gel. This gave us a homogenoius mixture which was analyzed instead (Figure 3.15). The amount of silica in the mixtures was up to 40 times more than the amount of sediment. In order to verify that the used silica was not contaminated with Hg 2-3 silica samples were analyzed with DMA.

Figure 3.15. Mixture of silica and sediments from Gunneklevfjorden.

The same measurement quality assurance as fish samples analysis used in sediment analysis. The used certified reference material was estuarine sediment for trace metals analysis (BCR-277R, European Commission).

Measured THg in dry fish and sediment samples are presented in Appendix E.

3.6 Microwave digestion of fish samples for heavy metal analysis

Microwave digestion technique uses microwaves which are absorbed by molecules with dipole moments such as water. This kinetic energy causes rotational movements of the molecules and as a result the temperature in solution would increase. (Bye 2009)

Since heavy metals bound inside the silicate crytal lattice in sediments is not exposured by humans and most likely are not a result of anthropogenic activities, there was no need to dissolve the silicate minerals in sediment. So hydrofluoric acid was not used to decompose the samples, instead samples required filteration after digestion.

The fish samples and DORM-4 fish protein as the certified reference material were digested in a mixture of 7 mL HNO_3 and 1 mL H_2O_2 using a microwave digestion instrument (ETHOS One milestone microwave oven). This microwave digestion completely decomposes the samples providing the metals readily available for elemental analysis.

Three fish samples from Gunneklevfjord, two from Flåte and three samples from Svanstulvatnet were digested together with the reference material.

3.7 Selected metal analysis of fish samples using ICP-MS

Aqueous digested fish samples were analyzed for their heavy metal content using inductively coupled plasma – mass spectrometry (ICP-MS). The digested samples were filtered through a 0.45 µm pore size filter (Cellulose acetate filter, VWR collection) and stored in polypropylene tubes at 4°C. The aqueous sample was diluted 25 times prior to analysis. It is recommended that dissolved solid samples with solid to liquid percentage higher than 0.2% w/v should not be

analyzed on ICP-MS, otherwise they will clogg the interface. Besides nitric acid content in samples should not exceed 5% v/v in order to prevent instruments filament destruction.

3.7.1 Calibration of the ICP-MS instrument

6 multi-element calibration solutions containing 11 elements were made by diluting standard reference solution (certified multi element standard, 50 µg/ml). For As, Mn, Cu, Co, Ni, Sr, Ba, Se and Zn the concentrations ranged from 0 to 100 µg/L, for Pb the concentrations ranged from 0 to 50 µg/L and for Cd the concentration ranged from 0 to 10 µg/L. The calibration solutions contained 3.5% nitric acid to have the same acid concentration as samples. The instrument was tuned using tuning solution, then calibrated and finaaly the samples were analyzed. The calibration approximation equation and correlation coefficiens are presented in Appendix G.3.

3.7.2 Analysis

Quantification of selected metals was in fish samples was done using the ICP-MS Nexion 300d (PerkinElmer Inc., Shelton, CT).

Using a peristaltic pump the sample solution was introduced to the nebulizer gas where it was converted to an aerosol. The aerosol was carried to the argon plasma which generated ions. Via the interface (three metal cones) and the ion optics (quadrupole ion deflector) of the instrument, the ions were directed to the quadrupole which is the mass separation device. The quadrupole allows ions of certain mass/charge ratio to reach the detector which recorded the number of electronic signals per seconds (Thomas 2008).

The selected isotope to be measured by the instrument was the most abundant isotope of the element with no or minimal interferences. The selected isotopes are given in Table G.2 in Appendix G.

After the analysis was done the concentrations of metals in the fish samples were calculated using Equation 3.6.

$$C_{sample} = \frac{C_{extract} . V_{extract}}{m_{sample}}$$

Where

$C_{sample} = \frac{mg}{kg\ fish}$

$C_{extract} = Concentration\ found\ in\ extract\ (\frac{mg}{L})$

$V_{extract} = Volume\ of\ extract\ (L)$

$m_{sample} = mass\ of\ digested\ fish\ sample$

Equation 3.6. Determination of metal concentration in fish samples.

A reference sample (DORM-4 fish protein) was analyzed to validate the accuracy of the method for digestion and quantification. The recovery of the elements in the reference material was calculated following Equation 3.7. A recovery of 90-110% of the reference value was accepted.

$$Recovery = \frac{C}{C_{certified}} \times 100$$

Where

$Recovery = Recovery\ of\ analyte\ (\%)$

$C = measured\ concentration\ in\ reference\ material\ (\frac{mg}{kg})$

$C_{certified} = concentration\ in\ reference\ material\ (\frac{mg}{kg})$

Equation 3.7. Reference material recovery calculation.

The recoveries (%) of different elements in reference material are shown in Figure 3.16.

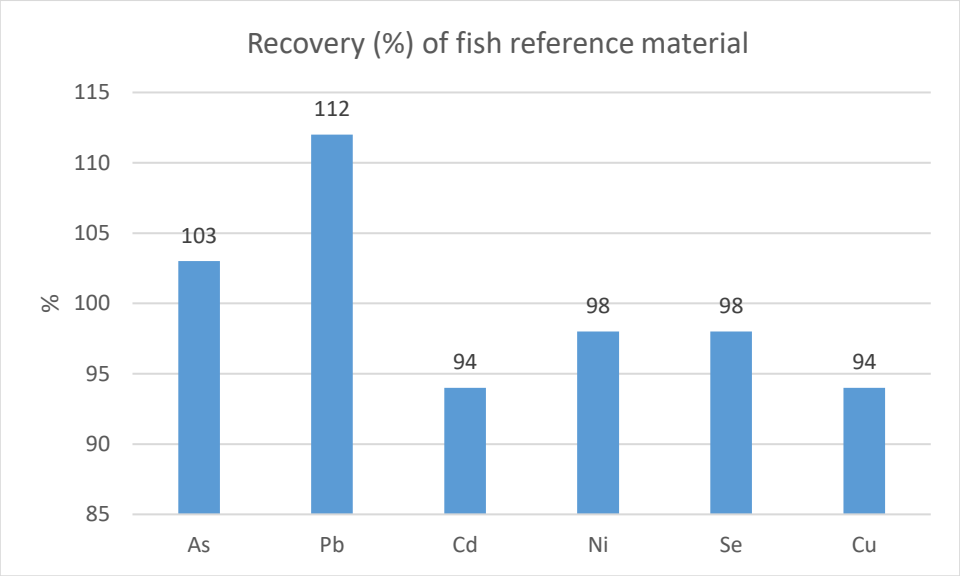

Figure 3.16. Recovery (%) of reference material (DORM-4 fish protein).

The recoveries for most elements are whithin the accepted limits (± 10%). In case of Pb the recovery is slightly higher than the accepted range, however the element was still included in the study.

The amount of cadmium (Cd) in all fish samples except S-22 was less that what could be detected by instrument. Since the digested fish samples contained relatively high amount of nitric acid (87%) they were diluted 25 times before injection to the instrument to prevent any damages to the filament inside the instrument. Low concentration of Cd in fish together with sample dilution is why no cadmium was detected in samples.

3.8 Students t-test

T-test was used to compare differences between the two groups. A t-test is a statistical test used to compare the means of two groups. It is often used in hypothesis testing to determine whether a process or treatment actually has an effect on the population of interest, or whether two groups are different from one another. The null hypothesis (H_0) is that the true difference between these group means is zero. The alternate hypothesis (H_a) is that the true difference is different from

zero. A t-test can only be used when comparing the means of two groups. The t-test is a parametric test of difference, meaning that it makes the same assumptions about the data as other parametric tests. The t-test assumes the data being compared are independent, are approximately but normally distributed, and have a similar amount of variance within each group. Trends are regarded as statistically significant at the 95% significance level ($p < 0.05$).

4 Results and discussion

4.1 Physicochemical properties of sediment samples

The pH and organic matter content in sediment samples are shown in Figures 4.1 and 4.2, respectively. Data on dry matter content and clay content of sediment samples are provided in Appendix C.

The clay fraction was rather constant between the samples, constituting about 27% of the mineral mass. The exceptions are for G-4, having only 15% clay, and G-5 & G-8 having approximately 35% clay.

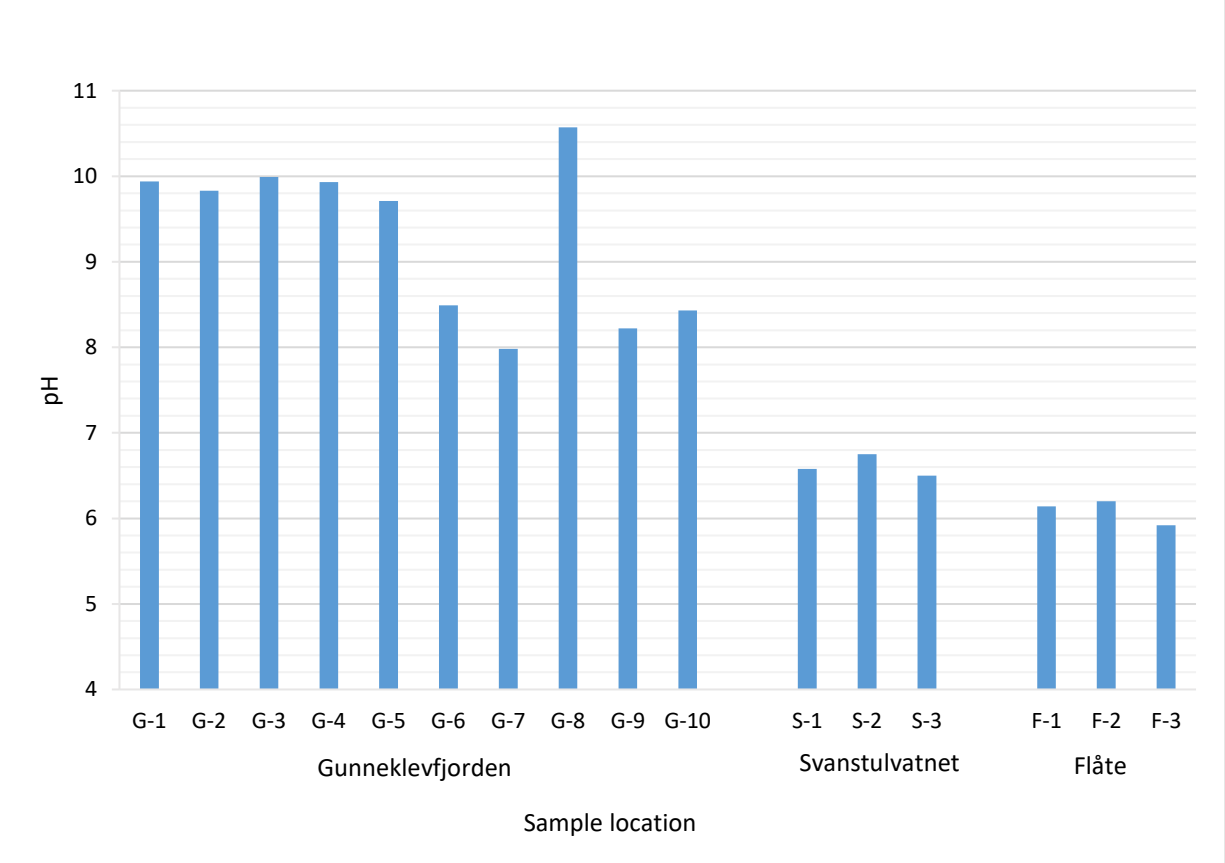

Figure 4.1. pH value of sediment samples in Gunneklevfjorden (G1-10), Svanstulvatnet (S1-3), and Flåte (F1-3). Data for S-4, S-5 and S-6 samples are missing due to lack of sufficient sample material.

Higher pH values are observed in sediment samples from Gunneklevfjorden compared to the two lakes (Figure 4.1). Unlike Flåte and Svanstulvatnet, water in Gunneklevfjorden is a mixture of sea and fresh water which results in higher pH. pH values above 9 (i.e., above seawater pH) in G-1 to G-5 and especially G-8 must be due to anthropogenic pollution. The sediment pH in

Flåte and Svanstulvatnet was higher than expected, especially for the dystrophic Svanstulvatnet with high DNOM levels (Table 4.2) and relatively lower pH in the water (Table 4.1).

