Biodegradability and Spectroscopic Properties of Dissolved Natural Organic Matter Fractions Linked to Hg and MeHg Transport and Uptake

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Abstract
To understand factors governing concentrations of the potent neurotoxin methyl mercury (MeHg) in surface waters, a study of the processes transporting total mercury (TotHg) and/or methyl mercury (MeHg) from the catchment soils to the surface waters, with dissolved natural organic matter (DNOM) as a transport vector, is needed. This study shows that high molecular weight (HMW) and low molecular (LMW) dissolved natural organic matter (DNOM) size fractions had differences in biodegradability, and differences in TotHg and MeHg concentrations. This is of large significance because DNOM stimulates microbial activity, which could lead to TotHg and/or MeHg being introduced to the food chain.

The use of tangential flow filtration (TFF) for size fractionation of DNOM was investigated and an optimal procedure was developed for it. The performance of the polysulfone membranes with a nominal molecular weight cut-off of 10 kDa for the summer samples, and 100 kDa for the fall samples, was examined on the Inlet and Outlet samples from the dystrophic lake Langtjern. In addition, a reference material collected from the same lake, was studied. After DNOM size fractionation, excitation-emission fluorescence and UV-VIS spectra confirmed that the HMW DNOM compounds were isolated in the Influent, i.e. < 0.2 µm, and Concentrate, i.e. 0.2 µm-10 kDa or 0.2 µm-100 kDa, size fractions. It was also confirmed that the LMW DNOM compounds were isolated in the Permeate size fraction, i.e. < 10 kDa or 100 kDa.

The highest relative amount of TotHg, i.e. \( \frac{\text{TotHg}(\text{ng L}^{-1})}{\text{DOC}(\text{mg L}^{-1})} \) ratio, was found in the HMW size fraction for both the Inlet and the Outlet samples in the fall. However, this was not observed in the summer samples. The reason for this was that the DOC in this LMW fraction was found to be below the method limit of quantification (MLOQ). The highest relative amount of methylated Hg, i.e. \( \frac{\text{MeHg}(\text{ng L}^{-1})}{\text{TotHg}(\text{ng L}^{-1})} \cdot 100 \) ratio, in the fall samples was found in the LMW DNOM fraction, i.e. < 100 kDa, in both the Inlet and Outlet samples, with values of 16% and 6%, respectively. For the samples collected in the summer, MeHg in the LMW fraction, i.e. < 10 kDa, was found to be below the method limit of detection (MLOD). Therefore, it was not possible to conclude whether this fraction also had the highest relative amount of MeHg. Since most of the MeHg in the fall sample was found in the most bioavailable fraction, although not to a high degree, this could cause the MeHg to be introduced to the food web.
Preface
The research study presented in this thesis was conducted as an integral part of the work package “Process-oriented studies of catchment and in-lake Hg cycling” in the research project “Climatic, Abiotic and Biotic Drivers of Mercury in Freshwater Fish in Northern Ecosystems” (CLIMER) in cooperation with the Norwegian Institute for Water Research (NIVA).

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Finally, I want to thank all my family and friends, especially my parents Rafael Martínez Díaz and Adela Francés Cachero for encouraging and supporting me during all my studies, not least when I decided to continue in Norway. Thank you to my grandparents Rafael, Pepita, Antonia and Antonio for your unconditional love and affection. Thank you abuelo Rafael for being who you are and for always been there for my brother and me. Thank you to my brother Rafael Martínez Francés, sister in law Laura Nieto López and my nephew Carlos Martínez Nieto for being part of my family. My last thanks go to Henrik Aksel Øien for helping me with everything, not only for listening to my problems and difficulties during this master thesis, but also for helping me with the formatting of the thesis, supporting and encouraging me. Thank you for being part of my life.
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List of abbreviations
Influent Fraction < 0.2 µm
Concentrate Fraction between 0.2 µm and 10 kDa (=0.001 µm) or 0.2 µm and 100 kDa (=0.01 µm)
Permeate Fraction < 10 kDa or < 100 kDa
NOM Natural organic matter
DNOM Dissolved natural organic matter
CDNOM Colour natural organic matter
Hg²⁺ Inorganic mercury
Hg⁰ Elemental mercury
MeHg Methyl mercury
TotHg Total mercury
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMW</td>
<td>High molecular weight</td>
</tr>
<tr>
<td>LMW</td>
<td>Low molecular weight</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse osmosis</td>
</tr>
<tr>
<td>TFF</td>
<td>Tangential flow filtration</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>MLOD</td>
<td>Method limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>MLOQ</td>
<td>Method limit of quantification</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
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1. Introduction

1.1 Background

The high concentration of the semi-volatile mercury and the persistent organic pollutants (POPs) at northern latitudes is attributed to a process known as global distillation. In this process pollutants, such as mercury (Hg), are transported by wind currents from warmer to colder areas, where they are subsequently trapped and accumulated due to low temperatures. Throughout this process, also referred to as grasshopper effect, chemicals repeatedly evaporate and condense on their journey toward the Arctic. They can condense directly on to the Earth’s surface, or on solid particles contained in the atmosphere (aerosols), which are then deposited with rain or snow (Wania, 2003). Moreover, the level of pollutants increase in cold and dark polar regions, where they are less likely to be degraded, resulting in high concentrations.

Hg emissions, predominantly in the long-lived gaseous elemental form (Hg\textsubscript{0}) are slowly oxidised to more reactive divalent forms, i.e. Hg\textsuperscript{2+} that readily deposit to marine and terrestrial ecosystems. The historical impact of natural and anthropogenic Hg emissions on deposition has been investigated using environmental archives such as ice cores (Schuster et al., 2002), lake sediments (Fitzgerald et al., 2005) and peat bogs (Martinez-Cortizas et al., 1999). On the basis of shallow lake sediments Hg deposition has increased by a factor 3 ± 1 since preindustrial times (Enrico et al., 2017). Deeper lake sediments and peat archives probing the Holocene Era also suggest an increase in Hg deposition in present times (Amos et al., 2015). Trends in Hg deposition are governed by a combination of anthropogenic emissions, re-volatilisation, atmospheric Hg concentrations and residence times (Enrico et al., 2017).

The primary natural sources of Hg emissions into the atmosphere are volcanoes, geothermal sources and topsoil enriched in Hg, whereas the re-emission of previously deposited Hg on vegetation, land or water surfaces is primarily related to land use changes, biomass burning and meteorological conditions (Pirrone et al., 2001; Mason, 2009). Hg can also be released to the atmosphere from a large number of anthropogenic sources. For instance, coal burning to generate electricity releases small amounts of mercury after the flue gas desulphurisation process, which is used to remove sulphur dioxide from exhaust flue gasses of fossil-fuel plants. The burning of oil also produces significant air pollution.
in the form of nitrogen oxides, carbon dioxide, methane, heavy metals such as mercury, and volatile organic compounds. Hg is also emitted from non-ferrous metal industries from copper, zinc, and lead smelters. Hg was also employed to assist with the extraction of gold and silver from ore because it readily forms alloys with gold and silver amalgams (Pirrone et al., 2010). All of these processes generating Hg, among other contaminants, occur world-wide, transporting Hg around the world by winds and ocean currents to northern latitudes.

High concentrations of Hg have been found in catchments soils rich in organic matter, for instance in Southern Norway (Jackson, 1997; Poste et al., 2015). This is because Hg is a B-type metal cation, and thus binds strongly to reduced sulphur functional groups in organic matter (Ravichandran, 2004). Moreover, dissolved natural organic matter (DNOM) plays an important role in the transport and fate of most metals, including Hg, from the catchment soil into the surface water. Photochemical reactions in surface waters break the molecule of DNOM containing Hg, i.e. DNOM-Hg$^{2+}$, down into smaller compounds making it more bioavailable for bacterial consumption (Graham et al., 2013). Thus DNOM-Hg$^{2+}$ increases the risk of methyl mercury (MeHg) production in soils and aquatic ecosystems by providing food for the methylation of Hg$^{2+}$ to MeHg. Methylating organisms such as sulphur reducing bacteria (SRB), iron reducing bacteria (IRB), methanogens or archaea are responsible for this process. This is because these organisms have been found to contain the hgcAB gene cluster, which is necessary for methylation in many organisms (Paranjape and Hall, 2017).

Mercury exists in several forms in the environment, but of particular concern is MeHg, an organic compound that is highly bio-accumulative and strongly neurotoxic (Bloom, 1992; Poste et al., 2015). The level of MeHg in freshwater fish is increasing in many regions in the Nordic countries (Braaten et al., 2014), despite apparent declines in atmospheric Hg deposition during recent decades (Braaten and de Wit, 2016). Therefore, it is important to understand the biogeochemical cycling of Hg in aquatic environment. In particular, the increase in terrestrial loading of DNOM to boreal aquatic environments (Monteith et al., 2007), which over the past 30 years has likely had a strong effect on MeHg loading to freshwater (Grigal, 2002), cycling (Ullrich et al., 2001) and bioaccumulation processes (French et al., 2014; Poste et al., 2015).
Bioaccumulation of MeHg in fish and its toxicity to humans are attributed to MeHg’s high affinity for sulphur-containing proteins, such as metallothionein and glutathione, making it available in the food web (Halbach, 1995). MeHg poisoning is a slow process that can take months or even years before the effects become noticeable, according to the U.S. National Institutes of Health (NIH). MeHg from food sources is absorbed into the blood stream through the intestinal wall, and then carried through the body. The kidneys, which filter the blood, can accumulate MeHg over time, and other organs can also be affected. Negative effects from MeHg contamination may include neurological and chromosomal problems having significant impacts. The toxicity of MeHg may also have consequences for pregnant women, with an increased risk of miscarriage. Moreover, the babies may develop deformities or severe nervous system diseases (Bradford, 2016). The awareness of Hg as a threat to human health and the environment has led to international agreements to reduce Hg emission through the Minamata Convention on Mercury of the United Nations Environmental Programme in Geneva, Switzerland, in 2013.

1.2. Aim of the study: DNOM Linked to TotHg and MeHg Transport and Uptake

The overarching aim of this master thesis is to examine the physico-chemical properties and biodegradability of DNOM, which is empirically and conceptually linked to processes governing transport, bioavailability and uptake of TotHg and MeHg.

The hypothesis that were set out to test were that temporal differences in the relative amount of DNOM size fractions can contribute to explain fluctuations in TotHg and MeHg levels in freshwaters, and thereby food web exposure of MeHg through differences in bioavailability. It was hypothesised that the main bulk of Hg (i.e. TotHg) would be found in the HMW DNOM size fraction and that the main amount of MeHg would be found in the LMW DNOM fraction, which at the same time is easily biodegradable by bacteria. Size fractionation of DNOM was conducted with the use of tangential flow filtration (TFF). Biodegradability of the different DNOM size fractions was tested by the use of a sensor dish reader monitoring the oxygen consumption.

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Minamata has a special position in mercury history because of the release of MeHg through the industrial wastewater from a factory into a river, from 1932 to 1968. This caused severe Hg poisoning in Minamata’s inhabitants and animals, whose diet was based on shellfish and fish having an accumulation of MeHg. This poisoning affected the nervous system of people and animals causing loss of peripheral vision, damage to hearing and speech, and in extreme cases insanity, paralysis, coma and even death (Withrow et al., 2007).
Outlet and Inlet water samples collected from the lake Langtjern\textsuperscript{2}, a long-term ecological monitoring station, were pre-treated at NIVA by size fractionation with TFF to produce different fractions based on their molecular sizes. DNOM fractions were characterised by measuring the amount of dissolved organic carbon (DOC), which is approximately 50\% of the DNOM (Schnitzer and Khan, 1972; Thurman, 1985), in each size fraction, and by spectroscopic methods measuring UV–VIS and molecular fluorescence.