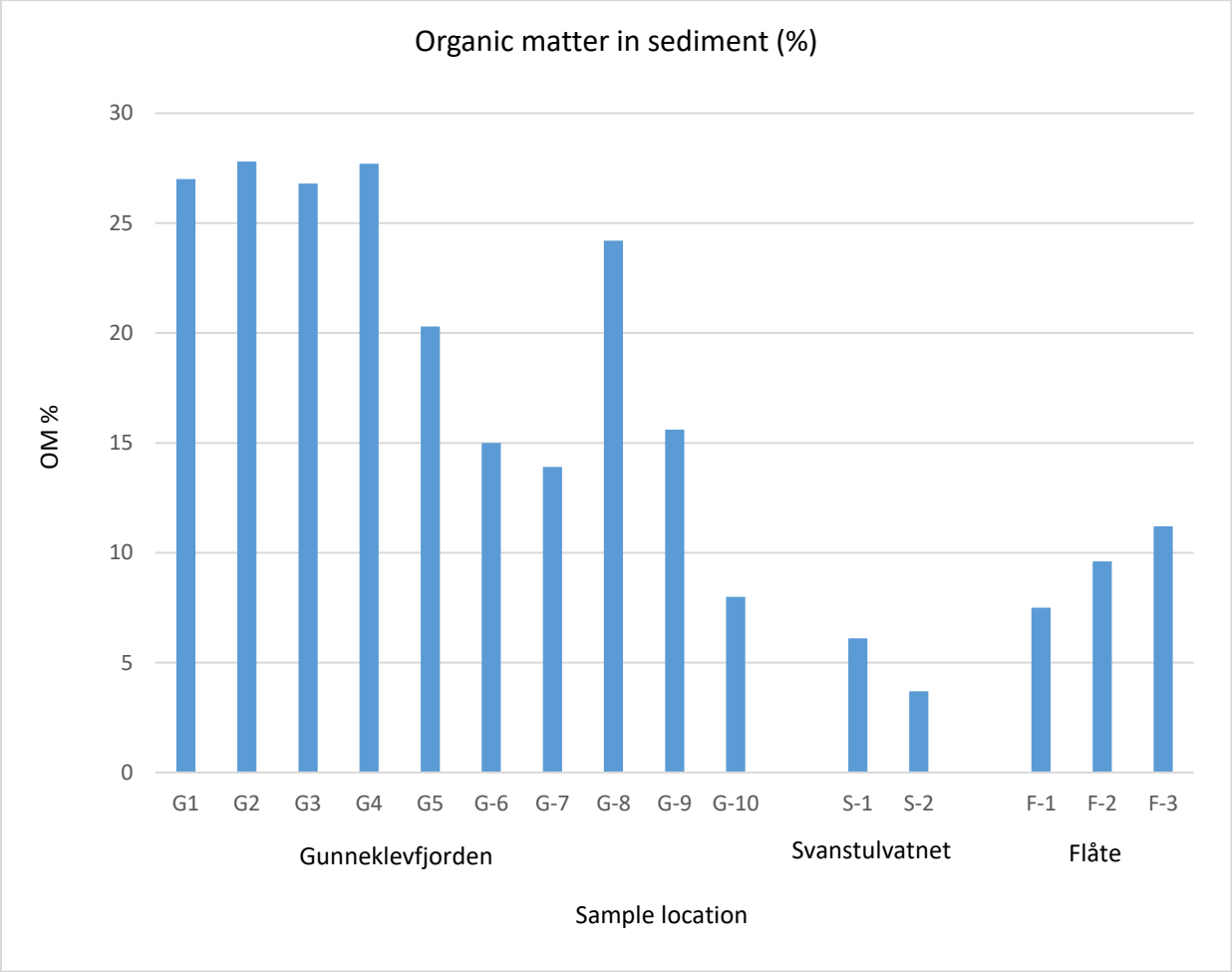

Figure 4.2. Organic matter content in sediment samples after LOI and clay content correction. S-3, S-4, S-5 and S-6 samples were not enough for OM measurements.

Highest amounts of organic matter were found in sediment samples from Gunneklevfjorden. This is mainly due to mixing of fresh water and sea water with high ionic strength which results in precipitation of humic matter. There is a general decreasing trend in organic matter content from G-1 to G-10 (Figure 4.2). Sediment samples G-1 to G-4, which are taken from the southwest shore of the fjord (location 12), have the highest concentration of organic matter. Water with DNOM from Skienselva enters Gunneklevfjorden from the inlet in the northwest and mixes with the saltwater mainly entering through a channel in the southeast. Average concentration of total organic carbon in Skienselva is around 2.6 mg/L in 2019, with more than 90% of that as dissolved organic carbon (Braaten, Gundersen, et al. 2020). Our speculation is

that the DNOM containing water flowing from Skienselva mixes with saltwater from the sea in location 12, constituting a mixing zone, where the DNOM flocculates and precipitates - resulting in higher levels of organic matter in sediments at this location.

Samples G-5 to G-8 are taken from the Deep mid-fjord (location 19) and Deep fjord-shore (location 23) part of the fjord where there is less resuspension by the tidal shifts and fluctuation in water fluxes from Skienselva. On the other hand, samples G-9 and G-10 are from the Shallow water part of the fjord (location 2), thus less amounts of organic matter is found in the sediments likely due to frequent resuspension by the tidal water flushing back and forth over the shallow water.

Sediments from Flåte had higher organic matter content compared to sediments from Svanstulvatnet. Higher sea salt concentration especially chloride in Flåte can be the reason for this finding. Seasalt concentration based on chloride in water from Flåte (3.43 mg/L) is at least three times higher than water from Svanstulvatnet (1.07 mg/L) (Braaten, Olsen, et al. 2017) (see Tables 4.4 – 4.6). The reason is that Flåte is located closer to the sea (Figure 3.5) and a large part of the catchment is below marine limit (Figure 4.3). This contributed to a greater flocculation and thus sedimentation of DNOM. So, assuming that the watersheds are similar the flux of DNOM to the surface waters are similar. Then the lower concentration in Flåte is due to that more DNOM is precipitated.

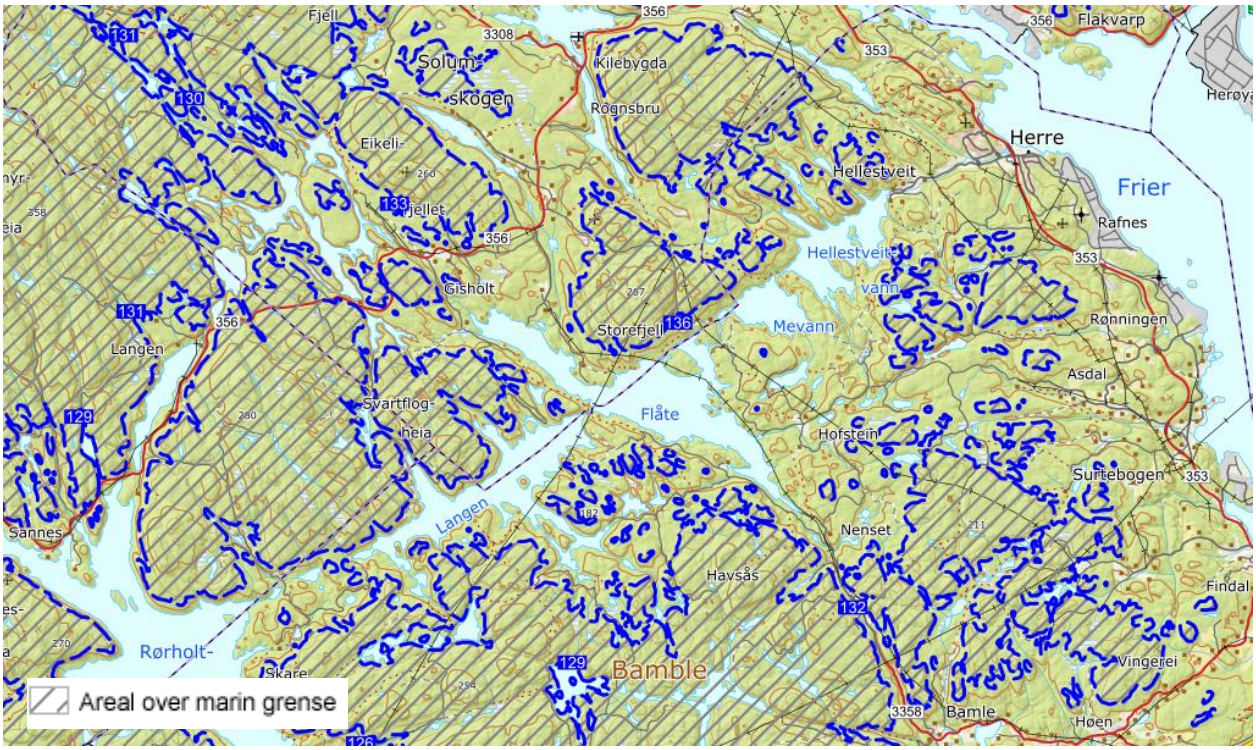

Figure 4.3. Unlike Svanstulvatnet large parts of Flåte catchment is located below the marine limit which causes elevated concentrations of carbonates and sea salt in water, marine limit is shown with blue dashed lines (http://geo.ngu.no/).

4.2 Physicochemical properties of water samples

Water samples from Gunneklevfjorden as the main studied water body and Flåte as the reference lake were collected in the summer of 2021 to compare their physicochemical properties. Dissolved Organic Carbon (DOC) content of water samples, their pH and UV-Vis absorptions were measured.

pH of the water samples from Gunneklevfjorden and Flåte in 2021, and as reported for 2016 and 2018 are shown in Table 4.1.

Table 4.1. Measured and previously reported values of pH in water samples from the three studied water bodies.

Water body	pH (measured)	pH (reported by NIVA[1])	pH (reported by NIVA[2])
Year	2021	2016	2017
Gunneklevfjorden	7.0	7.5	7.3
Flåte	6.3	6.9	6.9
Svanstulvatnet	_	5.8	6.0

[1] (Braaten, Olsen, et al. 2017), [2] (Braaten, Gundersen, et al. 2019)

Since water from Gunneklevfjorden is mixed with sea water (avg. pH 8.2) we expect to have higher pH value. The pH in the water at Svanstulvatnet and Flåte were higher than expected considering that both watersheds have bedrock of poorly weatherable igneous gneiss and granite. Svanstulvatnet has lower pH then Flåte due to that the watershed is located above the marine limit. There is thus less carbonates in the soil to buffer the pH at circumneutral values, such as in Flåte located mainly below the marine limit.

Since the DOC content of water affects the Hg level in the water and as a result of methylation the Hg uptake by biota, we measured the DOC levels of water samples collected from Gunneklevfjorden and Flåte in 2021. The results are shown along with previously reported data from 2006 in Table 4.2.

Table 4.2. Measured and previously reported DOC (mg/L) content of water samples.

Water body	DOC (measured)	DOC (reported by NIVA)[1]
Gunneklevfjorden	2.8	3.3
Flåte	2.0	4.4
Svanstulvatnet	-	9.4

[1](Braaten, Olsen, et al. 2017)

In case of Svanstulvatnet DOC is reported as 9.4 mg/L (Braaten, Olsen, et al. 2017). The samples from Gunneklevfjorden and Flåte have similar low levels of DOC. The concentration of DNOM in water from Flåte is low as large parts of the catchment are below the marine limit where the soil is richer in carbonates, thus the DNOM is not so mobile. Also the DNOM in Skienselva, which is leached from soils mainly above the marine limit, precipitate out due to mixing with water with higher ionic strength, such as seawater in Gunneklevfjorden. Both Gunneklevfjorden and Flåte are thus less dystrophic water bodies compared to Svanstulvatnet. In dystrophic lake in the boreal forest there is commonly a strong positive correlation between the concentration of DNOM and Hg in water as a result of transport of Hg by the DNOM from catchment area to the lake (Braaten et al. 2018; Braaten et al. 2014). In the presence of both Hg and DNOM the sulphur reducing bacteria may under slighty reducing conditions methylate the Hg to highly bioavailable MeHg. This can rationalize the higher concentration of THg in sediment and fish samples from Svanstulvatnet compared to Flåte. On the other hand, the DNOM has also an antagonistic effect on the bioavailability by complex binding the Hg to the high molecular weight refractory moieties of DNOM, rendering non-bioavailable (Braaten et al. 2018).

UV and Vis absorption spectra of DNOM in water samples collected in 2021 from Gunneklevfjorden and Flåte were recorded in order to assess the quality of DNOM. The results are presented in Appendix C.1. The sUVa (Abs254/DOC) and sVISa (Abs400/DOC) and SAR (sUVa/sVISa) values are given in Table 4.3.

Table 4.3. UV-Vis values of water samples.

Water body	sUVa (L mg^{-1} m^{-1})	sVISa (L mg^{-1} m^{-1})	SAR
Gunneklevfjorden	4.8	0.6	8.0
Flåte	4.9	0.7	7.0

The absorption values for water samples from Gunneklevfjorden and Flåte are very similar. This means that organic matter in the two water bodies have similar structural characteristics. Average sUVs and SAR in surface water in boreal lakes, as reported by (Vogt et al. 2001), are 5.1 L mg^{-1} m^{-1} and 7.1, respectively. Our measured values for sUVa and SAR are also very close to what is found in boreal lakes. The slightly higher SAR in Gunneklevfjorden was

expected due to the prevalence of lower molecular weight moieties (i.e. higher SAR) in these sites with higher ionic strength levels.

4.3 Water major anions and cations

4.3.1 Major anions

Major anion concentrations in water samples collected in 2021 from Gunneklevfjorden and Flåte are presented in Table 4.4.

Table 4.4. Concentration (µM) of major anions in water samples.

Water body	F^-	Cl^-	Br^-	SO_4^{2-}	NO_3^-
Gunneklevfjorden	63	28488	118	1436	118
Flåte	1.0	42	1.1	7.0	7.0
Sea water	53	535400	813	27565	50

Chloride (Cl^-) and sulfate (SO_4^{2-}) concentrations in water samples from three water bodies in 2016 and 2018 were previously measured in NIVA reports. The data are shown in Table 4.5.

Table 4.5. Concentration (µM) of chloride and sulfate in water samples reported by NIVA (Braaten, Olsen, et al. 2017) (Braaten, Gundersen, et al. 2019).

	Anions	Gunneklevfjorden	Flåte	Svanstulvatnet
2016	Cl^-	67695	96	22
	SO_4^{2-}	3123	20	8.1
2018	Cl^-	34975	113	25
	SO_4^{2-}	1946	23	8.1

Gunneklevfjorden is connected to the sea water. This results in brackish water with very high concentrations of anions, especially Cl^- and SO_4^{2-}, in water sample from this fjord. Water from Gunneklevfjorden has thus higher concentrations of all major anions compared to water from Flåte and Svanstulvatnet. The anions in the lake water from Flåte and Svanstulvatnet originates from the rain and weathering products from the watershed. As the Flåte catchment is partly below the marine limit the ionic strength is higher than in Svanstulvatnet, though has nevertheless low anion concentrations.