2. Theory

2.1 Natural Organic Matter

Natural Organic Matter (NOM) is a heterogeneous mixture of organic compounds comprised by the major elements carbon, hydrogen and oxygen. The major source of NOM is plant material and animal remains. It is mainly formed from dead organisms through incomplete biotic decay (microbial oxidation), abiotic oxidation and transformation processes (Thurman, 1985) followed by recombination (Hayes, 2009). NOM, also termed humus, affects numerous biochemical processes in soils (Stevenson, 1994)

NOM in soil and water exits as particles, colloids and dissolved molecules. It is appropriate to regard these distinctions dynamically, however, because organic matter can be inter-converted between these forms by dissolution or dissociation and precipitation, sorption and desorption, aggregation and disaggregation (Perdue and Ritchie, 2003).

NOM is classified into two different categories, non-humic and humic matter. Non-humic matter includes simple identifiable compounds, such as amino acids, carbohydrates, fats, waxes, resins, organic acids and other LMW dissolved organic matter (Schnitzer and Khan, 1972). Humic matter comprises complex large molecular weight compounds, i.e. HMW DNOM, which are mainly composed of aromatic units and aliphatic chains with functional groups such as carboxylic acid, phenolic and alcoholic hydroxyls attached to it (Gaffney et al., 1996). The HMW DOM is further classified according to three different categories or fractions: humic acids (HA), fulvic acids (FA) and humin. Generic

\textsuperscript{2} \url{http://www.niva.no/langtjern}. 

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molecular structures for HA and FA proposed by Stevenson (1982) and Buffle (1977), respectively, can be seen in Figure 1.

HA constitute the main fraction of NOM. HA are considered as degradation-resistant polyelectrolytic macromolecules of undefined structure. They have been found to contain larger content of fatty acids, which result in a more hydrophobic character (Beck et al., 1993). Moreover, HA is dominated by conjugated aromatic rings (Gaffney et al., 1996).

FA constitute the second largest moiety of NOM. They consist of more simple compounds than those found in HA (Choudhry, 1984; Stevenson, 1994). FA are further characterised by having a lower molecular weight and a higher O:C ratio than HA (Figure 1). The higher oxygen content of FA is attributed to a higher content of carboxylic (COOH) and phenolic (OH) functional groups, which results in a more acidic character (Stevenson 1985).

![Generic molecular structure of humic acids (Stevenson 1982) and fulvic acids (Buffle 1977).](image)

2.2 Dissolved Natural Organic Matter (DNOM)

Dissolved Natural Organic Matter (DNOM) is operationally defined as the fraction of NOM in solution not retained by 0.45 µm membrane filter, while the remaining fraction is termed as Particulate Organic Matter (POM). Concentration levels and physico-chemical properties of DNOM vary significantly in space and over time. On average roughly 50% of DNOM is carbon; the other main elements are oxygen, hydrogen, nitrogen and sulphur (Schnitzer and Khan, 1972; Thurman, 1985). The concentration of
DNOM is approximated by measuring the concentration of dissolved organic carbon (DOC) or the UV absorbance.

DNOM contains a variety of functional sites such as carboxylic, alcoholic and phenolic groups (Figure 1). It is a ubiquitous complexing agent of heavy metals, and its lipophilic moieties absorb persistent organic pollutants (POPs) (Tipping, 2002; Al-Reasi et al., 2011). DNOM therefore increases the mobility of heavy metals and organic contaminants by complexation and sorption, respectively, and thereby increases the loading of micro-pollutants from soils to surface waters. DNOM in aquatic systems is characterised by source of origin and classified as either allochthonous, coming from the terrigenous watershed, or autochthonous, derived within the aqueous lake itself. Allochthonous DNOM is thus produced on land and then washed into the water body (Thurman, 1985; Abbt-Braun and Frimmel, 1999; Tipping, 2002; Al-Reasi et al., 2011), whereas autochthonous DNOM is generated within the water column by microorganisms such as algae and bacteria (McKnight et al., 2001; Al-Reasi et al., 2011). In general, the allochthonous fraction of DNOM tends to be darker in colour, comprising more HA, while autochthonous DNOM is lighter and consists mainly of FA.

Due to their absorbance in the visible region, waters containing high concentration of humic matter are usually yellow to brown in colour, which is undesirable to tap water consumers. Moreover, the humic matter causes fouling in the drinking water distribution network. The removal of DNOM has thus been a major research interest for water treatment plants that use surface water as raw water sources. DNOM related studies have become more important due to increases in colour and concentration of DNOM in many of the water systems of the Northern Hemisphere over the last 20 years. Furthermore, shifts in DNOM levels and changes in its composition are of special concern due to its significance in aquatic ecosystems functioning. The main governing factors for this increase in DNOM concentrations seems to be related to the decrease in atmospheric acid deposition and the increasing impact of climate change agents (Pagano 2014).

2.3 Characterisation of DNOM using size fractionation techniques

Fifty years ago it was shown that dissolved humic substances (HS) in water could be separated into a number of different size fractions using gel filtration chromatography (Gjessing, 1965). Since then, different techniques and methods for DNOM fractionation have been studied to define and describe the physico-chemical properties and
composition of HS. The use of DNOM fractionation techniques, based on properties such as solubility, molecular size, charge and adsorption-desorption has extended the knowledge of molecular properties and characteristics of DNOM (Swift 1985).

In this study, tangential flow filtration (TFF) was used to size fractionate the DNOM based on molecular size. This was done to investigate the importance of HS both as a carrier and as a mediator of Hg transport from soil to surface water.

TFF is a technique used for fractionating colloids and dissolved compounds smaller than 0.2 µm in natural water systems. This technique is also referred to as cross-flow filtration where the solute flow, known also as Influent or Effluent, is tangential to the surface of the membrane. TFF enables the filtering of samples from 10 to 100 L, depending on the system, without clogging of the membrane. Accumulation of material on the membrane surface, known as fouling, can disturb the quantitative measurements of compounds associated with colloids and dissolved compounds. Fouling constitutes the main limitation of this technique, and depends on operational conditions (particularly cross-flow tangential velocity) and physico-chemical interactions of molecules with the membrane surface material (Yan-jun et al., 2000; Guéguen et al., 2002).

2.4 Characterisation of DNOM using spectroscopic techniques
Spectroscopic techniques comprise a range of proxies used to characterise the physico-chemical properties of DNOM. The results may be used to assess the role of DNOM and predict its fate in the environment. Absorption in the ultraviolet (UV) and visible (VIS) spectra and Fluorescence spectroscopic techniques were used in this study for this purpose.

2.4.1 UV-Visible absorbance
The UV-Visible (UV-VIS) spectrum refers to the electromagnetic radiation within 200 to 800 nm. The wavelength range of UV radiation starts at around 200 nm, and ends at the blue end of the visible light at approximately 400 nm (Figure 2). This radiation has enough energy to excite valence electrons in atoms and molecules; thus, UV radiation is involved in electronic excitation. Visible light is within a wavelength of 400 nm to 800 nm (Figure 2).
Absorption of UV-VIS radiation in surface waters is to a large degree attributed to aromatic chromophoric moieties in DNOM molecules, primarily in the humic fraction. Humic molecules are also thought to be largely responsible for the fluorescence in natural waters. UV–VIS spectra of DNOM are typically broad and nearly featureless. This is because the spectrum is the sum of a large number of different types of chromophores, and none possess an easily distinguishable spectrum (Leenheer and Croué, 2003). From about 200 nm DNOM’s absorbance always decreases with increasing wavelength (Appendix Section F.3 Figures 35-43).

Several UV-VIS absorbance indexes have been proposed to characterise the physico-chemical properties of DNOM, and in this study three such indexes were studied. The first, specific UV absorbance (sUVa), is defined as the absorbance at 254 nm normalized to the concentration of Dissolved Organic Carbon (DOC), i.e. \( \left( \frac{Abs_{254nm}}{DOC} \right) \times 100 \). This index is strongly related to the amount of aromatic moieties presented in DNOM (Vogt...
and Gjessing, 2008; Frimmel and Abbt-Braun, 2009). A high sUVa value indicates a large fraction of conjugated double bonds and aromatic ring moieties in DNOM. The second index, specific visible absorbance (sVISa), is defined as the absorbance at 400 nm normalised to the concentration of DOC, i.e. \( \left( \frac{Abs_{400\text{nm}}}{DOC} \right) \times 1000 \). This index is related to the amount of higher molecular weight chromophores (Vogt and Gjessing, 2008). In addition, the specific absorbance ratio (SAR\text{UV}), which is defined as the ratio of absorbance at 254 nm divided by 400 nm, was calculated. This index serves as a proxy for the relative contribution of lower to higher molecular weight chromophores (Vogt and Gjessing, 2008). A low SAR indicates more HMW organic compounds, and a low degree of conjugated aromatic rings.

2.4.2 Fluorescence Spectroscopy

Fluorescence may occur when an electron in an atom of a molecule is excited from its ground state to one of the various vibrational states, due to the absorbance of photon energy from the electromagnetic radiation. This excited electron may return to its ground state by emitting light energy in the form of fluorescence (Van Cleave 2011). The excitation and emission wavelength at which fluorescence occurs are characteristic to specific molecular structures (Fellman et al., 2010). Organic compounds that absorb and re-emit light are known as fluorophores (Mopper et al., 1996).

Characterisation of DNOM by fluorescence does not provide specific information on the chemical structure of DNOM or the concentration of organic compounds. The exact chemical compounds responsible for DNOM fluorescence are still undefined, but on a general basis fluorescence provides information regarding the content of fluorophore moieties of the DNOM, such as lignin, tannins, polyphenols, melanins, humic acids and fulvic acids. These aromatic compounds are usually responsible for the bulk of humic DNOM fluorescence in natural waters (Green and Blough, 1994; Del Vecchio and Blough, 2004; Fellman et al., 2010). Quinone moieties have also been suggested to contribute to humic DNOM fluorescence, and research has shown that more than half of DNOM fluorescence is potentially due to such structures (Cory and McKnight, 2005).

Molecular structure and fluorescence characterisation of DNOM

Fluorescence DNOM measurements are commonly collected as three-dimensional excitation emission matrix (EEM) contour plots. EEM contour plots are produced from
multiple emission spectra collected at successively increasing excitation wavelengths (Chen et al., 2003). Four general areas of excitation and emission wavelengths are constructed in which fluorescence is linked to ecologically meaningful characteristics of DNOM: humic-like peaks A and C, soluble microbial by product-like peak M, and protein-like peaks B and T (Figure 3 and Table 1).

![Figure 3 Sub-division of the EEM spectra of waters containing DNOM, with the position of the five primary fluorescence peaks A, C, M, B, and T. Modified from (Mohr, 2017).](image)

The group of peaks with humic-like components (A and C) are composed of a set of compounds referred to as humic acid-like (C) and fulvic acid-like (A) (Figure 3 and Table 1). In general peaks that exhibit emission at long wavelengths, such as A and C, are
referred, to as “red shifted ⁴”, and have broad emission maxima containing many conjugated fluorescence molecules. These compounds are aromatic, highly conjugated, and likely represent the HMW fraction of the DNOM pool (Coble et al., 1998). Such compounds are mainly derived from vascular plants, i.e. mainly of terrestrial origin. In contrast, peaks that exhibit emission at short wavelengths, such as peaks B, T and M (Figure 3 and Table 1), are referred to as “blue shifted ⁵”. These compounds are thought to be less aromatic and of lower molecular weight than peaks A and C (Fellman et al., 2010). The group of protein-like components (peaks B and T) are either tyrosine or tryptophan-like fluorescence components. These compounds are amino acids which are free or bound in proteins, or associated with LMW DNOM. These protein-like components may indicate more degraded peptide material in DNOM.