As is mentioned in Chapt. 2.2 production of MeHg takes place in the presence of sulfate reducing bacteria. The very high concentration of sulfate in Gunneklevfjorden means that the amount of sulfate in this water body is not a limiting factor for these bacteria. In other words if enough DOM is available, in slightly reducing conditions sulfate reducing bacteria can actively produce MeHg in such aquatic environment.

4.3.2 Major cations

Concentration of the four major cations calcium (Ca^{2+}), potassium (K^+), sodium (Na^+) and magnesium (Mg^{2+}) in water samples from Gunneklevfjorden and Flåte are shown in Table 4.6.

*Table 4.6. Concentration (*µM*) of major cations in water samples.*

Water body	Ca^{2+}	K^+	Na^+	Mg^{2+}
Gunneklevfjorden	2510	562	11227	3826
Flåte	26	4.3	4.8	7.8
Seawater [1]	9980	9719	459160	51923

[1] Data from https://www.lenntech.com

As was to be expected, higher concentrations of all four cations are observed in water sample from Gunneklevfjorden due to its connection to seawater. In case of Gunneklevfjorden concentration of Na^+ and Mg^{2+} is higher than Ca^{2+} and K^+. The high concentration of sodium is due to NaCl as the most abundant sea salt.

In water from Flåte as a dystrophic lake, with poorly weatherable mineral soils in its watershed, low concentration of all four cations is observed.

4.4 Mercury

4.4.1 Total Hg (THg) in sediment samples

Concentration of total Hg (THg) in sediments from different sites in Gunneklevfjorden are previously reported to range from 0.3 mg/kg to 307 mg/kg (Braaten, Johnson, et al. 2019). The highest average THg content have been observed in deeper sediment layers (20-25 cm) compare to the surface layer. The reference sediment samples from Svanstulvatnet had THg concentrations below detection limit (<0.4 mg/kg).

In this study we re-measured Hg content of several sediment samples from surface (0-5 cm) and deep (20-25 cm) sediments from Gunneklevfjorden as the main study location and Svanstulvatnet and Flåte as reference lakes. The results are shown in Figure 4.4. Hg concentration in sediment samples from Gunneklevfjorden ranged from 4.48 mg/kg to 245 mg/kg, which is similar to what was measured previously reported in Braaten et al. (Braaten, Johnson, et al. 2019).

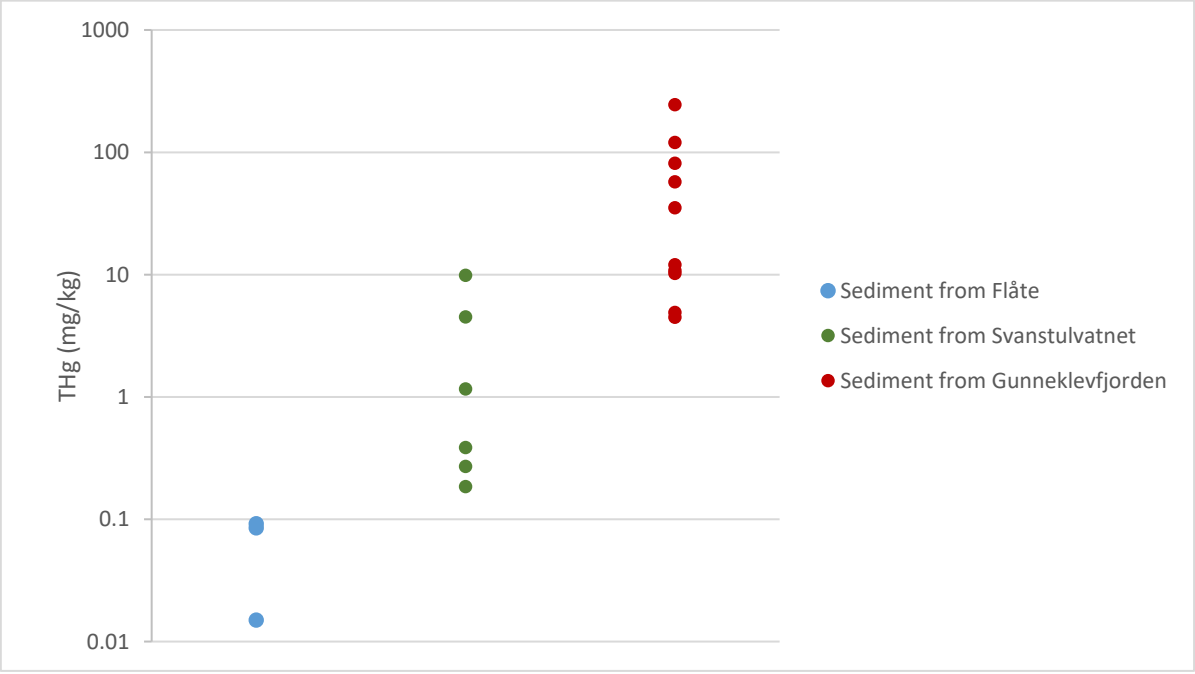

Figure 4.4. THg in sediment samples from three studied water bodies.

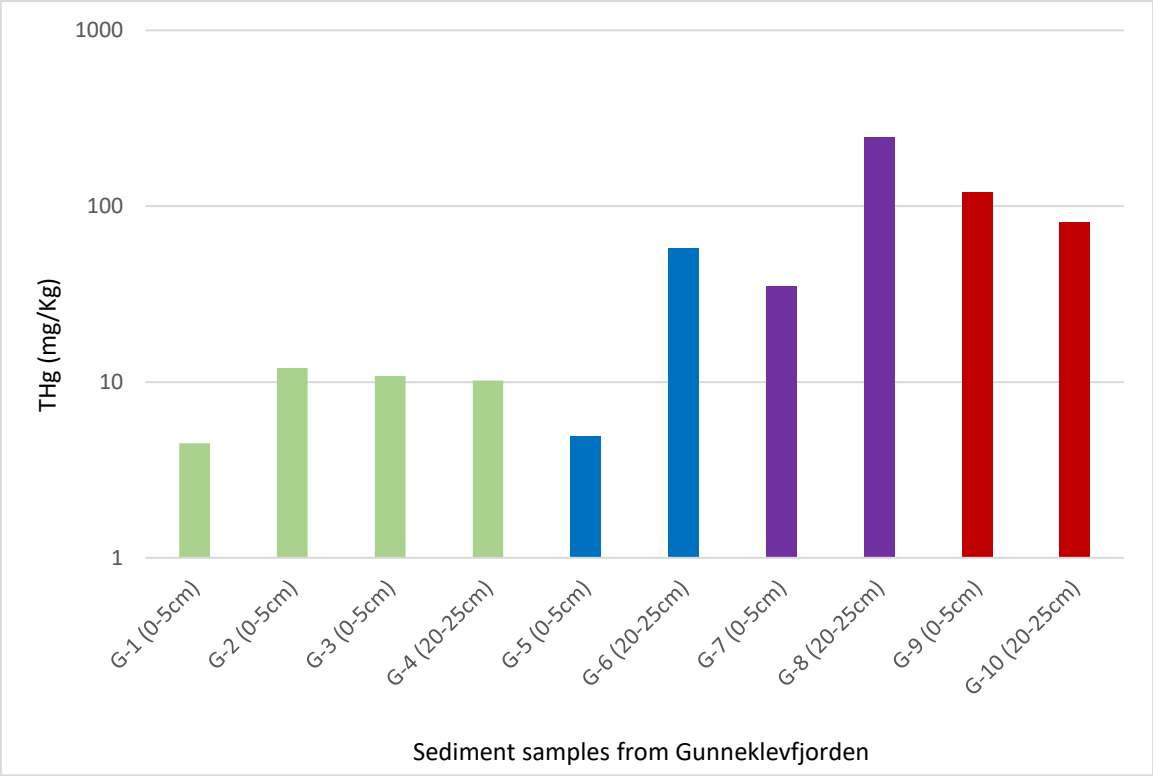

Figure 4.5. THg in sediment samples from Gunneklevfjorden, G-1-4, G-5-6, G-7-8 and G-9-10 are collected from Mixing zone (loc. 12), Deep mid-fjord (loc. 19), Deep fjord-shore (loc. 23) and Shallow water (loc.2), respectively. Sample depth is also shown for each sediment sample.

As it is clear from Figure 4.4 that the sediment samples from Gunneklevfjorden have significantly ($p<0.05$) higher Hg levels among the three water bodies. Gunneklevfjorden has, as is described in Section 2.8, been a major recipient of Hg in wastewater from Herøya industrial park several decades ago. As a result this fjord has had a local source of Hg in addition to the long-range transported atmospheric mercury. The Hg in sediments from Flåte and Svanstulvatnet originates mainly from atmospheric Hg and weathering in the catchments. In case of Gunneklevfjorden, the severe local pollution history from Herøya of this fjord is the main reason for the high sediment Hg levels.

The amount of THg in deep sediment (20–25 cm) at Deep fjord-shore was the highest (245 mg/kg), while surface sediments (0-5 cm) at the Mixing zone contained the lowest amount of mercury (Figure 4.5). THg in sediment samples from the Mixing zone (G-1 to G-4) as well as one surface sediment from the Deep mid-fjord (G-5) is not very different from THg in several samples from Svanstulvatnet. This implies that they have not been contaminated by a local pollution source, which supports the concept of relatively unpolluted water flowing into the

fjord from northwest from Skienselva to the Mixing zone along the southern shoreline of the fjord. The highest amount of Hg is found at deep G-8 sediment in the Deep fjord-shore region. This could be in a tailing from a possible pollution point source (wastewater pipe outlet). G-6 and G-7 in the Deep mid-fjord are possibly influenced by diffusion of Hg from G-8, thus also contain elevated THg.

Generally deeper (20-25 cm) sediment samples contain higher amounts of mercury. The exception is for the Shallow water where the G-10 from the deep sediment contained lower amount of Hg than G-9 collected from the surface sediment. Our speculation is that resuspension during tidal flux of the sediments in this shallow part of the fjord has eroded off the top layers of sediments leaving only the pre-industrial deposits as deeper sediments beneath a surface layer of more contemporary sediments. This would explain the higher amounts of THg as observed in surface sediments and lower levels in the deep sediments from this location.

4.4.2 THg in water samples

The concentrations of THg in water samples throughout Gunneklevfjorden are reported in (Schaanning et al. 2017). A summary of the findings at stations similar to the location of our sediment samples is described below.

From all sampling stations water sample was collected at depth 0.1 meter. Three water samples were collected at Deep mid-fjord (location 19) from 0.1, 1 and 3 meters depth. Finally, four samples were collected at Deep fjord-shore (location 23) at depth 0.1, 1, 3 and 5 meters.

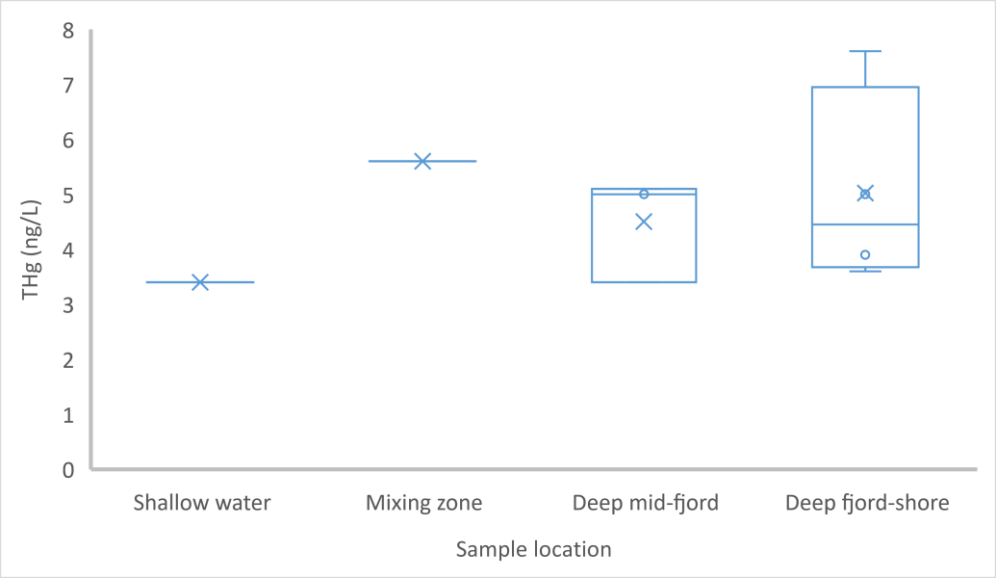

Figure 4.6 Statistical summary of total Hg (THg) concentration (ng/L) in water samples from all sampled depths at Gunneklevfjorden from locations 12, 19, 23 and 2, that are referred to as Mixing zone, Deep mid-fjord, Deep fjord-shore and Shallow water, respectively (Schaanning et al. 2017). The median and the mean are shown with a line and cross respectively.

The highest concentrations of Hg in water were observed close to the bottom at 5 m depth in the deepest part of the fjord (i.e., Deep fjord-shore location 23) (Figure 4.6). The highest Hg concentration in the water were found at the Deep fjord-shore, which is located on the opposite side of Herøya. This is likely due to leaching of Hg from these more contaminated sediments (see Chapter 4.4.1) facilitated by the longer residence time of these bottom layers of the fjord. The lowest concentration of Hg in water in Gunneklevfjorden was found in the Shallow water in the southeastern part of the fjord. This water is mainly comprised of seawater. Another reason for relatively low concentration of THg in the Shallow water part is that the water sample were only taken from upper parts of the water column. THg was higher in the water from the south in/outlet of the fjord compared to the water coming in from the northwest. As the present anthropogenic emissions of Hg to the fjord is very low these elevated levels must be due to mixing of surface and the Hg enrich deep water, as well as resuspension of contaminated sediments caused by turbulence from sea water flow.