**Table 1 Summary of the commonly observed natural fluorescence peaks of aquatic DNOM (Fellman et al., 2010).**

<table>
<thead>
<tr>
<th>Component</th>
<th>Excitation and emission maxima (nm)</th>
<th>Peak name</th>
<th>Probable source</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine-like</td>
<td>ex &lt; 250 nm</td>
<td>B, T</td>
<td>Terrestrial,</td>
<td>Amino acids, free or bound in proteins. May indicate more degraded peptide material.</td>
</tr>
<tr>
<td></td>
<td>em &lt; 350 nm</td>
<td></td>
<td>Autochthonous,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Microbial.</td>
<td></td>
</tr>
<tr>
<td>Tryptophan-like</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble microbial by-product-like material</td>
<td>ex 250-280 nm</td>
<td>M</td>
<td>Terrestrial,</td>
<td>LMW.</td>
</tr>
<tr>
<td></td>
<td>em &lt; 380 nm</td>
<td></td>
<td>Autochthonous,</td>
<td>Common in marine environments. Associated with biological activity. Found in wastewater,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Microbial.</td>
<td>wetland, and in agricultural environments.</td>
</tr>
<tr>
<td>Fulvic-like</td>
<td>ex &lt; 260 nm</td>
<td>A</td>
<td>Terrestrial.</td>
<td>HMW humic substances, but smaller than the molecular weight of humic-like components.</td>
</tr>
<tr>
<td></td>
<td>em &gt; 350 nm</td>
<td></td>
<td></td>
<td>They are widespread, being highest in wetlands and forested environments.</td>
</tr>
<tr>
<td>Humic-like</td>
<td>ex &gt; 250 nm</td>
<td>C</td>
<td>Terrestrial.</td>
<td>HMW humic substances.</td>
</tr>
<tr>
<td></td>
<td>em &gt; 380 nm</td>
<td></td>
<td></td>
<td>They are widespread, being highest in wetlands and forest environments.</td>
</tr>
</tbody>
</table>

⁴ Red shift: A spectra shift towards higher wavelengths, i.e. lower energy and lower frequency, is called red shift or bathochromic shift (Prens., 2015).

⁵ Blue shift: A spectra shift towards lower wavelengths, i.e. higher energy and higher frequency, is called blue shift or hypochromic shift (Prens., 2015).
2.5 Biodegradation of DNOM

The term biodegradability is described as a measurement of the degree of utilisation of organic compounds, in this case DNOM, by microorganisms. Biodegradation of organic compounds could be complete, giving CO$_2$ and H$_2$O as products, or incomplete leading to partial oxidation and fragmentation of the original compound (Marschner and Kalbitz, 2003).

DNOM is a heterogeneous and continuous mixture of a broad range of different organic molecules and therefore is assumed to comprise three different pools regarding its biodegradability.

1. The labile pool, which includes DNOM that is rapidly biodegradable. This fraction is mainly dominated by fulvic substances and LMW DOM, and presents a more aliphatic character. This pool consists of carbohydrates, amino acids, amino sugars, and LMW proteins (Lynch, 1982; Qualls and Haines, 1992; Guggenberger et al., 1994; Küsel and Drake, 1998; Kaiser et al., 2001; Koivula and Hänninen, 2001).

2. The moderately biodegradable pool includes a relatively stable DNOM fraction, which probably contains polysaccharides, and other degradation products which are more slowly biodegraded (Marschner and Kalbitz, 2003).

3. The pool of recalcitrant DNOM is mainly dominated by humic acids with aromatic and complex structures. This aromatic character renders it more difficult for bacteria to biodegrade (Marschner and Kalbitz, 2003).

Scientists use different methods to quantify the biodegradability of DNOM. This is because no general accepted standard methods are established, and parameters such as type and duration of incubation, initial DOC concentration, nutrient addition, type and amount of inoculum added to the sample, and temperature, among others, may affect the final result. Of these parameters, duration of the incubation during biodegradation experiments seems to be of high importance for the quantification of DNOM biodegradability. Addition of nutrients will accelerate DNOM biodegradation (Marschner and Kalbitz, 2003).

In this study, biodegradation of DNOM was investigated by monitoring oxygen consumption for 72 hours, in the different size fractionations, with a sensor dish reader (SDR). This principle is explained in detail in Section 3.4.5.2 Biodegradation experiment.
2.5.1 Factors controlling DNOM biodegradability

The biodegradability of DNOM is controlled by numerous factors that can be divided into three categories (Marschner and Kalbitz, 2003).

2.5.1.1 Intrinsic DNOM characteristics

Molecular size and chemical structure is frequently associated with biodegradability, probably because microorganisms have limitations in their capacity in degrading certain aromatic and larger molecules. Therefore, we could expect that non-humic LMW compounds and humic compounds with less complex organic structures (i.e. aliphatic and hydrophilic compounds, as well as some fulvic acids) are more biodegradable than compounds with more complex organic structures, i.e. aromatic compounds (humic acids) (Marschner and Kalbitz, 2003). Aromatic compounds with high sUVa, which tells us about the degree of aromaticity, are commonly found to be more persistent in the environment as they are less biodegradable. On the other hand sUVa also reflects the extent to which DNOM absorbs UV radiation, and thus the potential for photo-oxidation and degradation of the material (Marschner and Kalbitz, 2003).

Fluorescence spectroscopy has also been used to obtain information about the biodegradability of DNOM (Glatzel et al., 2003; Kalbitz et al., 2003; Marschner and Kalbitz, 2003). This is done by using the assumption that more condensed aromatic structures with a higher degree of conjugated fluorescent molecules, i.e. peaks A and C (Figure 3) are less biodegradable than structures with a low degree of condensation and conjugation, i.e. peaks B, M and T (Figure 3) (Marschner and Kalbitz, 2003).

2.5.1.2 Soil properties

Nutrients availability, microbial community, and the presence of toxic substances can influence the degradation process (Marschner and Kalbitz, 2003).

2.5.1.3 External factors

Temperature, rainfall regime and vegetation cycles will induce season variability of both DNOM inputs and microbial activity, which can affect intrinsic DNOM quality parameters and soil solution properties (Marschner and Kalbitz, 2003).
### 2.6 Biogeochemical cycling of mercury in soil-aquatic ecosystems

Mercury transport in the Environment can take several pathways. Mercury occurs in two stable oxidation states in the atmosphere: inorganic (Hg\(^{2+}\)) and mercuric (Hg\(^0\)). Hg\(^0\) is the dominant specie in the atmosphere, and can be easily transported for tens of thousands of kilometres followed by dry and/or wet deposition into terrestrial and/or aquatic ecosystems. Hg\(^{2+}\) deposited on terrestrial ecosystems may be lost either through volatilisation (Hg\(^0\)) back to the atmosphere, or in solution (Hg\(^{2+}\)) via stream flow having great implications for aquatic ecosystems and thus for public health (Schroeder and Munthe, 1998; Grigal, 2002).

This study is based on the fact that there is a large pool of Hg\(^{2+}\) accumulated in the organic forest floor in the southern part of the Nordic countries due to deposition of long range transported pollutants. This study relates to the biogeochemical processes governing the transport of mercury species from soils into water systems, which main mechanisms seem to be linked to DNOM acting as a transport vector for Hg\(^{2+}\) and MeHg from catchment soils into surface waters (Grigal, 2002).

DNOM is a complexing agent of heavy metals (Tipping, 2002; Al-Reasi et al., 2011), and its reduced organosulfur thiol groups (-SH) are in sufficient abundance to bind to all available Hg in natural terrestrial systems. Data from previous research (Aastrup et al., 1991) showed that the organic forest floor in Scandinavia act as sinks for atmospheric inputs of Hg\(^{2+}\) because of their strong binding to organic and mineral particles in the soils. Following this previous argument, concentrations of DOC show strong spatial correlations with concentrations of mercury in lakes and surface waters in Scandinavia (Meili et al., 1991).

During the biogeochemical cycling of Hg several organic species can be formed. MeHg formation is of special concern. It occurs when Hg\(^0\) is slowly oxidised to Hg\(^{2+}\), and subsequently deposited via through-fall or litter-fall into terrestrial or aquatic ecosystems. It can then be transformed (methylated) to MeHg being able to enter the food chain affecting humans and wildlife (Hightower and Moore, 2003; Wiener et al., 2003; Hall et al., 2008).
2.6.1 Methylation and demethylation processes

Methylation can occur in the soil, surface waters, wetlands, sediments and inundated environments, under slightly reducing conditions, among others. In terrestrial and aquatic ecosystems Hg$^{2+}$ can be methylated to MeHg through biotic and abiotic pathways.

It has been well established that DNOM, one of the main abiotic factors controlling mercury methylation, stimulates microbial activity and thus methylation. NOM acts as substrate in the methylation process because carbon acts as an electron donor when sulphate is reduced to sulphide by methylating organisms (Parks et al., 2013). Forest harvest plays an important role in MeHg production by reducing transpiration for a period of time, during which wetter soil conditions, with reducing conditions, can promote Hg methylation. DNOM can complex to both TotHg and MeHg (Ravichandran, 2004). When it complexes to TotHg and is transported to surface waters, it can be methylated and enter the food web. Other abiotic factors controlling methylation are the lack of oxygen availability, temperature, salinity, pH, and light.

Biotic methylation by microbes is the primary source of MeHg in aquatic ecosystems. Sulphate-reducing bacteria (SRB) were the first organisms identified as the primary bacteria responsible for methylation, however, iron-reducing bacteria (IRB) and methanogens have also been identified as significant sources of MeHg production (Gilmour et al., 2013b), contrary to past research asserting that methanogens only had a minor role in methylation (Ullrich et al., 2001). They may even be the primary methylator, especially in environments such as pluvial lakes (Hamelin et al., 2011). Studies have found that SRB, IRB and methanogens have in common the presence of hgcAB gene cluster, which is responsible for methylation. The presence of this gene in organisms living in methanogenic environments (such as rice paddies or animal digestive systems), extreme pH conditions or high salinity levels (Gilmour et al., 2013a), could broaden the range of environments at risk for Hg methylation. Potential environments in which methylation may occur, as suggested by hgcAB gene, include all areas with reducing conditions with DNOM available for methylating organisms, invertebrate digestive tracts, thawing permafrost soils, and extreme environmental conditions (Podar et al., 2015). The influence of flooding has also been demonstrated through the disproportionality high levels of MeHg found in rice compared with other crops; a result of its cultivation in flooded conditions increasing the anoxic environment in which methylating organisms thrive (Qiu et al., 2008).
Methylation in aquatic ecosystems (Figure 4) can take place in the sediments, in the water column, and on the periphyton\(^6\) (Li and Cai, 2013). The methylation of \(\text{Hg}^{2+}\) on the periphyton is of special concern because it can be the base of food for other microorganisms in aquatic environments, and thereby entering the food chain (Cleckner et al., 1999). Recent studies have confirmed the sediment and pore water of aquatic environments to be key locations of methylation, and have shown how methylation potential may change in proportion to depth within the sediments. Liu et al. (2015), found that methylation occurs mainly in the upper layers of the sediments where there is significant microbial activity. A similar effect has been observed in peatland porewaters, with higher MeHg concentrations being found close to the surface (Selvendiran et al., 2008). Methylation potential decreases with increasing distance from the sediment water interface. This may be due to bacteria from the sediment moving into the water column once oxygen is depleted (Eckley and Hintelmann, 2006) (Figure 4).

MeHg demethylation, the reverse process of \(\text{Hg}^{2+}\) methylation, occurs due to exposure to sunlight in the upper photic zone of the water column (Figure 4). UV radiations (UV-A and UV-B) have been confirmed to be the primary driver of MeHg photo-degradation (Lehnheerr and St. Louis, 2009). Demethylation can also proceed through biotic and abiotic pathways in which the same organisms responsible for methylation, SRB, IRB and methanogens, could be the primary microorganisms responsible for this process due to different redox conditions (Li and Cai, 2013). However, the chemical processes governing MeHg photodemethylation remains unclear. The variation of MeHg photodemethylation pathways in different aquatic systems may be caused by differences in their chemical characteristics, e.g. differences in DNOM concentration.