Elevated THg in water surface layer was also observed at the Mixing zone (location 12). This location is at the same side as Herøya. This is as discussed above the mixing zone of water from the Skienelva and the seawater. Leaching from the sediments and runoff from the land can thus be the reasons for this elevated THg value.

4.4.3 THg in fish muscle samples

THg in fish samples from Gunneklevfjorden, Flåte and Svanstulvetnet have been previously investigated and different changes was observed over time (Braaten, Olsen, et al. 2017). For Flåte, there was a significant decrease in average concentration from 2001 (0.25 ± 0.03 mg / kg) to 2008 (0.17 ± 0.08 mg / kg) and no change from 2008 to 2016 (0.15 ± 0.08 mg / kg). In Gunneklevfjorden there was a significant increase in mean concentration from 2013 (0.30 ± 0.20 mg / kg) to 2016 (0.47 ± 0.07 mg / kg), while the pattern in Svanstulvatnet shows that the average concentration increases from 1991 (0.17 ± 0.07 mg / kg) to 2008 (0.31 ± 0.15 mg / kg), but remains unchanged from 2008 to 2016 (0.31 ± 0.14 mg / kg).

Both Flåte and Svanstulvatnet are typically brown and dystrophic lakes. Increasing concentrations of organic matter in the water masses in these lakes, documented for the latter 20 years (Monteith et al. 2007), has been suggested as a possible factor for altered Hg concentrations in fish. This is because organic matter can affect Hg concentrations, accessibility, transport and transformation through several different processes, including transport from the catchment (Braaten et al. 2014), microbial production of MeHg (Ullrich et al. 2001), and abiotic degradation of MeHg (Poste et al. 2015)

In 2018, fish samples from the same water bodies were collected for a similar study (Braaten, Gundersen, et al. 2019). Same as the previous survey in 2017 the THg in samples from Gunneklevfjorden (0.48 ± 0.15 mg/kg) were found to be higher than Hg level in fish from Flåte (0.25 ± 0.04 mg/kg) and Svanstulvatnet (0.32 ± 0.07 mg/kg).

In this study the fish samples from 2018 were re-analyzed. Since organic Hg binds to the thiol groups in proteins it accumulates in the fish muscles (Polak-Juszczak 2018). The fish muscle was thus used as sample tissue to analyze the Hg content in fish.

THg was determined in 25 freeze dried fish muscle samples from Gunneklevfjorden, along with 25 fish samples from both Svanstulvatnet and Flåte as the reference lakes (Figure 4.7). Length and weight of the collected fish samples are summarized in Table 4.7. Fish samples from 2018 were both longer and heavier compared to fish from 2016.

Table 4.7. Length and weight of fish samples collected in 2018 from three water bodies.

Specification		Gunneklevfjorden	Svanstulvatnet	Flåte
Length (cm)	Mean	22	19	15
	STD	3	1	2
	Min	17	17	10.5
	Max	29	22	20
Weight (g)	Mean	145	69	34
	STD	90	13	16
	Min	51	51	10
	Max	422	102	80

The statistical summary of re-measured THg concentrations in fish samples from Flåte, Svanstulvatnet and Gunneklevfjorden are shown in Figure 4.8. In order to compare measured Hg content of dry fish samples with THg content in corresponding wet fish samples measured by NIVA (Braaten, Gundersen, et al. 2019), we need to correct the measured THg values. Dry fish muscle THg values were divided by 5 to give us approximate THg for wet samples (Cresson et al. 2017).

THg in fish samples measured by us and researchers at NIVA, is compared in Appendix E.8. The results were statistically similar ($p<0.05$) to the measured THg in NIVA report (Braaten, Gundersen, et al. 2019) for all three water bodies.

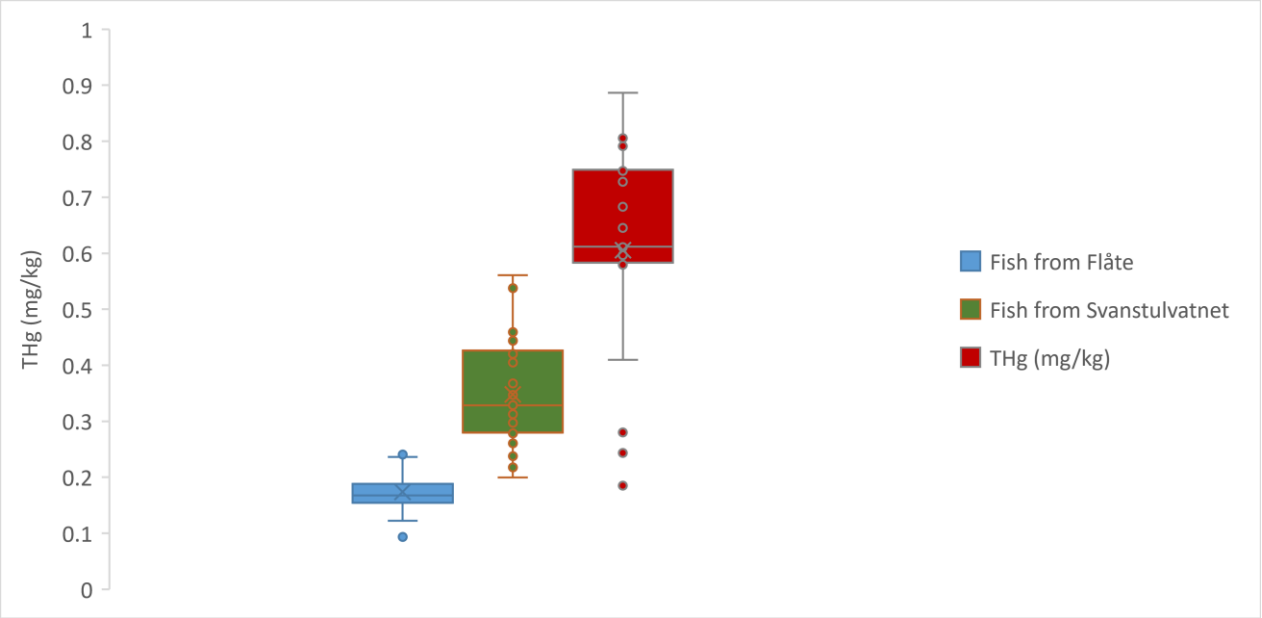

Figure 4.7. THg in wet fish samples (calculated by THg dry fish sample/5) from the three studied water bodies. The median and the mean are shown with a line and cross respectively within the box, the dots outside the lower limits are outliers.

As in 2018 the Hg level in samples from Flåte are the lowest compared to the two other water bodies and none of the samples from Flåte have concentration higher than the EU limit for protection of human health (0.5 mg/kg). The main source of Hg in fish from this lake is long-range transported atmospheric Hg as there is no local source of pollution. The variation observed in THg in fish from this water body is due to variety in length and weight of fish which were collected randomly by a fishing net.

Svanstulvatnet has long-range transported atmospheric Hg as the main source of this toxic element as well. However this lake is a more dystrophic water body with higher content of organic matter compared to Flåte (Table 4.2). Since organic matter has a key role in transportation of Hg from the forest floor in the catchment to the surface waters, we observed higher levels of Hg in fish samples from this lake compared to Flåte.

Samples from Gunneklevfjorden showed significantly ($p<0.05$) higher Hg levels compared to the two reference lakes, Flåte and Svanstulvatnet. This is due to the elevated THg levels in the sediments of Gunneklevfjorden (Chapter 4.4.1).

In conclusion, since Flåte and Svanstulvatnet only have long-range transported atmospheric Hg as their main source of mercury, the THg in fish samples from these two water bodies have significantly ($p<0.05$) lower concentrations of Hg (0.09-0.25 mg/kg and 0.2-0.56 mg/kg) than samples from Gunneklevfjorden (0.24-0.89 mg/kg). This implies that this Hg in the fish at from Gunneklevfjorden mainly originates from both atmospheric Hg and sediments polluted by local Hg point sources.

4.5 Selected metal concentration in fish samples

Total concentration of selected rare earth elements in fish samples from Flåte, Svanstulvatnet and from Gunneklevfjorden are presented in Table 4.8. The amount of arsenic (As) and lead (Pb) in one of the samples from Flåte (F-8) was below the detection limit of the instrument. The cause for this was that lower amount of this fish (150 mg) sample was available for microwave digestion. Therefore, the instrument could not detect some of the elements due to the very low concentration.

The highest concentration of Pb and As is found in fish from Gunneklevfjorden. This is likely due to anthropogenic contaminated sediment. Fish absorbs free aqueous heavy metals through the gills and through their diet.

Both Pb and As are elements with high covalent index (i.e., type B or soft elements) which makes them appropriate to bind the DNOM. Thus, DNOM increases the solubility and thereby flux of type B elements from forest floor in the watershed to surface waters. This allows them to be transported to surface waters where they may be made bioavailable and taken up in the food chain.

Table 4.8. Concentration of selected metals in fish muscle samples.

Sample	mg/kg									
	As	Pb	Mn	Cu	Co	Ni	Sr	Ba	Se	Zn
F-5	0.063	0.0252	0.852	2.552	0.17	0.383	2.4	5.748	3.561	36.45
F-8	_	_	0.64	0.513	0.314	0.823	1.964	0.92	4.021	52.97
S-16	0.112	0.038	0.411	0.689	0.188	0.345	0.768	6.234	3.6	41.7
S-19	0.174	0.128	0.656	1.645	0.215	0.316	2.253	5.38	3.739	39.46
S-22	0.19	0.082	1.878	1.872	0.19	0.422	1.515	0.42	5.544	35.03
G-1	1.718	0.643	0.594	3.624	0.167	0.56	1.39	5.55	1.798	36.87
G-2	1.215	0.927	0.72	1.048	0.246	0.516	1.831	0.222	2.097	39.82
G-13	1.28	0.459	0.658	1.074	0.148	0.288	1.21	10.43	1.671	33.42

The concentration of manganese (Mn), zink (Zn), selenium (Se) and to some extent copper (Cu) in fish from the locally polluted Gunneklevfjorden was slightly lower than the fish from the two background fresh water reference bodies. These elements are borderline metals which show the characteristics of both class A and class B metals. Higher pH in Gunneklevfjorden could cause more hydrolysis of these elements, thus precipitation and less availability of them for fish. Besides the uptake of heavy metals decreases when the concentration of base cations increases.

The amount of heavy metals in 15 different fresh water fish species including perch in a border region between Norway and Russia was investigated (Amundsen et al. 1997). Concentration of Zn, Ni, Hg and Cu in different organs of fish such as muscle, liver and gills was reported. Considering all fish species used in this study the mean concentration of heavy metals in muscle varied from 0.01-0.81 µg/g Cd, 1.6-12.3 µg/g Cu, 0.16-0.89 µg/g Hg, 0.48-3.1 µg/g Ni, and 17-63 µg/g Zn. Very high concentrations of Ni in fish from this region is due to Ni mines at Nikel in Russia. In our measurements, the amount of several elements such as Cd, Cu and Ni were to some extent lower than what observed in this study.

It is worth mentioning that in order to have a better understanding of the trends in heavy metal concentration in fish from three water bodies we need to analyze the heavy metal content in a larger fish population.

4 Conclusion

The levels of Hg in a population of perch from Gunneklevfjorden was significantly higher than perch from two background fresh water reference lakes, Flåte and Svanstulvatnet. Among 25 analyzed fish samples from Gunneklevfjorden, 20 of them had Hg levels more than EU limit value for Hg to protect human health (0.5 mg/kg). Hg concentration in fish from the three water bodies appears to be mainly reflecting the amount of Hg in sediments. The main source of Hg in sediments from reference lakes are long range atmospheric Hg and Hg from the catchment. This is to some extent in the form of MeHg, which is partly allochthonous and partly autochthonous from the sediments. Gunneklevfjorden has a long history of pollution from Herøya industrial site. Although the Hg pollution from industry at this area ended in 1987, the effect of this local Hg source in the sediments is still noticeable in biota from this fjord.

The physicochemical properties of the sediments from the three water bodies were as expected. Highest organic matter content was found in samples from Gunneklevfjorden, which is due to the precipitation of DNOM rich water from Skienselva when mixing with high salinity of water in the Gunneklevfjorden. However, no correlation was found between organic matter and Hg content of sediments. This is probably because the Hg contamination is unevenly distributed from a likely point source and that the deposition of OM is unevenly distributed due to the spatial distribution of the mixing with saltwater determined by the different runoff fluxes and tidal fluctuations.

As we expected the levels of DNOM has effects on Hg uptake in biota. In a dystrophic lake such as Svanstulsvatnet transport of Hg by DNOM from catchment area to the lake causes increased concentration of Hg in sediments and thus fish. However, DNOM is known to have both synergistic and antagonistic effect on the bioconcentration of Hg into the food chain (Braaten et al. 2018). The complexation of the more refractory Hg and MeHg to high Mw and aromatic DNOM render the Hg less bioavailable.

Relatively higher amounts of the heavy metals Pb and As were also found in fish from Gunneklevfjorden. Since this is not seen in the reference site this is also likely coming from locally anthropogenic contaminated sediments. On the other hand, the concentration of some other rare earth metal ions, such as Zn and Mn, were lower in fish from Gunneklevfjorden. This

can be due to reduced heavy metals uptake in the presence of high concentration of base cations in the fjord. Elevated hydrolysis of these elements and their precipitation at high fjord water pH can be another reason for lower concentration of these elements in fish from Gunneklevfjorden.