\(^6\) Periphyton: Aquatic organisms such as certain algae, cyanobacteria, microbes or detritus that live attached to the rocks or other surfaces (Collings English Dictionary 2014).
2.6.2 Bioavailability of mercury species in aquatic ecosystems

Production of MeHg requires Hg$^{2+}$ to be available to methylating organisms. Deposition of Hg$^{2+}$, although decreasing globally, is not expected to decline to zero. In addition, legacy mercury deposits currently sequestered in sediments, wetland soils, and forests may become mobile during disturbances of these systems, such as forest fires, harvest activities or erosion, thus increasing the Hg$^{2+}$ available for MeHg production (Paranjape and Hall, 2017). The bioavailability of mercury species in aquatic ecosystems is mainly determined by the speciation of mercury in the water phase, and its distribution between the soil and aqueous phase. Hg$^{2+}$ distribution between the soil and aqueous phase is expected to affect the bioavailability of Hg$^{2+}$ because only dissolved forms of Hg$^{2+}$ can be transported through cell membranes and be methylated or de-methylated (Li and Cai, 2013). Hg$^{2+}$ and MeHg in aquatic environments are generally not free ions, but complexed to various inorganic or organic anion ligands, including hydroxide, chloride, sulphides, and DNOM (Morel et al., 1998; Li and Cai, 2013).
3. Materials and methods

Three sets of fresh water samples (Section 3.1.2) from Langtjern forested lake catchment were used for this study. Two of them, corresponding to the second and the third sets of water samples, were completely analysed, characterised and assessed. The first set of water samples, collected in March 2016, was used for method development of TFF, described in detail in Section 3.2.2. This set of samples was also used to implement and test all the analytical methods before analysing the samples from sets 2 and 3.

Complementing these three sets of water samples from Langtjern, a reference material previously obtained by freeze drying Reverse Osmosis (RO) DNOM from the lake was also characterised, and used for this study. The reference material is described in more detail in Section 3.3.

Figure 5 provides a complete overview of the sample preparation, fractionation, treatment and characterisation conducted in this study.

![Flow chart showing the sample pre-treatment, fractionation, treatment and characterisation carried out in this master thesis.](image)
3.1 Study site

3.1.1 Sampling site description
Langtjern (Figure 6) is a forested boreal humic lake catchment located in South-Eastern Norway (60°37’N; 9°73’E), at approximately 80 km northwest of Oslo, with an elevation of 500-710 meters. The lake covers a surface area of 0.23 km², with a maximum and mean depth of 12 and 2 meters, respectively. The summer thermocline is located at approximately 3 m. The catchment area comprises 4.69 km², most of which consists of sparse coniferous pine forest (63%) on thin podzolic mineral soils with granitic gneiss bedrock outcrops, and peat bogs (16%) (Wright, 1983). The area is acid sensitive and acid deposition has driven the original trout population to extinction (Braaten, 2015).

Langtjern has been the research site for numerous studies of precipitation, stream-water and lake-water chemistry and biology since 1973. From 1973 to 1978 these studies were included in the SNSF project: Norwegian Interdisciplinary Research project “Acid precipitation - effect on forest and fish”, although SNSF continued until 1980. From this year these studies were continued by the Norwegian Institute for Water Research (NIVA), and Langtjern became one of 5 field ecological monitoring stations in the Norwegian National Environmental Monitoring Program. Figure 7 shows a map of Langtjern modified from (de Wit et al 2014) with its Outlet LAE01, and Inlets LAE02 and LAE03.
Figure 6 Langtjern catchment (NIVA 2010).
3.1.2 Water sampling

The following sets of water samples are included in this study.

1. The 1st water sample was collected in March 14th, 2016. This was a 54 L sample from the Outlet stream (LAE01) of Langtjern. The temperature of the Outlet during sampling was approximately 0°C. There were no intense precipitation periods registered for the meteorological station Gulsvik II, 132 m elevation, (60°38’N; 9°60’E) (www.aquamonitor.no/Langtjern) in March prior to sampling. This sample was mainly used for TFF method development and familiarization with the analyses (Figure 5). A trial attempt for the size fractionation with a membrane cut-off of 10 kDa was conducted. All the trials and size fractionation procedures for the different samples using TFF are described in Section 3.2.2.2.

2. The 2nd set of water samples was collected in June 6th, 2016. This sampling includes 20 L from both the Outlet (LAE01) and the Inlet (LAE03) of Langtjern. This set of samples was used to test the TFF and it also constituted the first real samples analysed.
For size fractionation with TFF a membrane cut-off of 10 kDa was used. The temperature of the Inlet and Outlet registered in the monitoring data from Langtjern during sampling was approximately 9°C and 19.5°C, respectively. No intensive precipitation periods were registered prior to sample collection.

3. The 3rd set of samples was collected in September 15th, 2016. This sampling includes 18 L from both the Outlet (LAE01) and Inlet (LAE03) of the lake. For size fractionation, a new membrane cut-off of 100 kDa was used. The reason for this change in the membrane cut-off is explained in detail in Section 3.2.2. The temperature of the Inlet and Outlet during sampling was about 9.9°C and 16.5°C degrees, respectively. Four moderate precipitation periods were registered from the end of June towards the end of August.

4. The 4th sample comprises a 20 L sample from a RO and freeze dried DNOM isolate from Langtjern, which was filtered through 0.2 µm filters prior to characterisation.

For the 2nd and 3rd set of water samples, samples from the Inlet and Outlet were collected in four separated 10 L high density polyethylene containers (two containers for the Inlet, and two for the Outlet), transported from the lake and stored in a dark and cold room for less than 24 hours prior to filtration.

Before filtration samples from the same sampling site were bulked and homogenized in 25 L containers, one container for each sample. All sample containers were thoroughly acid washed with a solution of 7% HNO₃ beforehand. Containers were covered with aluminium foil to avoid any possible photochemical reaction.

3.2 Sample pre-treatment

3.2.1 Filtration

After less than 24 hours of sample storage, samples were filtered through 0.7 µm glass fibre filters, and subsequently through 0.2 µm membrane filters. Colloids are defined as suspended particles smaller than 0.2 µm. By pre-filtering the sample through 0.2 µm filters, bacteria (according to this criterion) in the samples are removed, and the sample is sterilised (Figure 8).
The glass fibre filters were previously pre-combusted in a furnace (Naber Industrieofenbau D-2804 Lilienthal/Bremen) for approximately 5 hours at 450°C. This was done in order to remove any potential contamination coming from the filters, which may otherwise release components such as carbon, nitrogen or mercury during filtration into the sample.

Filtration through a pore size of 0.45 µm is commonly used to separate between particulate and dissolved constituents in a water sample (Figure 8) (Thurman, 1985). Particulate organic matter (POM) is thus considered as the organic matter fraction that is retained on the 0.45 µm membrane filter. Nevertheless, the filters used in this thesis had pore sizes of 0.7 µm and 0.2 µm. Pre-filtration through 0.7 µm filters was conducted in order to remove the larger particles, thereby speeding up the 0.2 µm filtration process.

Filtration was conducted using two water vacuum pumps. Filters were pre-rinsed using 150 mL of Milli-Q Type I water, and conditioned with approximately 100 mL of the sample prior to filtration. Substantial removal of particulate matter was observed during the first filtration step (0.7 µm filters) (Figure 9).

The Outlet samples presented more particulate material than the Inlet sample, and thus filtration through 0.7 µm filters was slower. Figure 9 shows an example of how the filters

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*Figure 8 Size range of particulate (POM) and dissolved organic matter (DOM) and organic compounds in natural waters. AA, amino acids; CHO, carbohydrates; CPOM, coarse particulate organic matter; FA, fatty acids; FPOM, fine particulate organic matter (Nebbioso and Piccolo 2012).*
appeared after filtrating the first 900 mL of the Outlet and Inlet samples collected in September through 0.7 and 0.2 µm membrane filters.

![Image](image.png)

Figure 9 0.7 and 0.2 µm filters after filtrating the first 900 mL of sample.

### 3.2.2 Size fractionation with Tangential Flow Filtration (TFF)

TFF membranes used to fractionate colloids in aquatic environments are usually made of regenerated cellulose or polysulfone. The latter is usually used to filtrate water collected from estuaries, lakes, seawaters and river waters (Guéguen et al., 2002). In this study two different polysulfone membranes were used for the size fractionation procedure. For the first fractionation, a polyethersulfone (PES) membrane supplied by GE with a cut-off of 10 kDa was used. This membrane has a maximum operating pressure of 13 bars. For the second fractionation, a GR40PP polysulfone membrane from Alfa Laval, with a cut-off of 100 kDa and a maximum operating pressure of 15 bars, was used. The 100 kDa membrane needed a special chemical cleaning with a 0.2% Na-EDTA and NaOH alkaline wash to remove the protective coating material that it presented on the surface. The increase of membrane cut-off from 10 kDa to 100 kDa was done because the DOC concentration in the Permeate fractions < 10 kDa were close the limit of detection (LOD), 0.56 mg C/L, and below the limit of quantification (LOQ), 1.86 mg C/L. This implies that there was no significant amount of DNOM below 10 kDa. This fraction is usually referred to as LMW DNOM and is thus the fraction that is most bioavailable.

Figure 10 shows the membrane fractionation principle of TFF. The Effluent or the Influent sample is the pre-filtered sample containing DNOM < 0.2 µm to be fractionated
using TFF. Concentrate is the retentate fraction that does not pass through the membrane, and thus is comprised of DNOM with the size between 0.2 µm and 10 kDa or 0.2 µm and 100 kDa. The term Concentrate reflects that more water molecules passes through the membrane than DNOM, leading to an up-concentration of the fraction not passing through the membrane. Permeate is the fraction passing through the TFF membrane. In this case through a membrane cut-off of 10 kDa or 100 kDa.

![Figure 10 Membrane fractionation.](image)

### 3.2.2.1 Fractionation procedure

Figure 11 depicts the TFF system used for the fractionation procedure in this master thesis. TFF consists of an ultrafiltration membrane and a peristaltic pump (Watson Marlow 701S), which ensures the tangential circulation of the fluid in the membrane. Two modes of ultrafiltration can be used; the recirculation mode and the concentrate mode. In the recirculation mode, the Permeate and the Concentrate are recycled. Therefore, the sample volume remains constant. Recirculation mode is normally used for the membrane cleaning and conditioning process. In the concentration mode, the Permeate and Concentrate are collected in separate reservoirs (Figure 9).

Three replicates for the Inlet and Outlet samples, previously filtered through 0.7 and 0.2 µm membrane filters, were size fractionated. Prior to fractionation, the system was flushed out with a large volume of RO water for approximately 1 hour in order to remove any possible residual organic carbon in the system. Blanks for the feed tank (Influent), Permeate and Concentrate were collected prior to sample fractionation. A DOC balance
between the amount corresponding to Concentrate, Permeate and Influent was calculated to see if the fractionation procedure by using ratio 4:1, i.e. by introducing 4 L of the bulk solution (< 0.2 µm) into the feed tank and producing 1 L of Permeate and 1 L of Concentrate, gave reliable results.

Figure 11 Tangential Flow Filtration system (TFF).

3.2.2.2 TFF Method development

3.2.2.2.1 Trial attempt with the Outlet sample using a membrane cut-off of 10 kDa

Procedure

Samples for fractionation method development were collected in March and fractionated in April 2016, i.e. 1st sample, Chapter 3.1.2. Three replicates were made to test the repeatability of the fractionation method by using ratio 4:1. The three fractionations took place on three different days, April 15th, 18th and 20th, under the same conditions at a pressure of 10 mbars, and by using a new membrane for each replicate.

Results

The DOC balance can be seen in the Table 2.
Table 2 DOC balance of the fractionation method development using 10 kDa membrane cut-off.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>MWCO range</th>
<th>DOC (mg/L)</th>
<th>DOC± error (%)</th>
<th>Sample volume (L)</th>
<th>DOC mass (mg)</th>
<th>DOC distribution (%)</th>
<th>Feed to permeate ratio</th>
<th>DOC gain/loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Influent &lt; 0.2 µm</td>
<td>11.37</td>
<td>0.23</td>
<td>4.10</td>
<td>46.62</td>
<td></td>
<td>4.10</td>
<td>-0.37</td>
</tr>
<tr>
<td></td>
<td>Concentrate (10 kDa-0.2 µm)</td>
<td>11.41</td>
<td>0.23</td>
<td>3.10</td>
<td>35.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 10 kDa</td>
<td>11.07</td>
<td>0.22</td>
<td>1.00</td>
<td>11.07</td>
<td></td>
<td>23.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>Influent &lt; 0.2 µm</td>
<td>12.35</td>
<td>0.25</td>
<td>4.00</td>
<td>49.38</td>
<td></td>
<td>8.51</td>
<td>-6.58</td>
</tr>
<tr>
<td></td>
<td>Concentrate (10 kDa-0.2 µm)</td>
<td>12.80</td>
<td>0.26</td>
<td>3.53</td>
<td>45.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 10 kDa</td>
<td>1.99</td>
<td>0.04</td>
<td>0.47</td>
<td>0.93</td>
<td></td>
<td>2.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>R3</td>
<td>Influent &lt; 0.2 µm</td>
<td>10.32</td>
<td>0.21</td>
<td>4.25</td>
<td>43.86</td>
<td></td>
<td>3.94</td>
<td>-10.97</td>
</tr>
<tr>
<td></td>
<td>Concentrate (10 kDa-0.2 µm)</td>
<td>11.78</td>
<td>0.24</td>
<td>3.17</td>
<td>37.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 10 kDa</td>
<td>1.56</td>
<td>0.03</td>
<td>1.08</td>
<td>1.68</td>
<td></td>
<td>4.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

A good fractionation requires low loss of DOC during the procedure. During size fractionation, some problems were encountered. For instance, Replicate 1 could not be taken into account due to poor performance of the membrane, most likely because of a leakage. This can be seen in Table 2 where the Permeate size fraction presented a concentration of 11.1 mg C/L, which is much higher than the concentration obtained in the Permeate size fraction in Replicates 2 and 3. After size fractionation, the Permeate typically presents the lowest DOC concentration as can be seen in Replicates 2 and 3. In Replicate 2 the Feed to Permeate ratio obtained was ~ 8:1, which was not the desired ratio. This high ratio can be explained because the Permeate production was extremely slow and therefore the Permeate production had to be reduced to half of the volume, i.e. ~ 0.5 L. The loss of DOC in Replicate 2 was approximately 7%, which is acceptable. The only satisfactory fractionation was achieved with Replicate 3, where the Feed to Permeate ratio was ~ 4:1 and the loss of DOC was ~ 11%. The loss of DOC can be explained by fouling of carbon on the membrane, which is a general problem encountered with TFF.
Conclusion
It seemed that size fractionation with a membrane cut-off of 10 kDa and ratio 4:1 could work, however, only Replicate 3 showed satisfactory results. For the upcoming experiments, we decided to continue using the 4:1 ratio and keep three replicates for testing the repeatability of the fractionation method.

3.2.2.2 First sample fractionation; Inlet and Outlet samples size fractionated using a membrane cut-off of 10 kDa

Procedure
Water samples from the Inlet and Outlet of Langtjern were collected in the beginning of June 2016. Triplicates of the Outlet sample were size fractionated on June 10th, 11th and 12th, and triplicates of the Inlet samples were fractionated on June 13th, 14th and 15th.

The main aim of this experiment was to test the repeatability of the fractionation method. For this, all replicates were used. In addition, Replicate 2 for both Inlet and Outlet samples was also used for the assessment of the biodegradability and the spectroscopic properties of DNOM fractions linked to Hg and MeHg transport and uptake. Therefore, during the size fractionation of Replicate 2 a higher volume of sample was used in order to obtain the desired volume to carry out all the analyses (Table 3 and 4).

Results
DOC balance for the Inlet and Outlet can be seen in Table 3 and 4, respectively.
Table 3 DOC balance of the fractionation method using 10 kDa membrane cut-off on the Inlet sample in the summer of 2016.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>MWCO range</th>
<th>DOC (mg/L)</th>
<th>DOC± error (mg/L)</th>
<th>Sample volume (L)</th>
<th>DOC mass (mg)</th>
<th>DOC distribution %</th>
<th>Feed to permeate ratio %</th>
<th>DOC gain/loss %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent &lt; 0.2 µm</td>
<td>12.11</td>
<td>0.24</td>
<td>1.40</td>
<td>16.95</td>
<td>98.16</td>
<td>4.67</td>
<td>-12.42</td>
</tr>
<tr>
<td>R1</td>
<td>Concentrate (10 kDa-0.2 µm)</td>
<td>13.25</td>
<td>0.27</td>
<td>1.10</td>
<td>14.58</td>
<td>98.44</td>
<td>4.67</td>
<td>-12.42</td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 10 kDa</td>
<td>0.91</td>
<td>0.02</td>
<td>0.30</td>
<td>0.27</td>
<td>1.84</td>
<td>4.67</td>
<td>-12.42</td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Influent &lt; 0.2 µm</td>
<td>12.55</td>
<td>0.25</td>
<td>6.00</td>
<td>75.30</td>
<td>98.44</td>
<td>4.00</td>
<td>-21.27</td>
</tr>
<tr>
<td>R2</td>
<td>Concentrate (10 kDa-0.2 µm)</td>
<td>12.99</td>
<td>0.26</td>
<td>4.50</td>
<td>58.46</td>
<td>98.44</td>
<td>4.00</td>
<td>-21.27</td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 10 kDa</td>
<td>0.55</td>
<td>0.01</td>
<td>1.50</td>
<td>0.82</td>
<td>1.39</td>
<td>4.00</td>
<td>-21.27</td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Influent &lt; 0.2 µm</td>
<td>12.70</td>
<td>0.25</td>
<td>1.20</td>
<td>15.24</td>
<td>97.57</td>
<td>4.00</td>
<td>-16.59</td>
</tr>
<tr>
<td>R3</td>
<td>Concentrate (10 kDa-0.2 µm)</td>
<td>13.78</td>
<td>0.28</td>
<td>0.90</td>
<td>12.40</td>
<td>97.57</td>
<td>4.00</td>
<td>-16.59</td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 10 kDa</td>
<td>1.03</td>
<td>0.02</td>
<td>0.30</td>
<td>0.31</td>
<td>2.43</td>
<td>4.00</td>
<td>-16.59</td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4 DOC balance of the fractionation method using 10 kDa membrane cut-off on the Outlet sample in the summer of 2016.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>MWCO range</th>
<th>DOC (mg/L)</th>
<th>DOC± error (mg/L)</th>
<th>Sample volume (L)</th>
<th>DOC mass (mg)</th>
<th>DOC distribution %</th>
<th>Feed to permeate ratio %</th>
<th>DOC gain/loss %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent &lt; 0.2 µm</td>
<td>8.58</td>
<td>0.17</td>
<td>1.20</td>
<td>10.30</td>
<td>95.58</td>
<td>4.00</td>
<td>-18.97</td>
</tr>
<tr>
<td>R1</td>
<td>Concentrate (10 kDa-0.2 µm)</td>
<td>8.86</td>
<td>0.18</td>
<td>0.90</td>
<td>7.97</td>
<td>95.58</td>
<td>4.00</td>
<td>-18.97</td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 10 kDa</td>
<td>1.23</td>
<td>0.02</td>
<td>0.30</td>
<td>0.37</td>
<td>1.55</td>
<td>4.00</td>
<td>-18.97</td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Influent &lt; 0.2 µm</td>
<td>8.78</td>
<td>0.18</td>
<td>6.00</td>
<td>52.68</td>
<td>98.45</td>
<td>4.00</td>
<td>+11.85</td>
</tr>
<tr>
<td>R2</td>
<td>Concentrate (10 kDa-0.2 µm)</td>
<td>12.89</td>
<td>0.26</td>
<td>4.50</td>
<td>58.01</td>
<td>98.45</td>
<td>4.00</td>
<td>+11.85</td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 10 kDa</td>
<td>0.61</td>
<td>0.01</td>
<td>1.50</td>
<td>0.92</td>
<td>1.55</td>
<td>4.00</td>
<td>+11.85</td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Influent &lt; 0.2 µm</td>
<td>8.70</td>
<td>0.17</td>
<td>1.40</td>
<td>12.18</td>
<td>96.61</td>
<td>4.67</td>
<td>-17.09</td>
</tr>
<tr>
<td>R3</td>
<td>Concentrate (10 kDa-0.2 µm)</td>
<td>8.87</td>
<td>0.18</td>
<td>1.10</td>
<td>9.76</td>
<td>96.61</td>
<td>4.67</td>
<td>-17.09</td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 10 kDa</td>
<td>1.14</td>
<td>0.02</td>
<td>0.30</td>
<td>0.34</td>
<td>3.39</td>
<td>4.67</td>
<td>-17.09</td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Discussion**

The DOC recovery during the fractionation procedure is not stable between replicates: For the Inlet sample (Table 3) it can be seen a DOC loss between 12 and 21%, which is not ideal. For the Outlet sample (Table 4) a loss of DOC of approximately 20% is observed, as well as a DOC gained of about 12% in Replicate 2. A gain in DOC after fractionation can be explained by a possible source of contamination coming either from the TFF system or from the glassware used during fractionation procedure (i.e. beakers or sample containers), despite the fact that all the material was previously washed and combusted in the furnace in order to avoid contamination. Moreover, the low DOC values in the Permeate fraction (Table 3 and 4), close to the LOD, are highly uncertain and thus could explain the poor reproducibility in the DOC balance between replicates.

**Conclusion**

Due to the low concentration of DOC in the < 10 kDa Permeate fraction it was decided to change the membrane to a bigger molecular cut-off.

**3.2.2.2.3 Trial attempt with the Langtjern RO isolate using a molecular cut-off of 100 kDa.**

**Procedure**

Prior to fractionation of the real samples with the membrane cut-off of 100 kDa, a test with the RO Langtjern isolate was done to verify the repeatability of the fractionation.

**Results**

The DOC balance for the trial attempt with the RO Langtjern isolate can be seen in Table 5.
Table 5 DOC balance of the fractionation method using 100 kDa membrane cut-off on the RO isolate sample.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>MWCO range</th>
<th>DOC (mg/L)</th>
<th>DOC±error</th>
<th>Sample volume (L)</th>
<th>DOC mass (mg)</th>
<th>DOC distribution %</th>
<th>Feed to permeate ratio</th>
<th>DOC gain/loss %</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Influent &lt; 0.2 µm Concentrate (100 kDa-0.2 µm) Permeate &lt; 100 kDa Total mass = Permeate+Concentrate Recovery</td>
<td>9.96</td>
<td>0.20</td>
<td>1.20</td>
<td>11.96</td>
<td>11.27</td>
<td>84.76</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.53</td>
<td>0.29</td>
<td>0.90</td>
<td>11.27</td>
<td>15.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.75</td>
<td>0.14</td>
<td>0.30</td>
<td>2.03</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13.30</td>
<td>111.23%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>Influent &lt; 0.2 µm Concentrate (100 kDa-0.2 µm) Permeate &lt; 100 kDa Total mass = Permeate+Concentrate Recovery</td>
<td>10.56</td>
<td>0.21</td>
<td>1.20</td>
<td>12.67</td>
<td>12.90</td>
<td>86.53</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.33</td>
<td>0.29</td>
<td>0.90</td>
<td>12.90</td>
<td>13.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.69</td>
<td>0.13</td>
<td>0.30</td>
<td>2.01</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.90</td>
<td>117.61%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>Influent &lt; 0.2 µm Concentrate (100 kDa-0.2 µm) Permeate &lt; 100 kDa Total mass = Permeate+Concentrate Recovery</td>
<td>10.59</td>
<td>0.11</td>
<td>1.20</td>
<td>12.71</td>
<td>13.01</td>
<td>88.41</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.45</td>
<td>0.29</td>
<td>0.90</td>
<td>13.01</td>
<td>11.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.68</td>
<td>0.21</td>
<td>0.30</td>
<td>1.71</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.71</td>
<td>115.74%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

A DOC gain ranging from 11% to 18%, was found in all replicates. As previously explained, this gain can be due to a possible source of contamination coming either from the system or from the glassware used during the fractionation. Even though perfect results in the DOC balance were not obtained, it was decided to continue with this approach because the DOC concentrations in the Permeate fraction increased from 0.6 and 1.2 mg C/L to 5.7 and 6.7 mg C/L, respectively, and therefore better results could be expected.