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Appendix A Sample locations

Appendix A contains GPS coordinates for sediment and water sample locations.

A.1 GPS coordinates for sediment sample locations

Table A 1. Coordinates for sediment sampling locations.

Water body	Latitude (°*N*)	Longitude (°*E*)
Gunneklevfjorden	59.1228	9.6335
	59.1241	9.6378
	59.1249	9.6401
	59.1200	9.6455
Svanstulvatnet	59.3942	9.4254
Flåte	59.0618	9.4631

A.2 GPS coordinates for water samples

Table A 2. Coordinates for water sampling locations.

Water body	Latitude (°*N*)	Longitude (°*E*)
Gunneklevfjorden	59.1286	9.6308
Flåte	59.0710	9.4237

Appendix B Freeze drying of samples

Freeze drying was used for drying sediment and fish samples. This gentle drying method results in more porous material that is easier to homogenize. To make the drying process faster, the samples should be left in the freezer at -18 °C for 24 hours before freeze-drying. The thickness of the drying material should not be more than 1-2 cm.

The instruments used for freeze-drying were two pieces Lyovac GT2 with drying chamber (Asterix and Obelix) and two-stage vacuum pump Trivac D4A or D4B. The samples were frozen at -18 °C, then placed in an acrylic glass container which is empties of air by means of a vacuum pump. The moisture sublimates from the sample and condenses to ice on a cooling coil in a condensation tank. Drying can take from 1 to 7 days, depending on the amount of material.

Appendix C Physicochemical properties of the sediment samples

Appendix C contains results for the physicochemical properties of the sediments. The results for pH is mentioned in C.1, LOI in C.2 , clay estimates and correction factors for OM in C.3, dry matter content in C.4 and OM in C.5. The flow chart followed to estimating the clay content is found in C.6.

C.1 pH

Table C 1. pH of sediment samples.

Sample	Water body	pH
F_1	Flåte	6.1
F_2	Flåte	6.2
F_3	Flåte	5.9
S_1	Svanstulvatnet	6.6
S_2	Svanstulvatnet	6.7
S_3	Svanstulvatnet	6.5
G_1	Gunneklevfjorden	9.9
G-2	Gunneklevfjorden	9.8
G_3	Gunneklevfjorden	10.0
G_4	Gunneklevfjorden	9.9
G_5	Gunneklevfjorden	9.7
G_6	Gunneklevfjorden	8.5

G_7	Gunneklevfjorden	7.9
G_8	Gunneklevfjorden	10.6
G_9	Gunneklevfjorden	8.2
G_10	Gunneklevfjorden	8.4

C.2 Loss on ignition

Table C 2. Temperature program used for loss on ignition measurement.

Time	Temperature (°C)
00:10:00	300
06:00:00	600

Figure C 1. Oven used for loss on ignition measurements of sediment samples.

C.3 Clay estimates

Table C 3. Estimates of clay content in sediment samples and the corresponding correction factors.

Sample	Clay content (%)	Correction factor for OM
F_1	27	2.5
F_2	27	2.5
F_3	27	2.5
S_1	27	2.5
S_2	27	2.5
G_1	27	2.5
G_2	27	2.5
G_3	27	2.5
G_4	15	2
G_5	35	2.5
G_6	27	2.5
G_7	27	2.5
G_8	35	2.5
G_9	27	2.5
G_10	27	2.5

C.4 Dry matter content of sediment samples

Table C 4. Dry matter content of sediment samples.

Sample	Dry matter content (%)
F_1	97
F_2	97.6
F_3	97.3
S_1	98
S_2	98.5
G_1	96.6
G_2	95.9
G_3	97.4
G_4	97.6
G_5	95.8
G_6	96.0
G_7	97.2
G_8	97.3
G_9	95.4
G_10	97.7

C.5 OM in sediment samples

Table C 5. Organic matter content of sediment samples.

Sample	Organic matter (% LOI after clay content correction)
F_1	7.5
F_2	9.6
F_3	11.2
S_1	6.1
S_2	3.7
G_1	27
G_2	27.8
G_3	26.8
G_4	27.7
G_5	20.3
G_6	15
G_7	13.9
G_8	24.2
G_9	15.6
G_10	8

C.6 Flow chart used for estimation of clay content

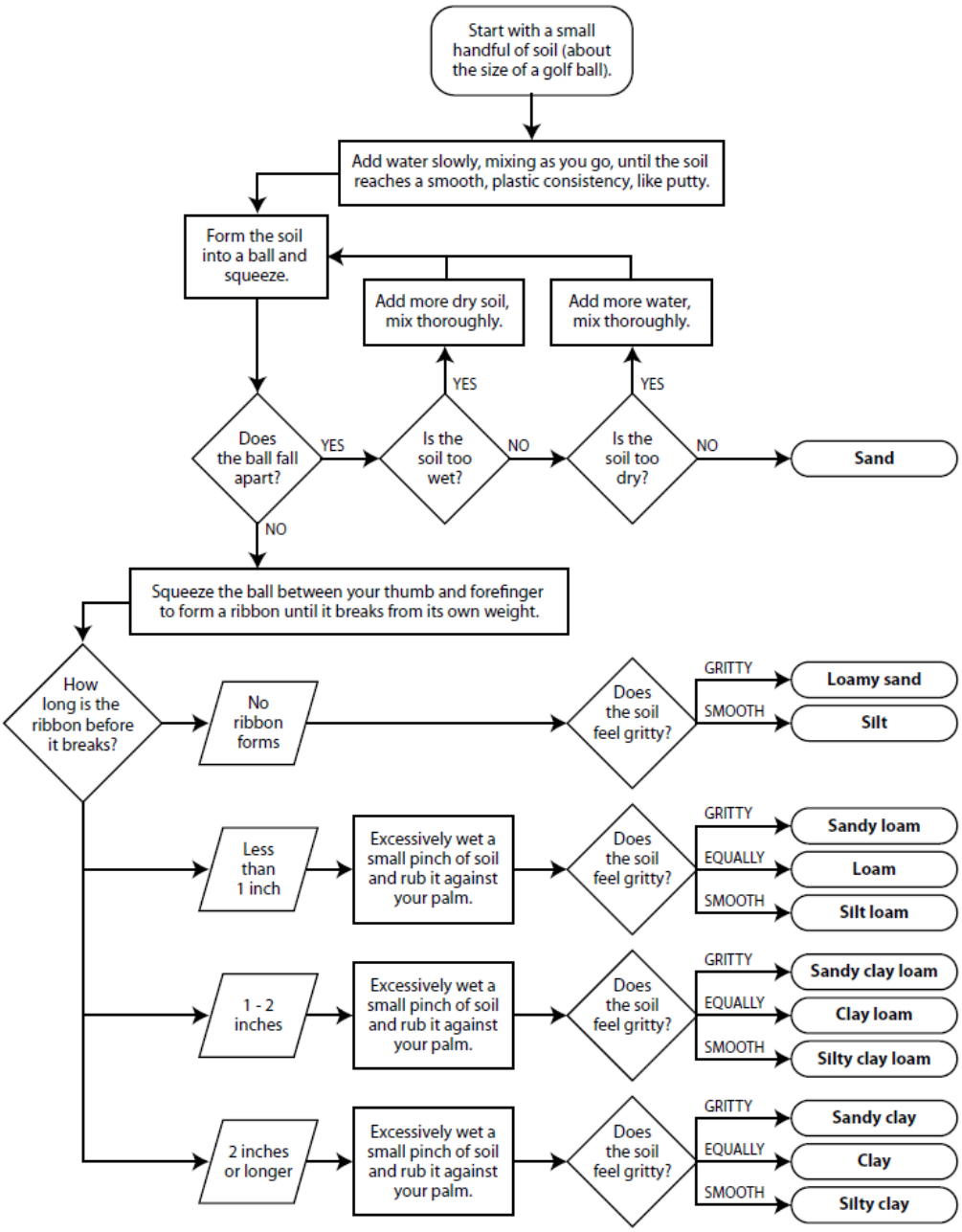


Figure C 2. Flow chart used to determine the sediment textures by feel.

Appendix D Physicochemical properties of water samples

In Appendix C results for physicochemical properties of water samples are presented. Sections D.1 and D.2 and D.3 contain results for spectrophotometry, major anions and major cations measurements respectively.

D.1 UV-Vis absorption of water samples

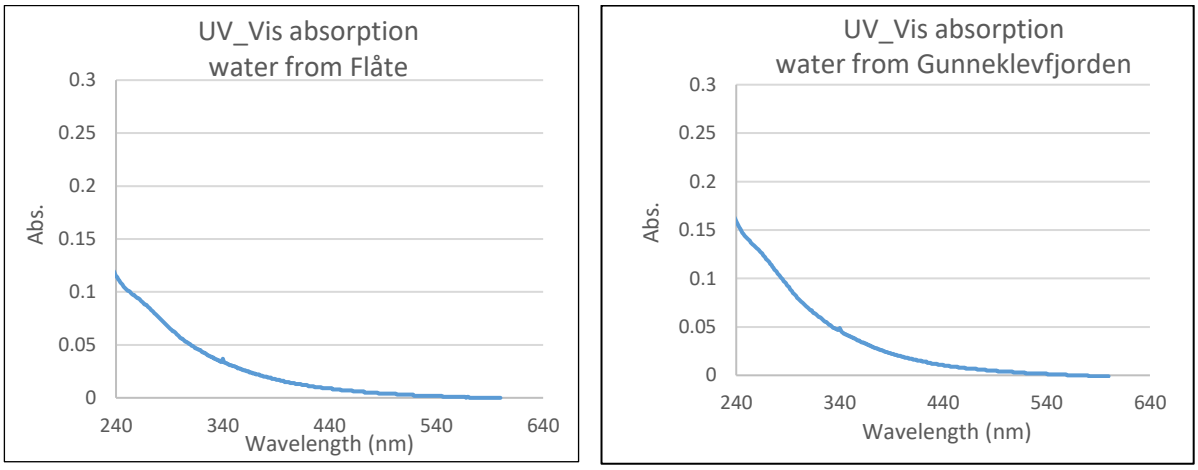


Figure D 1. UV-Vis absorption spectra of water samples from Gunneklevfjorden and Flåte.

D.2 Major anions measurements by IC

Thermo Fisher Scientific Dionex Integrion HPICTM instrument was used to measure major anions concentration in water samples.

D.2.1 General procedure

Before starting any analysis we need to replace the water in the eluent bottle with fresh type one water. Then the pump needs to be primed in order to remove any air bobbles in the tubes to prevent them from entering the analysis column. After priming the flow rate needs to be entered and the pump, eluent generator and suppressor current must be switched on. We need to monitor pressure and the base line to make sure they are stable before any analysis. After the pressure become stable and the conductivity of the eluate is below one we make a sequence

consist of the calibration solutions and the samples to be analyzed. Then we chose the method of interest and start the analysis. The detailed procedure has been described in the instrument SOP.

Figure D 2. Thermo Fisher Scientific Dionex Integrion HPICTM instrument.

D.2.2 Instrument set up

Table D 1. Instrument set up in major anions measurements.

Elution type	Isocratic
Flow rate (ml/min)	0.3
Cartridge type	EGC 500 KOH
Eluent Generator concentration (mM)	36
Column temperature (°C)	35
Compartment temperature (°C)	30

D.2.3 Dinox standard

The calibration curve was made using Thermo Fisher Scientific Dionex Seven Anion standard solution. The ion concentrations in the original solution are presented in table 7.

Table D 2. The ion concentrations in the Dionex seven anion standard solution.

Element	Concentration (mgL^{-1})
Fluoride (F^-)	20
Chloride (Cl^-)	100
Nitrite (NO_2^-)	100
Bromide (Br^-)	100
Nitrate (NO_3^-)	100
Phosphate ($PO4_3^-$)	200
Sulphate ($SO4_2^-$)	100

D.2.4 Calibration graphs

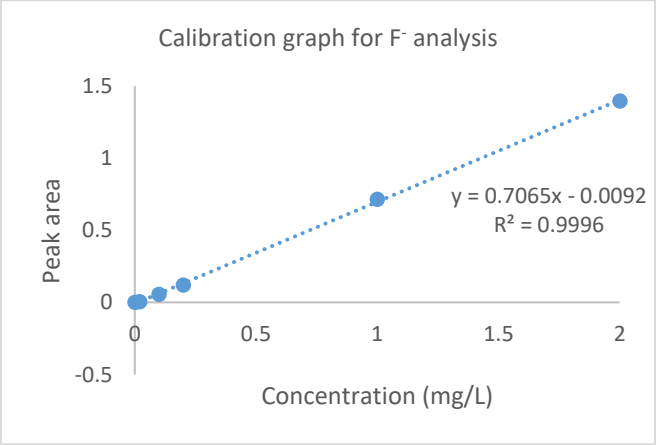


Figure D 3. Calibration graph for F^- measurements in water samples.

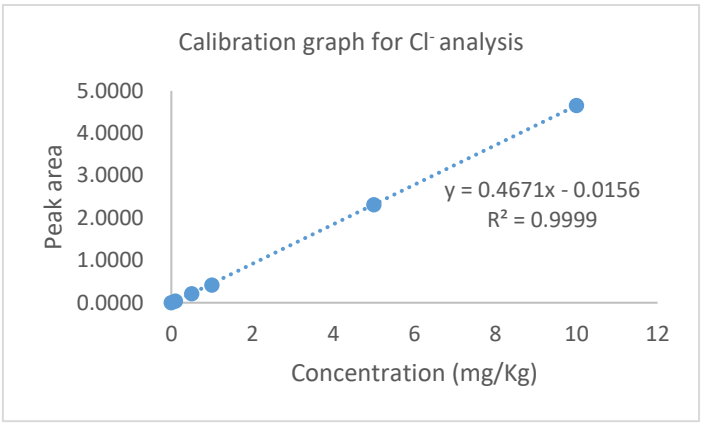


Figure D 4. Calibration graph for Cl^- measurements in water samples.