**Conclusion**

Because of the increase in DOC concentration in the Permeate fraction, and after considering that the gain in DOC was not exceeding 20%, it was decided to continue using the 100 kDa membrane cut-off for the next fractionation.
3.2.2.4 Second sample fractionation; Inlet and Outlet samples size fractionated using a membrane cut-off of 100 kDa

Procedure
Samples from Langtjern Outlet and Inlet, were collected in September 15\textsuperscript{th}, 2016. Three replicates from the Inlet were fractionated on October 19\textsuperscript{th} and 20\textsuperscript{th}, and three replicates from the Outlet on October 21\textsuperscript{th} and 24\textsuperscript{th}. As a result of changing the membrane to a bigger cut-off, the fractionation procedure was much faster, allowing fractionation of 2 replicates with the same membrane and on the same day (Replicate 1 and 2). The membrane was changed for Replicate 3, which was the sample used for the assessment of the biodegradability and the spectroscopic properties of DNOM fractions linked to Hg and MeHg transport and uptake.

Results
The DOC balance for the Inlet and Outlet can be seen in Table 6 and 7, respectively.

Table 6 DOC balance of the fractionation method using 100 kDa membrane cut-off on the Inlet sample in the fall in 2016.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>MWCO range</th>
<th>DOC (mg/L)</th>
<th>DOC error</th>
<th>Sample volume (L)</th>
<th>DOC mass (mg)</th>
<th>DOC distribution %</th>
<th>Feed to permeate ratio</th>
<th>DOC gain/loss %</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Influent &lt; 0.2 µm</td>
<td>15.49</td>
<td>0.31</td>
<td>1.25</td>
<td>19.36</td>
<td>91.73%</td>
<td>4.17</td>
<td>-5.71%</td>
</tr>
<tr>
<td></td>
<td>Concentrate (100 kDa-0.2 µm)</td>
<td>17.63</td>
<td>0.35</td>
<td>0.95</td>
<td>16.75</td>
<td>8.27%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 100 kDa</td>
<td>5.03</td>
<td>0.10</td>
<td>0.30</td>
<td>1.51</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>Influent &lt; 0.2 µm</td>
<td>15.54</td>
<td>0.31</td>
<td>1.25</td>
<td>19.43</td>
<td>93.52%</td>
<td>4.17</td>
<td>-2.83%</td>
</tr>
<tr>
<td></td>
<td>Concentrate (100 kDa-0.2 µm)</td>
<td>18.58</td>
<td>0.37</td>
<td>0.95</td>
<td>17.65</td>
<td>6.48%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 100 kDa</td>
<td>4.08</td>
<td>0.08</td>
<td>0.30</td>
<td>1.22</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>Influent &lt; 0.2 µm</td>
<td>15.47</td>
<td>0.07</td>
<td>5.40</td>
<td>83.54</td>
<td>94.79%</td>
<td>4.50</td>
<td>+0.68%</td>
</tr>
<tr>
<td></td>
<td>Concentrate (100 kDa-0.2 µm)</td>
<td>18.76</td>
<td>0.38</td>
<td>4.25</td>
<td>79.73</td>
<td>5.11%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permeate &lt; 100 kDa</td>
<td>3.65</td>
<td>0.31</td>
<td>1.20</td>
<td>4.38</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7 DOC balance of the fractionation method using 100 kDa membrane cut-off on the Outlet sample in the fall in 2016.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>MWCO range</th>
<th>DOC (mg/L)</th>
<th>DOC± error</th>
<th>Sample volume (L)</th>
<th>DOC mass (mg)</th>
<th>DOC distribution %</th>
<th>Feed to permeate ratio %</th>
<th>DOC gain/loss %</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Influent &lt; 0.2 µm Concentrate (100 kDa-0.2 µm) Permeate &lt; 100 kDa</td>
<td>12.17</td>
<td>0.24</td>
<td>2.16</td>
<td>26.31</td>
<td>92.25%</td>
<td>7.21</td>
<td>+6.70%</td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td>13.91</td>
<td>0.28</td>
<td>1.86</td>
<td>25.90</td>
<td>7.75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>Influent &lt; 0.2 µm Concentrate (100 kDa-0.2 µm) Permeate &lt; 100 kDa</td>
<td>11.94</td>
<td>0.24</td>
<td>1.20</td>
<td>14.33</td>
<td>89.14%</td>
<td>4.00</td>
<td>+4.86%</td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td>14.88</td>
<td>0.30</td>
<td>0.90</td>
<td>13.39</td>
<td>10.86%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>Influent &lt; 0.2 µm Concentrate (100 kDa-0.2 µm) Permeate &lt; 100 kDa</td>
<td>11.94</td>
<td>0.11</td>
<td>3.30</td>
<td>39.40</td>
<td>85.74%</td>
<td>104.86%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total mass = Permeate+ Concentrate Recovery</td>
<td>14.33</td>
<td>0.29</td>
<td>2.30</td>
<td>32.96</td>
<td>14.26%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>5.48</td>
<td>0.24</td>
<td>1.00</td>
<td>5.48</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>5.48</td>
<td>0.24</td>
<td>1.00</td>
<td>5.48</td>
<td>97.56%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

DOC concentrations in the three replicates of the 100 kDa Permeate fraction were all well above the MLOD and MLOQ. The DOC recovery in all three replicates after fractionation ranged from -6% to +1%, for the Inlet (Table 6), and from -2% to +7%, for the Outlet (Table 7). These values suggest that the change of membrane cut-off was satisfactory.

Conclusion

For future size fractionation experiments the use of 100 kDa cut-off is recommended.

3.3 Langtjern isolate produced by Reverse osmosis (RO)

In addition to the water samples, the size fraction < 0.2 µm of a RO isolate material from Langtjern was studied and characterized for comparison with the same size fraction of the fresh lake water samples collected in June and September in 2016.

The study of the RO isolate also contributed to the TFF method development, previously explained in Section 3.2.2.2.3.
3.3.1 RO General Principle

RO is a common technique to isolate DNOM from aquatic ecosystems. A feed solution consisting of water and aqueous solutes is separated into a Permeate and a Concentrate (Retentate) solution (Figure 12). The sample reservoir is filled with the feed solution, which is normally water previously filtered through 0.45 µm membrane filter. The feed solution is passed through a cation exchange resin with Na⁺ to avoid the fouling of the RO membrane by precipitation with calcium carbonate, or iron (III) hydroxide among others. As this water is pumped into the RO system, the Concentrate returns back to the sample reservoir and the Permeate (virtually clean water) is discarded. In order to maintain a constant water level in the sample reservoir, more feed water is added (IHSS, 2016).

The concentration of the solutes that are rejected by the RO membrane, i.e. retentate, gradually increase in the sample reservoir, as more feed solution is processed.

This process continues until a sufficient volume of feed solution has been processed. After this the concentrated, partially desalted DNOM, is freeze-dried in order to preserve the integrity of the DNOM sample (IHSS, 2016).

![Figure 12 General schematic diagram for RO process (IHSS, 2016).](image)
3.3.2 Preparation and storage of the RO Langtjern isolate

On April 5th, 2006, 675 L of water were collected downstream of the Outlet (LAE01) of Langtjern and transported in 5 L containers to the University of Agder, Kristiansand, Norway. There DNOM was isolated by Professor Dag Olav Andersen using a RO isolation system. As a result, 21.6 g of isolate were produced. DNOM isolation was conducted in collaboration with Biochemistry Research Project in Northern Watersheds (BNW) (Bodding 2007).

A Langtjern DNOM sample was prepared from the RO isolate on February 19th, 24th and 29th. The amount of RO isolate weighted for this purpose was based on the fact that Langtjern has a mean DOC concentration of about 10 mg C/L in April, month in which this sample was collected and isolated in 2006 (Bodding 2007).

On February 19th, two solutions of 2 L each were made and kept in the stirring device for 48 hours to reach complete dissolution. These solutions were covered in aluminium foil to avoid photochemical reactions. The same procedure was done on February 24th and 29th, however, this time three and five solutions of 2 L each were made, respectively. After this, the 10 solutions were bulked together in a 25 L container and homogenised prior to filtration through 0.2 µm cellulose acetate filters. After filtration, the sample was kept in a dark room at 4°C until further analysis.

3.4 Chemical analyses

The following chemical analyses were conducted on all filtered, and size fractionated water samples.

3.4.1 Water sample treatment

Conductivity and pH measurements were conducted at the Department of Chemistry, at the University of Oslo (UiO), in accordance with ISO 7888 (1985) and ISO 10523 (2008) methods, respectively. A volume of approximately 15 mL per sample was first measured for conductivity using a Mettler-Toledo AG FiveGo™ electrode, followed by pH analysis, using an Orion pH-meter equipped with a combined Ross electrode. Both instruments were previously calibrated with a standard solution of 85 µS cm⁻¹, and with a buffer solution with pH 4.01 and pH 7.00 for conductivity and pH, respectively.
3.4.2 Elemental composition and speciation

3.4.2.1 Total Organic Carbon (TOC)

Samples for TOC were analysed at the Department of Biology, UiO, using a TOC-VC_{PH} analyser with an ASI-V auto-sampler (both from Shimadzu corporation), following the ISO 8245 (1999) method. The instrumental setting called Non-Purgeable Organic Carbon (NPOC) was applied to remove inorganic carbon by acidifying the samples to pH 2-3 by adding 1.5% 2M of HCl in the samples. The remaining NPOC was decomposed to CO_2 over a titanium oxide catalyst at 680°C, and measured after removing all the water vapour by a nondispersive infrared sensor (NDIR) detector. In Table 5 in Appendix B.3, the instrument settings of the TOC-analysers are presented, and the calibration curve is presented in Figure 1 in Appendix B.1. The TOC instrument measures each sample 3 to 5 times, until the SD of the signal area is < 0.1 or the RSD is < 2%. MLOD and MLOQ are 0.56 mg C/L and 1.88 mg C/L, respectively.

3.4.2.2 Major Anions:

The concentration of major anions fluoride (F^-), chloride (Cl^-), sulphate (SO_4^{2-}), and nitrate (NO_3^-) was determined at the Department of Chemistry, UiO, in accordance with ISO 10304-1 (2007) method using a Dionex Integrion high performance ion chromatograph (HPIC) system equipped with an AS 4 µm anion analytical (AS4) column, an anion electrolytically regenerated suppressor (AERS), and a conductivity detector. The calibration curves for all major anions are presented in Figures 2-5 in Appendix C.1, and the instrument settings are presented in Table 7 in Appendix C.2.

The analytes are transported through the AS4 column using a mobile phase, and partition between the stationary and the mobile phase takes place. These analytes are thereby separated from each other based on their charge and ionic radius. The ions to be separated must be negatively charged, i.e. anions, to interact with the positively charged stationary phase of the AS4 column. Moreover, the greater the anions’ charge the greater the interaction with the stationary phase and consequently the longer the retention time (Figures 6-20 C.3). A suppressor (AERS) is placed after the column because the eluent contains relatively high amounts of salts and thus has high background conductivity. Therefore, to be able to detect small differences in the conductivity of the eluate with the presence of the analyte ions, a suppression process is desirable. The AERS principle is based on removing all major base cations (mainly Ca^{2+}, Mg^{2+}, Na^+ and K^+) from the eluent and replaces them with hydronium ions formed by electrolysis of the eluent. These
hydronium ions combined with the hydroxyl or carbonate ions from the eluent lead to the formation of water and carbonic acid, respectively, which have very low conductivity compared to the hydroxyl or carbonate. Moreover, the analyses are associated with \( \text{H}^+ \), which has a very high specific conductivity, upon passing through the detector enhancing thus the sensibility of the detector towards the anionic analytes.