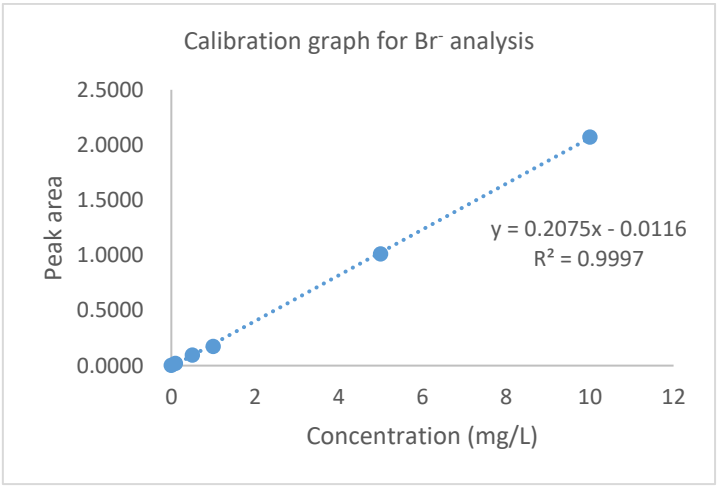


Figure D 5. Calibration graph for Br^- measurements in water samples.

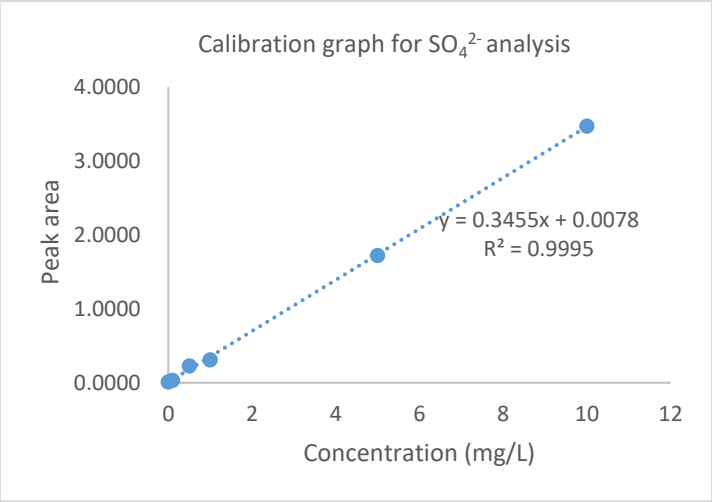


Figure D 6. Calibration graph for SO_4^{2-} measurements in water samples.

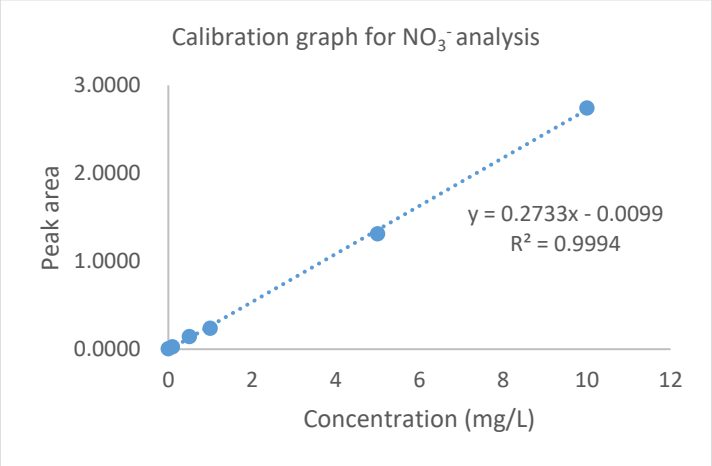


Figure D 7. Calibration graph for NO_3^- measurements in water samples.

D.3 Major cations measurements by MP-AES

Agilent 4100 MP-AES was used to measure concentrations of four cations including Ca^{2+}, Na^{+}, K^{+} and Mg^{2+} in water samples.

D.3.1. General procedure

First, we need to inspect the plasma torch to make sure it is not broken or does not have any stains on from the previous measurements. The pre-optic window must be inspected as well. After switching on the plasma and the gas flows (both nitrogen and argon) we need to wait 30 minutes until the instrument warms up. After that we need to rinse the plasma chamber with Type 2 water with help of the autosampler and the pump (the details are mentioned in the SOP). In addition, the flow coming out of the instrument must always be checked to make sure that we have a constant flow. For each element that we analyze we need to optimize the nebulizer pressure and viewing position of the instrument. Then we need to set up a program for each element measurement that consists of the appropriate emission wavelength for that element, the concentration of the standards, the calibration error and some other parameters which are mentioned in detail in the instrument's SOP. After setting the program, the instrument first analyzes the standard solutions, creates a calibration curve and then measures the metal content of the samples.

Figure D 8. Agilent 4100 MP-AES.

D.3.2. Instrument set up

Table D 3. Instrument set up in major cations measurements.

Replicates	3
Pump speed (rpm)	15
Sample introduction	Autosampler
Uptake time (sec)	15
Stabilization time (sec)	20
Rinse time (sec)	30
Correlation coefficient limit	0.99

D.3.3 Calibration graphs

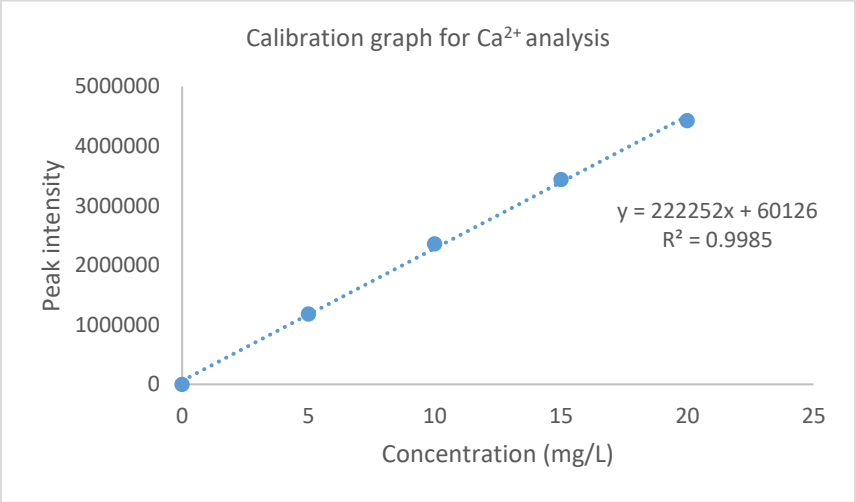


Figure D 9. Calibration graph for Ca^{2+} measurements (emission at 422.6 nm).

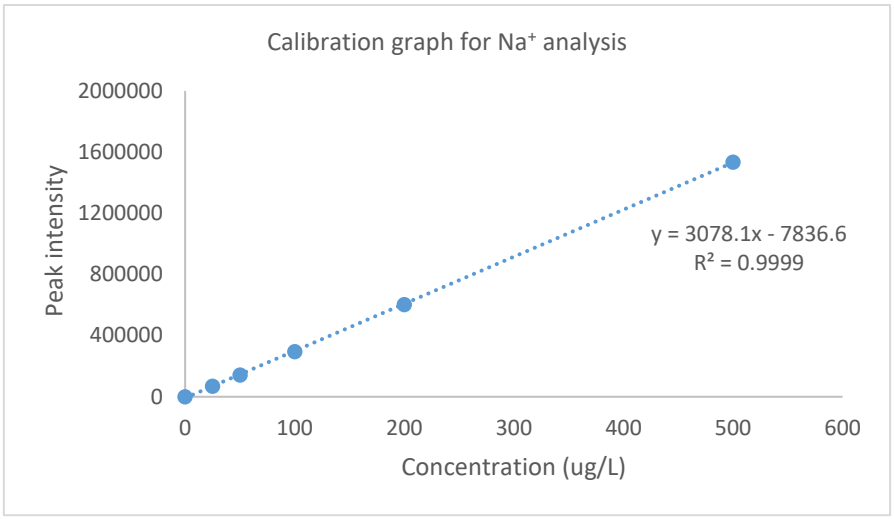


Figure D 10. Calibration graph for Na $^{+}$ measurements (emission at 589.5 nm).

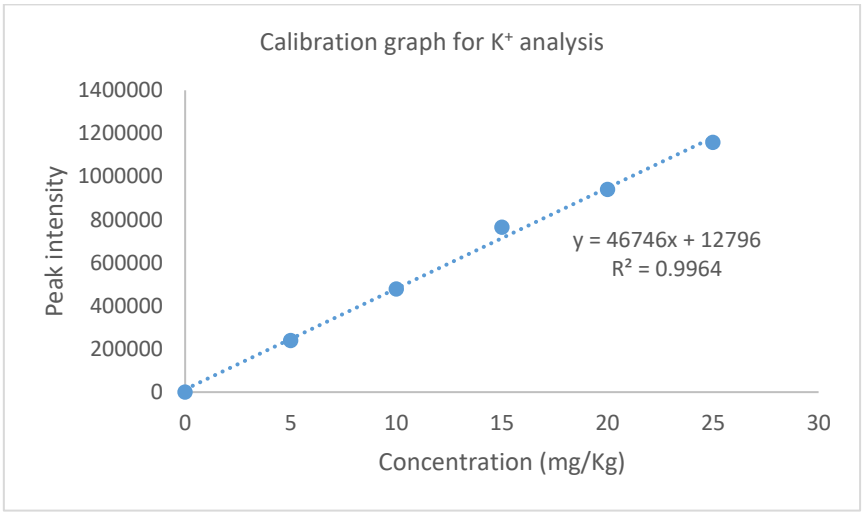


Figure D 11. Calibration graph for K $^{+}$ measurements (emission at 769.8 nm).

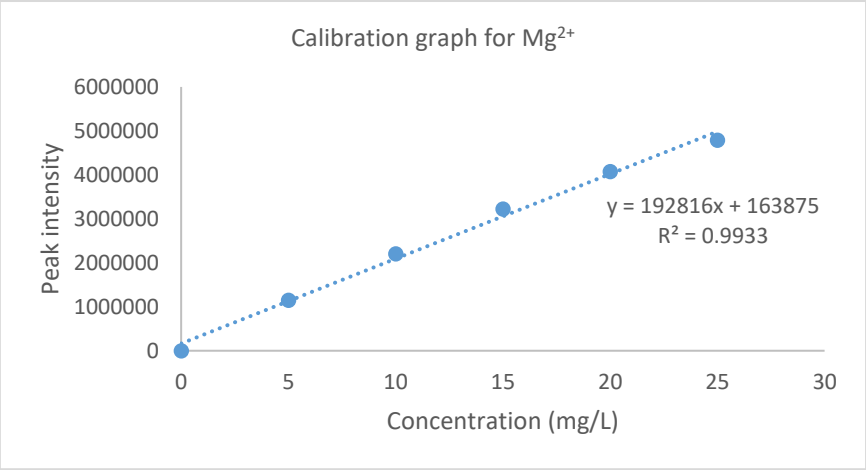


Figure D 12. Calibration graph for K^+ measurements (emission at 285.2 nm).

Appendix E THg measurements

Appendix E contains information about the DMA analysis procedure (E.1, E.2 and E.3), as well as the measured concentrations in dry fish and sediment samples (E.4), recovery of reference materials (E.5), RSD of measurements (E.6) and certificate of analysis for the reference materials (E.7). Finally measured THg in same fish samples by us and researchers at NIVA is presented (E.8).

E.1 Concentration of standard solutions and calibration graphs

Both low and high concentration of Hg standard solutions were prepared to build a calibration graph. The concentrations and the calibration graphs are shown below.

Table E 1. Low concentration standard solutions for Hg analysis.

Low Concentration solutions	V (mL) 1000 ng/mL Hg stock solution	Total volume (mL)	C (ng/mL) Mercury	m (ng) Mercury
#0	0	100	0	0
#1	0.2	100	2	1
#2	0.4	100	4	2
#3	1	100	10	5
#4	2	100	20	10

#5	4	100	40	20
#6	6	100	60	30

Table E 2. High concentration standard solutions for Hg analysis.

High Concentration solutions	V (mL) from 10000 ng/mL Hg stock solution	Total volume (mL)	C (ng/mL) Mercury	m (ng) Mercury
#0	0	100	0	0
#1	2	100	200	100
#2	4	100	400	200
#3	6	100	600	300
#4	8	100	800	400
#5	10	100	10000	500

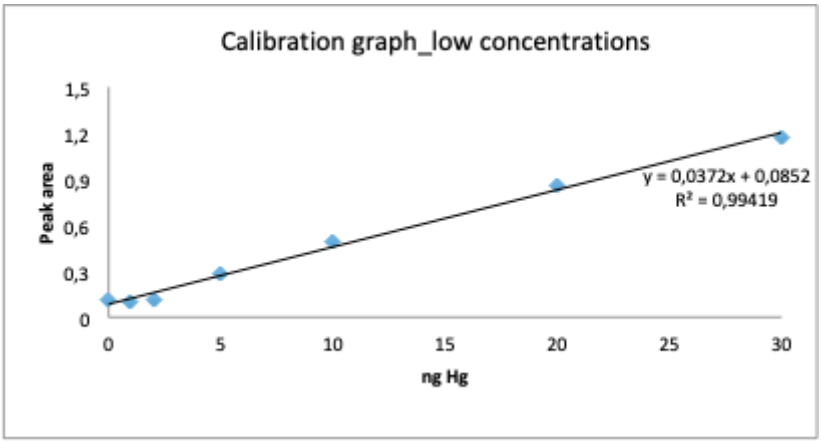


Figure E 1. Calibration graph for low concentration Hg standard solutions.

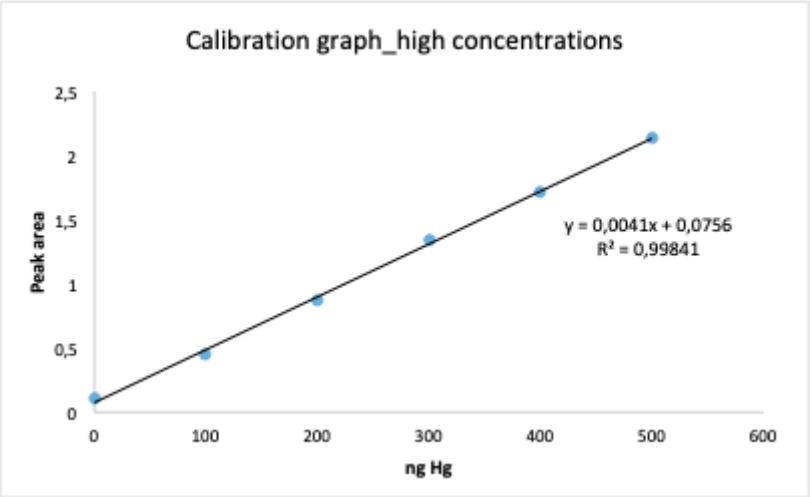


Figure E 2. Calibration graph for high concentration Hg standard solutions.

E.2. General procedure to measure Hg by DMA-80

The instrument requires at least 20 minutes to heat up. Prior any analysis, the boats need to be cleaned using a cleaning program. To prepare calibration curves, blank and standard solutions were added to the boats (0.5 mL ≈ 0.5 g) and their Hg content was measured. To analyze Hg content of an unknown sample we can use either the high or low concentration calibration curves depending on their approximate concentrations.

Figure E 3. Milestone DMA-80.

E.3. Instrument set up

E.3.1 Cleaning boats

Table E 3. DMA-80 instrument set up for cleaning the boats.

Parameter	Value
Max start T (°C)	500
Purge time (sec)	30
Amalgamator time (sec)	12
Signal recording time (sec)	24

Table E 4. DMA-80 temperature program for cleaning the boat.

Step	Temperature (°C)	Time (hour:min:sec)
Drying	850	35
Decomposition	850	45

E.3.2 Calibration graphs

Table E 5. DMA-80 instrument set up for THg measurements in calibration solutions.

Parameter	Value
Max start T (°C)	400
Purge time (sec)	60
Amalgamator time (sec)	12
Signal recording time (sec)	30

Table E 6. DMA-80 temperature program for THg measurements in calibration solutions.

Step	Temperature (°C)	Time (hour:min:sec)
Ramp to drying step	300	00:00:30
Drying	300	00:05:50
Ramp to decomposition step	850	00:02:00
Decomposition	850	00:03:00

E.3.3 Fish samples

Table E 7. DMA-80 instrument set up for THg measurements in fish samples.

Parameter	Value
Max start T (°C)	300
Purge time (sec)	60
Amalgamator time (sec)	12
Signal recording time (sec)	30

Table E 8. DMA-80 temperature program for THg measurements in fish samples.

Step	Temperature (°C)	Time (hour:min:sec)
Ramp to drying step	300	00:00:10
Drying	300	00:01:00
Ramp to decomposition step	750	00:01:00
Decomposition	750	00:02:00

E.3.4 Sediment samples

Table E 9. DMA-80 instrument set up for THg measurements in sediment samples.

Parameter	Value
Max start T (°C)	500
Purge time (sec)	30
Amalgamator time (sec)	12
Signal recording time (sec)	24

Table E 10. DMA-80 temperature program for THg measurements in sediment samples.

Step	Temperature (°C)	Time (hour:min:sec)
Ramp to drying step	300	00:00:30
Drying	300	00:00:10
Ramp to decomposition step	850	00:02:00
Decomposition	850	00:04:20

E.4 Measured THg

E.4.1 Fish samples

Table E 11. Measured THg in dry fish samples from Flåte (mg/Kg).

Sample ID	Sample weight (g)	THg	Sample ID	Sample weight (g)	THg
F_1	0.02076	1.408	F_14	0.01226	2.295
F_2	0.02226	1.486	F_15	0.01163	2.025
F_3	0.0230	1.564	F_16	0.01204	2.803
F_4	0.02251	1.571	F_17	0.02136	0.998
F_5	0.0249	1.734	F_18	0.02506	1.089
F_6	0.01442	1.839	F_19	0.01466	1.440
F_7	0.02148	1.303	F_20	0.01602	1.261
F_8	0.0290	1.391	F_21	0.01307	2.223
F_9	0.02644	1.597	F_22	0.01228	1.643
F_10	0.02492	2.101	F_23	0.01454	2.218
F_11	0.0193	1.190	F_24	0.01595	1.773
F_12	0.0208	1.701	F_25	0.01396	2.688
F_13	0.01995	2.165	-	-	-

Table E 12. Measured THg in dry fish samples from Svanstulvatnet (mg/Kg).

Sample ID	Sample weight (g)	THg	Sample ID	Sample weight (g)	THg
S_1	0.02076	1.408	S_14	0.01226	2.295
S_2	0.02226	1.486	S_15	0.01163	2.025
S_3	0.0230	1.564	S_16	0.01204	2.803
S_4	0.02251	1.571	S_17	0.02136	0.998
S_5	0.0249	1.734	S_18	0.02506	1.089
S_6	0.01442	1.839	S_19	0.01466	1.440
S_7	0.02148	1.303	S_20	0.01602	1.261
S_8	0.0290	1.391	S_21	0.01307	2.223
S_9	0.02644	1.597	S_22	0.01228	1.643
S_10	0.02492	2.101	S_23	0.01454	2.218
S_11	0.0193	1.190	S_24	0.01595	1.773
S_12	0.0208	1.701	S_25	0.01396	2.688
S_13	0.01995	2.165	-	-	-

Table E 13. Measured THg in dry fish samples from Gunneklevfjorden (mg/Kg).

Sample ID	Sample weight (g)	THg	Sample ID	Sample weight (g)	THg
G_1	0.00861	3.044	G_14	0.00723	3.639
G_2	0.00785	2.983	G_15	0.00813	1.245
G_3	0.0109	3.735	G_16	0.00880	3.757
G_4	0.00792	2.937	G_17	0.00947	3.474
G_5	0.01072	2.049	G_18	0.01125	3.790
G_6	0.01063	3.415	G_19	0.01134	0.925
G_7	0.00909	3.955	G_20	0.00890	3.225
G_8	0.00919	4.029	G_21	0.00855	3.779
G_9	0.01031	2.960	G_22	0.00860	3.015
G_10	0.01157	2.896	G_23	0.01154	3.058
G_11	0.00971	1.401	G_24	0.00924	3.056
G_12	0.00712	1.217	G_25	0.00780	3.680
G_13	0.00929	4.434	-	-	-

E.4.2 Sediment samples

Table E 14. Measured THg (mg/Kg) in sediment samples from three water bodies.

Sample ID	Water body	Sample weight	THg
F-1	Flåte	0.034	0.092
F-2	Flåte	0.039	0.085
F-3	Flåte	0.033	0.015
S-1	Svanstulvatnet	0.014	4.50
S-2	Svanstulvatnet	0.015	9.86
S-3	Svanstulvatnet	0.016	1.16
S-4	Svanstulvatnet	0.010	0.270
S-5	Svanstulvatnet	0.012	0.386
S-6	Svanstulvatnet	0.010	0.185
G-1	Gunneklevfjorden	0.011	4.48
G-2	Gunneklevfjorden	0.008	12.0
G-3	Gunneklevfjorden	0.008	10.8
G-4	Gunneklevfjorden	0.014	10.2
G-5	Gunneklevfjorden	0.010	4.90
G-6	Gunneklevfjorden	0.010	57.2
G-7	Gunneklevfjorden	0.009	35.1
G-8	Gunneklevfjorden	0.006	245
G-9	Gunneklevfjorden	0.007	120
G-10	Gunneklevfjorden	0.006	81.4

E.5 Recovery of reference material in THg measurements

E.5.1 Fish samples

Three Standard Reference Material (DORM-4 fish protein) samples were analyzed at the beginning of each sequence. After every 10th sample one SRM sample was analyzed and recovery of reference material was measured.

Table E 15. Recovery of reference material in THg measurements in fish samples from Flåte.

	Recovery (%)
DORM-4-1	106
DORM-4-2	100
DORM-4-3	107
DORM-4-4	103
DORM-4-5	106

Table E 16. Recovery of reference material in THg measurements in fish samples from Svanstulvatnet.

	Recovery (%)
DORM-4-1	98
DORM-4-2	102
DORM-4-3	101
DORM-4-4	101
DORM-4-5	109

Table E 17. Recovery of reference material in THg measurements in fish samples from Gunneklevfjorden.

	Recovery (%)
DORM-4-1	91
DORM-4-2	109
DORM-4-3	104
DORM-4-4	101
DORM-4-5	109

E.5.2 Sediment samples

Three Standard Reference Material (BCR-277R) samples were analyzed at the beginning of the sediment sequence. After every 10th sample one SRM sample was analyzed and recovery of reference material was measured.

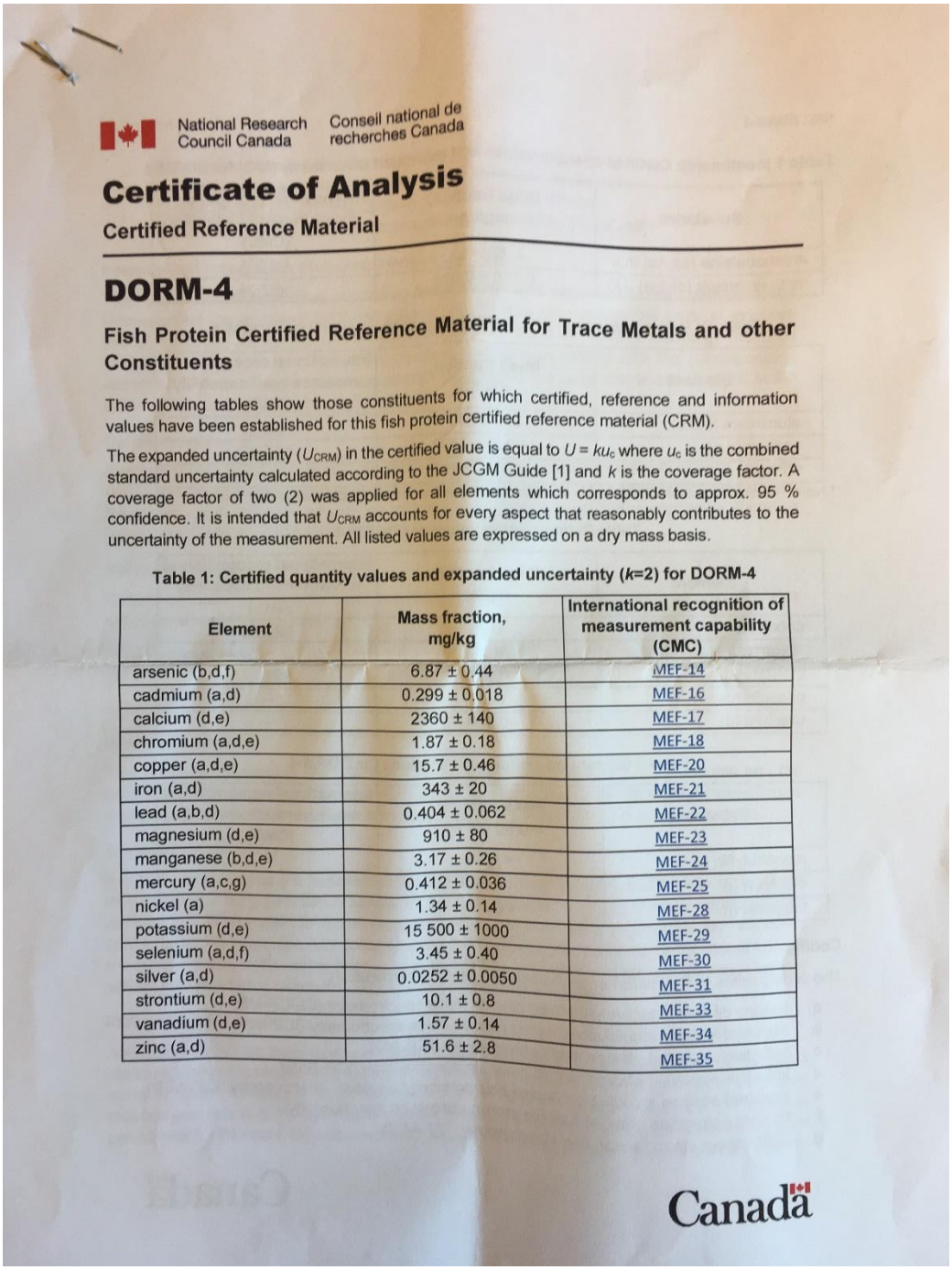
Table E 18. Recovery of reference material in THg measurements in sediment samples samples from three water bodies.

	Recovery (%)
BCR-277R-1	101
BCR-277R-2	101
BCR-277R-3	99
BCR-277R-4	104
BCR-277R-5	98

E.6 Calculated RSD of doublets in THg measurement

For quality assurance of THg measurements in fish and sediment samples 10% of samples were analyzed as duplicates and RDS was calculated. RSD for fish sample doublets was 2.29, 5.84, 0.36, 0.25, 7.78 and 1.67%. In case of sediment sample duplicates RSD was 2.7 and 7.73.