### 3.4.2.3 Major cations

In addition to the major cations calcium (\( \text{Ca}^{2+} \)), magnesium (\( \text{Mg}^{2+} \)), potassium (\( \text{K}^+ \)) and sodium (\( \text{Na}^+ \)), and the concentration of aluminium (\( \text{Al}^{3+} \)), iron (\( \text{Fe}^{3+} \)) and manganese (\( \text{Mn}^{2+} \)) was determined at the Department of Chemistry, UiO, in accordance with ISO 22036 (2008) method using a Varian Vista AX CCD simultaneous axial viewing inductively coupled plasma optical emission spectrometer (ICP-OES), with a cone spray nebulizer and a Sturman Master spray chamber. The calibration curves for the cations are presented in Figures 22-28 in Appendix D.1, and the LOD and LOQ are presented in Tables 10 and 11 in Appendix D.3. The sample was introduced by a hose connected to a peristaltic pump, which pushes the sample solution through the hose and into the nebulizer. In the nebulizer, the sample is converted into a mix of finely divided droplets, i.e. aerosols. The aerosols are separated into the spray chamber, where the large droplets go to drain (~ 99%), and the fine droplets are carried to the plasma (~1%). Due to the high temperature of the plasma (6000-10000°K), the analytes are excited leading to photon emission and ionization. The wavelength of the emitted line spectrum is specie-specific and by using the plasma both atom and ion lines can be obtained. Prior to analysis, all samples and standard solutions were matrix matched adding 65% \( \text{HNO}_3 \) to a concentration of 0.3M in the samples and in the standards. A rinsing solution of 0.3M \( \text{HNO}_3 \) was also made and used for rinsing between measurements in order to avoid carryover. In Table 9 in Appendix D.2 the instrument settings for ICP-OES are presented.

### 3.4.3 Total mercury and methyl mercury

**Principle for total mercury determination**

The method for total mercury (TotHg) determination follows EPA 1631(2002) protocol. The calibration curve is presented in Figure 29 in Appendix E.2.

CVAFS systems consist of a Hg cold vapour (CV) generator and an atomic fluorescence spectrometry (AFS) detector (Figure 14). The AFS detector determines total elementary mercury (\( \text{Hg}^0 \)) at 253.7 nm. The first requirement is to release all inorganic \( \text{Hg}^{2+} \)
compounds from the sample matrix and convert all organic forms of Hg to Hg$^{2+}$ by
digestion or oxidation processes. In this case bromide monochloride (BrCl), an oxidant
and preservative for TotHg species, was added to the samples. Subsequently, stannous
chloride (SnCl$_2$) was added to reduce Hg$^{2+}$ to elemental mercury vapor (Hg$^0$). The Hg$^0$
produced is purged out of the sample and carried to the AFS detector by argon gas (Ar).
The following reaction shows the reduction of Hg$^{2+}$ to Hg$^0$ by the addition of SnCl$_2$.

$$\text{Hg}^{2+} + \text{Sn}^{2+} \rightarrow \text{Hg}^0 + \text{Sn}^{4+}$$

Principle of methyl mercury determination

The analytical method for determining MeHg concentrations in water samples was based
on EPA 1630 (1998) protocol. Water samples were distilled to remove potential matrix
interferences. Prior to distillation, a chelating solution of 1% ammonium pyrrolidine
dithiocarbonate (APDC $^7$) was added to each sample. After distillation, the distillate
samples containing Hg were ethylated using an acetate buffer sodium tetraethyl borate
(NaBEt$_4$$^8$). Figure 13 shows the distillation equipment. The MeHg calibration curve can
be seen in Figure 30 in Appendix E.2.

$^7$ APDC is a chelating agent that is used to complex the species from the matrix (EPA 1630, 1998).
$^8$ NABEt$_4$ is an acetate buffer that serves to derivate the two remaining ionic Hg species after distillation (inorganic
Hg$^{2+}$, and CH$_3$Hg$^{+}$ to their ethylated forms, diethyl-Hg and methyl-ethyl-Hg, respectively) (EPA 1630, 1998).
Figure 13 Hg distillation equipment.

The ethylated mercury species in the distillate sample are purged out with nitrogen gas (N₂) for approximately 20 minutes and collected on a carbon sample trap. The ethylated mercury species are desorbed thermally from the sample trap, separated using a gas chromatography (GC) column, reduced using a pyrolytic column, and detected using CVAF. In Figure 14 it can be see the schematic diagram of CVAFS.
The method’s limit of detection (MLOD) was 0.02 ng/L and 0.1 ng/L (3 standard deviations of the method blanks) for MeHg and Total Hg, respectively. For both species, automated systems were used for analysis (Brooks Rand Labs MERX automated systems with Model III AFS Detector).

In Tables 16 and 17 in Appendix E.1 the instrument settings for TotHg and MeHg analysers are presented.
3.4.4 Structural characterization of DNOM

3.4.4.1 UV-/VIS Absorbance

A full absorbance spectrum of the samples was measured at irradiance of light at wavelengths from 200 nm to 800 nm, using 1cm quartz cuvettes on a Varian Cary 100 Bio UV-VIS spectrophotometer at the UiO. The UV-VIS photometer was background corrected prior to analysis using two cuvettes containing type I water. During the scan, one cuvette, containing type I water, was kept as a reference. Table 26 Appendix F.1 the instrument settings of the photometer are presented.

3.4.4.2 Fluorescence Spectroscopy

Fluorescence analysis was performed using a quartz cuvette on a Varian Cary Eclipse fluorescence spectrophotometer at the Norwegian Institute for water Research (NIVA). EEM spectra were obtained by scanning the emission from 200 to 600 nm by increasing the excitation wavelength by 25 nm increments from 250 to 450 nm. Excitation and emission slit widths were set to 10 and 5 nm, respectively. Scan speed was set to 600 nm/min. Data were processed using Varian Cary Eclipse fluorescence software.

3.4.5 Biodegradation experiment

3.4.5.1 Inoculum preparation

Two water samples, one for each set of samples (summer and fall), containing indigenous bacteria from Langtjern were used for this purpose.

The preparation of the inoculum was made by filtering 100 mL of raw water through a pore size filter of 2.0 µm. This pore size filter was selected to exclude any microorganism longer than bacteria, including the main competitors: zooplankton and phytoplankton (Figure 8). It has been previously proved that the addition of phosphorous (PO$_4^{3-}$) as a nutrient, increases microbial community growth in drinking water (Miettinen et al., 1997). Therefore, a solution of 10 mM PO$_4^{3-}$ was made and consequently added to the inoculum and the samples. The 100 mL of filtered water for the inoculum preparation was put in a 250 mL Erlenmeyer flask and 1mL of 10 mM phosphorous (PO$_4^{3-}$) was added to favour bacterial growth (Miettinen et al., 1997). The inoculum, covered in aluminium foil, was kept in a shaker device at room temperature of approximately 21 degrees for 48 hours allowing the bacteria to grow before the biodegradation experiment.
3.4.5.2 Sample preparation

A solution of glucose (C₆H₁₂O₆) containing a concentration of 10 mg C/L was used as reference material to test the microbial activity, and also to compare it with the rest of the samples.

All samples, including the reference material and blank, were prepared in 25 mL volumetric flasks (Figure 15). 250 µL of nutrients (10 mM PO₄³⁻) and 200 µL of inoculum were added to 24.55 mL of each sample.

![Sample preparation for the biodegradation experiment.](image)

3.4.5.2 Sensor dish reader (SDR) to monitor oxygen consumption by bacteria during the biodegradation experiment.

Biodegradation in the samples was followed during incubation by measuring the oxygen consumption using a small 24-channel sensor dish reader (SDR) situated under a set of multi-dishes with vials (Figure 16).

![Sensor dish reader (SDR) to monitor oxygen consumption by bacteria during the biodegradation experiment.](image)

Every vial has a sensor located at the bottom containing a luminescent dye. This dye is excited by the SDR system located under the multi-dish, and its luminescence lifetime is then detected non-invasively through the transparent bottom (Figure 17). The luminescence lifetime of the dye depends on the oxygen partial pressure in the sample. Here the O₂ acts as a quencher, reducing the luminescent lifetime. This relationship can be calculated by the computer by converting the sensor response to an O₂ value. This is done by using a variant of the stern-Volmer equation (Presens 2012). Where I₀ denotes
the luminescence lifetime with no $O_2$, $I$ the actual luminescence lifetime of the sample, and $KSV$ the quenching rate.

\[
\frac{I_0}{I} = 1 + KSV * (O_2)
\]

The SDR system can be used in incubators and on shakers, being an ideal tool for cell cultivation. In this master thesis, the biodegradation experiment was carried out in a Thermax incubator located at the Department of Biology, UiO, (Figure 18). The SDR system together with the samples to be degraded was placed inside the Thermax incubator, and kept for approximately three days, at 20°C for the summer samples and at 18°C for the fall samples, connected to a computer recording the oxygen consumption.

Figure 16 Sensor dish reader (SDR) equipment with a small 24 channel reader to the left, and a multi-dish located with the vials on the top of the SDR to the right (Presens 2012).
Figure 17 Sensor located at the bottom of the vial (Presens 2012).

Figure 18 Thermax incubator.
4. Results and discussion

4.1 pH

pH in the different size fractions, including the Raw water, for the Inlet and Outlet samples fractionated with 10 or 100 kDa membrane cut off are presented in Figure 19 and 20, respectively.

The lake was found to be acidic, as expected (Section 3.1.1), with pH values less than 6. pH and conductivity measurements for each size fraction using 10 or 100 kDa cut-offs, respectively, are given in Tables 1 and 2 in Appendix A.

The pH in the Influent and Concentrate in the Inlet and Outlet samples collected in the summer were within the same range, with values around 5 (Figure 19). The Permeate size fraction (Inlet and Outlet samples), which was expected to contain the most acidic DNOM fraction (i.e. low molecular weight and hydrophilic compounds with high density of weak acid functional groups) and thus lower pH, actually had a slightly higher pH. This can be explained by that the amount of DNOM in this fraction was so low that it was practically distilled water, with pH values of 5.54 and 6.02 for the Inlet and Outlet, respectively.

The measured pH of the Outlet Raw water sample taken in the summer, 5.17, was lower than the values obtained by the Aqua Monitor station at Langtjern. In that day, a maximum pH of 5.5 and a minimum pH of 5.42 were registered. The reason for the difference can be attributed for instance to the use of different pH-meters in the field and in the laboratory.

The pH values for all the size fractions of the Inlet and Outlet samples collected in the fall and fractionated with 100 kDa are shown in Figure 20. All the fractions had pH values ranging from 5.05 to 5.49 (Table 2 in Appendix A). The pH value of the Outlet Raw water sample, 5.04, was within the pH values obtained by the Aqua Monitor station at Langtjern. In that day, a maximum pH of 5.14 and a minimum pH of 5.04 were registered.
4.2 Dissolved Organic Carbon (DOC)

DNOM is comprised of about 50% of carbon (Schnitzer and Khan, 1972; Thurman, 1985). Therefore, by measuring DOC it is possible to have a good approximation of the amount of DNOM in the sample.

Measured and estimated\(^9\) DOC concentration (mg C/L) in the samples that were size fractionated with 10 kDa membrane cut-off, i.e. Inlet and Outlet samples collected in the summer in 2016, are given in Table 3 in Appendix B.2. The estimated DOC concentration in each size fraction is presented in Figure 21.

The DOC in the < 10 kDa Permeate fraction accounted for only 1.4% and 1.5% of the DOC in the Inlet and Outlet samples, respectively (Section 3.2.2.2.2). This implied that there was no significant amount of DNOM below 10 kDa. These DOC values were also found to be close the MLOD and below the MLOQ of the instrument.

\[^9\] The estimated DOC concentration is calculated based on the DOC percent distribution in the different size fractions after fractionation, considering that the Influent size fraction contains 100% of the DOC (Table 3 in Appendix B.2).
The estimated DOC concentration in the samples that were size fractionated with 100 kDa, i.e. Inlet and Outlet collected in the fall in 2016, are shown in Figure 22.