E.7. Reference materials



National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Certified Reference Material

DORM-4

Fish Protein Certified Reference Material for Trace Metals and other Constituents

The following tables show those constituents for which certified, reference and information values have been established for this fish protein certified reference material (CRM).

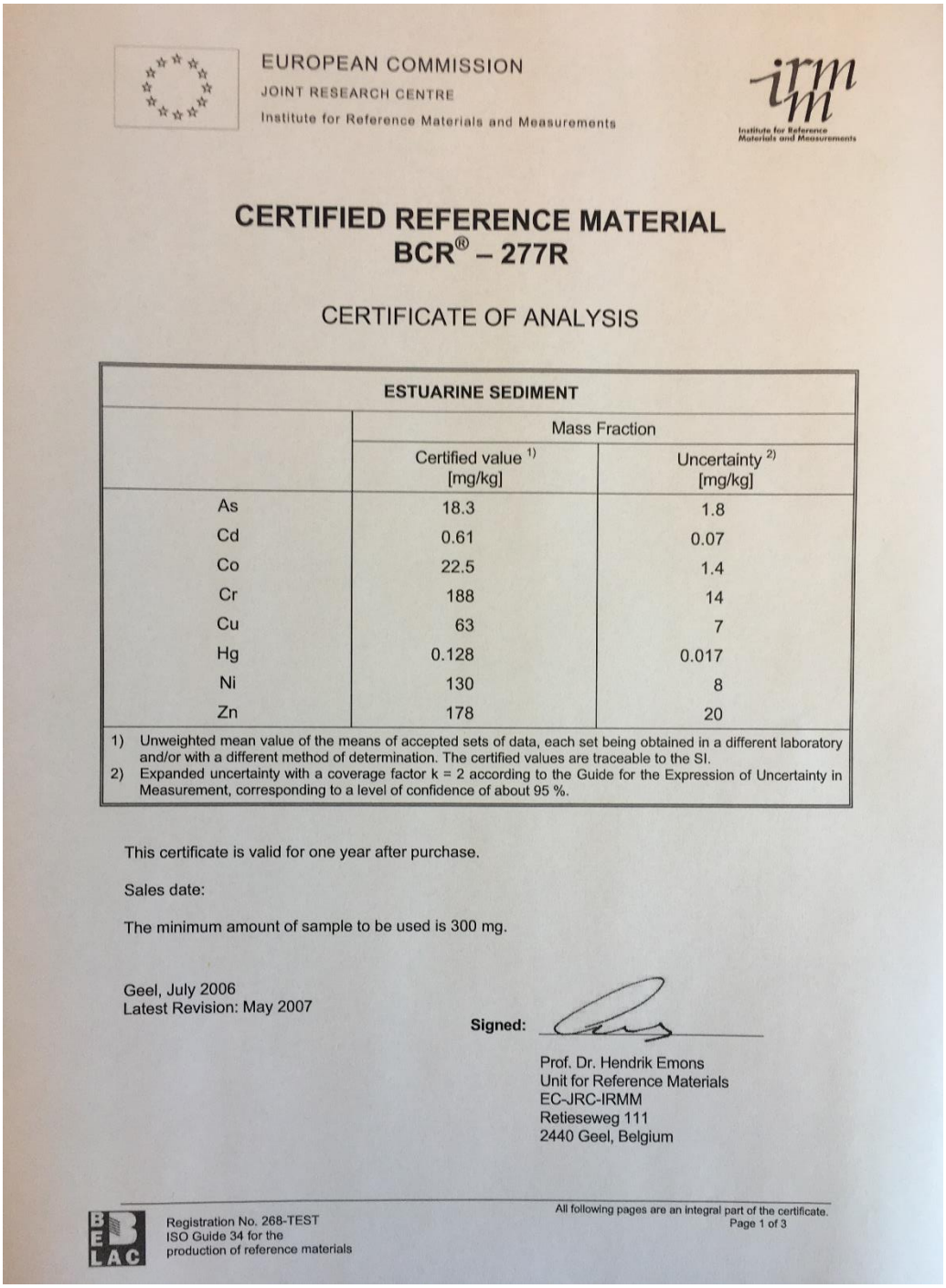
The expanded uncertainty (U_{CRM}) in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM Guide [1] and k is the coverage factor. A coverage factor of two (2) was applied for all elements which corresponds to approx. 95 % confidence. It is intended that U_{CRM} accounts for every aspect that reasonably contributes to the uncertainty of the measurement. All listed values are expressed on a dry mass basis.

Table 1: Certified quantity values and expanded uncertainty (k=2) for DORM-4

Element	Mass fraction, mg/kg	International recognition of measurement capability (CMC)
arsenic (b,d,f)	6.87 ± 0.44	MEF-14
cadmium (a,d)	0.299 ± 0.018	MEF-16
calcium (d,e)	2360 ± 140	MEF-17
chromium (a,d,e)	1.87 ± 0.18	MEF-18
copper (a,d,e)	15.7 ± 0.46	MEF-20
iron (a,d)	343 ± 20	MEF-21
lead (a,b,d)	0.404 ± 0.062	MEF-22
magnesium (d,e)	910 ± 80	MEF-23
manganese (b,d,e)	3.17 ± 0.26	MEF-24
mercury (a,c,g)	0.412 ± 0.036	MEF-25
nickel (a)	1.34 ± 0.14	MEF-28
potassium (d,e)	15 500 ± 1000	MEF-29
selenium (a,d,f)	3.45 ± 0.40	MEF-30
silver (a,d)	0.0252 ± 0.0050	MEF-31
strontium (d,e)	10.1 ± 0.8	MEF-33
vanadium (d,e)	1.57 ± 0.14	MEF-34
zinc (a,d)	51.6 ± 2.8	MEF-35

Canada

Figure E 4. DORM-4 fish protein as the reference material for fish THg analysis.



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements

CERTIFIED REFERENCE MATERIAL
BCR® – 277R

CERTIFICATE OF ANALYSIS

ESTUARINE SEDIMENT		
	Mass Fraction	
	Certified value [1] [mg/kg]	Uncertainty [2] [mg/kg]
As	18.3	1.8
Cd	0.61	0.07
Co	22.5	1.4
Cr	188	14
Cu	63	7
Hg	0.128	0.017
Ni	130	8
Zn	178	20

1) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The certified values are traceable to the SI.
2) Expanded uncertainty with a coverage factor k = 2 according to the Guide for the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 300 mg.

Geel, July 2006
Latest Revision: May 2007

Signed:

Prof. Dr. Hendrik Emons
Unit for Reference Materials
EC-JRC-IRMM
Retieseweg 111
2440 Geel, Belgium

Registration No. 268-TEST
ISO Guide 34 for the
production of reference materials

All following pages are an integral part of the certificate.
Page 1 of 3

Figure E 5. Sediment reference material for THg analysis in sediment samples.

E.8. Comparison of measured THg in wet fish samples

Table E 19. THg in wet fish samples from Flåte.

Sample ID	THg $_{\text{wet fish muscle}}$ ($THg_{\text{dry fish muscle}}$/5)	THg measured by NIVA
F_1	0.167	0.179
F_2	0.093	0.117
F_3	0.135	0.152
F_4	0.181	0.18
F_5	0.192	0.225
F_6	0.218	0.267
F_7	0.254	0.318
F_8	0.236	0.299
F_9	0.169	0.179
F_10	0.144	0.198
F_11	0.173	0.188
F_12	0.165	0.204
F_13	0.202	0.197
F_14	0.185	0.191
F_15	0.149	0.183
F_16	0.184	0.18
F_17	0.240	0.255
F_18	0.167	0.174
F_19	0.167	0.214
F_20	0.169	0.178
F_21	0.122	0.149
F_22	0.151	0.154
F_23	0.157	0.158
F_24	0.163	0.193
F_25	0.163	0.185

Table E 20. THg in wet fish samples from Svanstulvatnet.

Sample ID	THg $_{\text{wet fish muscle}}$ ($THg_{\text{dry fish muscle}}$/5)	THg measured by NIVA
S_1	0.282	0.288
S_2	0.297	0.289
S_3	0.313	0.357
S_4	0.314	0.307
S_5	0.347	0.356
S_6	0.368	0.348
S_7	0.261	0.257
S_8	0.278	0.301
S_9	0.319	0.297
S_10	0.420	0.35
S_11	0.238	0.237
S_12	0.340	0.336
S_13	0.433	0.458
S_14	0.459	0.437
S_15	0.405	0.367
S_16	0.561	0.511
S_17	0.199	0.183
S_18	0.218	0.219
S_19	0.288	0.304
S_20	0.252	0.232
S_21	0.445	0.426
S_22	0.329	0.307
S_23	0.444	0.417
S_24	0.355	0.331
S_25	0.538	0.483

Table E 21. THg in wet fish samples from Gunneklevfjorden.

Sample ID	$THg_{\text{wet fish muscle}}$ ($THg_{\text{dry fish muscle}}/5$)	THg measured by NIVA
G_1	0.609	0.525
G_2	0.597	0.573
G_3	0.747	0.623
G_4	0.587	0.513
G_5	0.409	0.352
G_6	0.683	0.71
G_7	0.791	0.72
G_8	0.806	0.777
G_9	0.592	0.548
G_10	0.579	0.559
G_11	0.280	0.307
G_12	0.243	0.254
G_13	0.887	1.008
G_14	0.728	0.63
G_15	0.249	0.246
G_16	0.751	0.707
G_17	0.695	0.649
G_18	0.758	0.729
G_19	0.185	0.18
G_20	0.645	0.605
G_21	0.756	0.616
G_22	0.603	0.545
G_23	0.612	0.576
G_24	0.611	0.526
G_25	0.736	0.598

Appendix F Microwave digestion of fish samples

In order to measure heavy metal content in fish samples we used microwave digestion instrument to dissolve the fish at high pressure and high temperature. Appendix F information about fish samples digestion procedure.

F.1. General procedure

The vessels were cleaned with a mixture of HNO_3, H_2O_2 and Type 1 water using a cleaning program with the microwave oven. After the cleaning step, the samples are weighed directly in the vessels and the digestion solution is added to them. The volume of the digestion solution in the vessels should always be below 10 mL. After installation of the vessels into the instrument, a digestion method was defined as below.

Table F 1. Temperature program used in microwave digestion of fish samples.

No.	Time	E (W)	Temperature (°C)	Step
1	00:10:00	1000	50	Ramp
2	00:05:00	1000	50	
3	00:10:00	1000	100	Ramp
4	00:05:00	1000	100	
5	00:05:00	1000	150	Ramp
6	00:01:00	1000	150	
7	00:05:00	1000	200	Ramp
8	00:15:00	1000	200	
9	00:20:00	0	20	Cooling

F.2 Microwave digestion reagents

Table F 2. Reagents used for microwave digestion of fish samples.

Reagent	Concentration (%)	Volume (mL)	Grade
HNO_3	65	7	Suprapur
H_2O_2	35	1	Purum Pa.

F.3 Cleaning of microwave vessels

Table F 3. Reagents used for cleaning microwave vessels.

Reagents	Concentrations (%)	Volume (mL)	Grade
HNO_3	65	5	Suprapur
H_2O_2	35	1	Purum Pa.
Water	-	4	Type 1

Table F 4. Temperature program used for cleaning microwave vessels.

Time	Temperature (°C)	Power (watt)	Step
00:05:00	180	1000	Ramp
00:10:00	180	1000	
00:10:00	-	0	Cooling

F.4 Digested fish samples

Table F 5. Selected fish samples for microwave digestion.

Fish sample ID	Sample amount (mg)
DORM-4 fish protein	449
F_5	317
F_8	150
S_16	259
S_19	233
S_22	305
G_1	327
G_2	329
G_13	334

Appendix G ICP-MS

Appendix G contains information about the ICP-MS analysis procedure (G.1, G.2, G.3). The concentrations measured of the reference material are found in G.4.

G.1 Setup

Table G 1. Instrumental setup of ICP-MS.

Component/Parameter	Type/value/Mode
Nebulizer	Meinhard glass concentric
Spray chamber	Glass cyclonic
Triple cone interface material	Nickel/Aluminum
Plasma gas flow	17.0L/min
Auxiliary gas flow	1.2L/min
Nebulizer gas flow	Ca. 0.96L/min
Peristaltic pump	24rpm
RF power	1000W
Integration time	1000ms
Replicates per sample	3
Mode of operation	Standard

G.2 Isotopes measured

Table G 2. Isotopes selected for ICP-MS analysis.

Analyte	Mass (amu)
As	75
Pb	208
Mn	55
Cu	63
Co	59
Ni	60
Sr	88
Ba	138
Se	78
Zn	64
Cd	114

G.3 Calibration curves

Table G 3. Calibration approximation equations and R^2.

Elements	Linear through zero	R^2
As	3024.7x	0.9994
Pb	19572x	0.9983
Mn	43997x	0.9990
Cu	15680x	0.9995
Co	36051x	0.9987
Ni	7723x	0.9995
Sr	50170x	0.9991
Ba	49230x	0.9992
Se	721.8x	0.9996
Zn	4939.5x	0.9987
Cd	10860x	0.9986

G.4 Concentrations measured of reference material

Table G 4. Concentrations measured of reference material.

	As (mg/kg) Ref. Conc.	Pb (mg/kg)	Cd (mg/kg)	Ni (mg/kg)	Se (mg/kg)	Cu (mg/kg)
BCR-277R	6.351	0.527	0.275	1.338	3.483	13.52