DOC in the Inlet Permeate after fractionation with 100 kDa, accounted for 5.5% of the total DOC. Similarly, the DOC in the Outlet Permeate comprised 14.3% of the Influent DOC. These DOC values in the Permeate size fraction were well above the MLOQ. The measured and estimated DOC concentration in the samples size fractionated with 100 kDa membrane cut-off are presented in Table 4 in Appendix B.2.

The Inlet samples from the summer and the fall had higher DOC concentrations compared to the Outlet (Figures 21 and 22). For instance, the Inlet Influent in the summer had a DOC concentration of 12.6 mg C/L, whereas the Outlet Influent had a concentration of 8.78 mg C/L. This decrease in DOC from the Inlet to the Outlet of the lake could be due to partial mineralization of the DNOM by biological and abiotic (photo-oxidation) processes. However, the Inlet might not be representative of all the water flowing into the catchment, and therefore have higher or lower DOC concentration than that found in the lake itself. This is because the concentration of DOC in the lake is an average of what comes into the reservoir. Therefore, it could be affected by the concentration of DOC in the other tributaries, or by groundwater seepage with low DOC concentration.
The DOC concentration was higher in the fall compared to the summer samples. For instance, the values in the Inlet Influent increased from 12.6 mg C/L in the summer to 15.5 mg C/L in the fall. Similarly, the DOC in the Outlet Influent increased from 8.78 mg C/L to 11.9 mg C/L. The higher DNOM concentrations in the fall sample, can be explained by that the fall sampling time was preceded by four moderate precipitation periods, lasting from the end of June towards the end of August (www.aquamonitor.no/Langtjern). This has led to more water flowing through shallow sub-lateral flow-paths, richer in DNOM, by passing the adsorptive capacity of the deeper mineral soil layers, causing more transport of organic matter from the soil directly into the surface water.

Typical seasonal fluctuations in DOC concentrations in the Inlet and Outlet at Langtjern are shown in Figure 23 (Austen pers. comm., 2015). Likewise, unpublished data from 2016 (Garmo pers. comm., 2016), i.e. the year in which the samples were collected, showed a very similar fluctuation pattern. The pattern observed in Figure 23 may partly be explained by the stratification of the lake water taking place in winter and summer. In winter, an ice layer forms on the surface of the lake. This becomes a barrier to wind-induced mixing of the slightly warmer surface water layer beneath the ice with the rest of the water column. In the spring, the ice melts and the water column becomes uniform, reaching a temperature of approximately 4°C, from top to bottom. The water column mixes completely in a process called spring turnover. A similar situation occurs in the summer, when light energy is absorbed by DNOM and converted to heat energy, resulting in warming of the surface water. This creates thermal stratification with warmer water in the epilimnion and colder in the hypolimnion, which usually lasts all summer. During this time, the thermocline creates a strong and effective barrier to water-column mixing. In the late summer and fall, the epilimnion begins to cool and the temperature zonation begins to break down. Once the thermal barrier is gone, the lake reaches a uniform temperature, and completely mixes, or turns over, from top to bottom. This destratification process is often called fall turn over (Davenport, 2017).

The variation in DOC concentration from June to December was higher in the Inlet compared to the Outlet of the catchment (Figure 23). This can be attributed to a shift from slow groundwater seepage, i.e. low in DOC, during the winter to a high flux of DNOM due to more allochthonous production, and more water flowing through shallow flow-paths into the lake during the summer. During the spring and the late fall turnovers, the
reservoir acts as a buffer balancing the concentration of DOC in the Inlet and Outlet of the lake. This can be seen in Figure 23 when the two curves intersect.

Figure 23 DOC concentrations in the Inlet and Outlet of Langtjern in 2015, modified from Austnes pers. comm., 2015.

4.3 Water characterization: Major anions and cations

The concentration (µeq/L) of the major anions (DNOM-A\textsuperscript{-}, Cl\textsuperscript{-}, SO\textsubscript{4}\textsuperscript{2-}, NO\textsubscript{3}\textsuperscript{-} and F\textsuperscript{-}) and cations (Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, Na\textsuperscript{+}, and H\textsuperscript{+}) are presented in Figures 24 and 25 for the Inlet and Outlet Influent (< 0.2 µm) samples collected in the summer and in the fall of 2016. The concentration of DNOM-A\textsuperscript{-} was estimated based on charge balance and using a model by Oliver et al (1983) with an adapted charge density, the DOC and pH of the water sample.

Tables 14 and 15 in Appendix D.3 list the concentration of each ion in each size fraction (Influent, Concentrate and Permeate) for the Inlet and Outlet samples. All samples from both seasons were analysed together presenting therefore the same LOD and LOQ for each ion.

The composition of the major anions and cations in the Influent (< 0.2 µm) summer sample is shown in Figure 24. A significant difference between the concentration of inorganic anions and cations was observed in the Inlet and the Outlet samples, where the inorganic anionic concentration was lower than the cationic concentration. This difference is accounted for by a large contribution of organic anions (DNOM-A\textsuperscript{-}) balancing the charge in this dystrophic lake.

In order to acquire charge balance, the charge density of the DNOM-A\textsuperscript{-}, used in the model of Oliver et al. 1983, had to be adjusted to 7.8 and 8.5 µeq/mg C for the Inlet and Outlet,
respectively. The concentrations of ions from the Inlet to the Outlet of the lake were within the same range. A two-sample t-test of the set of water samples collected in the summer, assuming unequal (p < 0.05) and equal (p > 0.05) variance for Ca$^{2+}$ and Mg$^{2+}$, respectively, was calculated. This t-test was carried out in order to test the hypothesis that the Outlet of the lake contained more water that had seeped into the lake from groundwater sources than the Inlet stream. This groundwater has higher concentrations of Ca$^{2+}$ and Mg$^{2+}$ compared to the Inlet, as it is primarily originated from the deeper mineral soil deposits and from water saturated peat bogs at Langtjern. This t-test confirmed that there were statistically significant differences (p < 0.05) in the concentration of these cationic species, being higher in the Outlet compared to the Inlet.

The concentration of the ionic species in the Influent (< 0.2 µm) samples collected in the fall are presented in Figure 25. A higher sum of equivalent concentration of cationic species compared to anionic species was again observed for both Inlet and Outlet samples. However, to a less degree compared to the summer samples despite higher concentrations of DNOM. In order to acquire charge balance, the density of the DNOM-A$^{-}$ had to be adjusted to 3 and 2.5 µeq/mg C for the Inlet and the Outlet, respectively. This is rather strange since the typical DNOM-A$^{-}$ density used to obtain charge balance ranges between 5 and 10 µeq/mg C.

A two-sample t-test, assuming equal variance (p < 0.05), was carried out again to test the previous hypothesis that the Outlet of the lake comprised more water seeping into the lake than the Inlet stream. This t-test showed statistical significant difference (p < 0.01) in the concentration of Mg$^{2+}$, and non-statistical differences (p > 0.05) in the concentration of Ca$^{2+}$ in the Outlet compared to the Inlet.
By comparing the water chemistry in the summer and in the fall (Figure 24 and 25), it can be seen that the ionic species were more diluted (~30%) in the fall compared to the summer. This could be due to the four moderate precipitation periods registered at Langtjern from the end of June and towards the end of August (Section 4.2).

### 4.4 Total mercury in the different DNOM size fractions

The estimated total Hg (TotHg) concentrations in the different 10 kDa size fractions for the Inlet and Outlet summer samples are shown in Figure 26. The estimated TotHg concentration was calculated based on the distribution percent of TotHg in each size fraction (Table 18 Appendix E.3).

The Outlet sample contained higher TotHg concentrations in all the size fractions compared to the Inlet. The measured absolute and the estimated relative ($\frac{\text{TotHg}(\text{ng})}{\text{DOC} \text{ (mg/L)}}$) ratio (Table 22 and 23 in Appendix E.7), showed that the LMW DNOM fraction (i.e. Permeate) contained more TotHg per DOC compared to the HMW DNOM fraction (i.e. Influent and Concentrate) for both Inlet and Outlet samples. However, previous studies (Hintelmann et al., 2000; Wu et al., 2004) had shown otherwise.

Estimated concentrations of TotHg in the different 100 kDa size fractions in the samples collected in the fall are shown in Figure 27. The corresponding data are given in Table
19 in Appendix E.3. The Outlet sample had a non-significantly higher concentration (p < 0.01) of TotHg compared to the Inlet sample. The measured absolute and estimated relative \( \frac{\text{TotHg (ng/L)}}{\text{DOC (mg/L)}} \) ratio (Table 24 and 25 in Appendix E.7) showed the opposite trend from what was observed with the 10 kDa membrane. For both the absolute and the relative, the HMW DNOM had more TotHg per DOC compared to the LMW DNOM fraction, which is in agreement with previous studies by (Hintelmann et al., 2000; Wu et al., 2004).

Summary

The \( \frac{\text{TotHg (ng/L)}}{\text{DOC (mg/L)}} \) ratio in all the size fractions in the Outlet samples, for the 10 and 100 kDa cut-off, were higher compared to the Inlet samples. The highest absolute and relative \( \frac{\text{TotHg (ng/L)}}{\text{DOC (mg/L)}} \) ratio was found in the LMW DNOM fraction in the samples fractionated with 10 kDa, whereas for those fractionated with 100 kDa it was found in the HMW DNOM fraction. The inconsistency of these results can be explained by the low concentration of LMW DNOM, i.e. < 10 kDa, causing the DOC concentration to be below the MLOQ.
therefore resulting in inaccurate \( \frac{\text{TotHg} (\text{ng L}^{-1})}{\text{DOC} (\text{mg L}^{-1})} \) ratios. In this study, there was insufficient data to consistently prove the hypothesis that the HMW DNOM fraction contains more TotHg per DOC (Hintelmann et al., 2000; Wu et al., 2004), and further studies must be conducted to confirm that theory.

**TotHg and DOC correlation from January to December 2016**

TotHg concentrations in the Inlet and the Outlet of Langtjern were not found to differ significantly \( (p > 0.05) \) in the monitoring data from 2015 and 2016, assuming equal variance (Unpublished data: de Wit and Braaten, pers. comm., 2016). This suggests that there is not net loss of TotHg in the lake.

Eklöf et al. (2012), in a study carried out from 2000 to 2010 in 19 watercourses in Sweden, found strong spatial correlations between TotHg and DOC. It was therefore expected that the concentration of TotHg would follow that of DOC. However, no temporal correlation between TotHg and DOC was found in 2016, neither in the Inlet nor the Outlet of the lake. Such lack of temporal correlation was also observed by Eklöf et al., (2012). Even if DOC increased in most watercourses, the TotHg increased significantly in just one. This implies that a temporal increase in DOC does not necessarily results in an increase in TotHg, despite the strong spatial correlations between these two substances. In fact, Eklöf et al. (2012) observed a stronger temporal correlation between TotHg and colour of organic matter (CDNOM), measured as absorbance at 420 nm, during these 10 years.

### 4.5 Methyl mercury in the different DNOM size fractions

The estimated concentrations of methyl mercury (MeHg) in the 10 kDa and 100 kDa cut-off size fractions for the Inlet and Outlet samples are shown in Figures 28 and 29. The data for MeHg concentrations are given in Tables 20 and 21 in Appendix E.4. The \( \frac{\text{MeHg} (\text{ng L}^{-1})}{\text{Total Hg} (\text{ng L}^{-1})} \) ratio was also calculated, and is presented in Tables 22 to 25 in Appendix E.5 for both 10 and 100 kDa. Although the concentration of MeHg was found to be below MLOD (0.02 ng/L) in the summer Permeate samples, values were reported. This was done in order to estimate how MeHg was distributed in the different size fractions.

In Figure 28, it can be seen that the Inlet had higher estimated concentration of MeHg compared to the Outlet. The \( \frac{\text{MeHg} (\text{ng L}^{-1})}{\text{DOC} (\text{mg L}^{-1})} \) ratio was also found to be higher in the Inlet.
samples. The uncertainty in the MeHg data, being below MLOD, in the Permeate fraction made it impossible to test whether this LMW DNOM fraction presented the highest 
\[ \frac{MeHg(n_g)}{TotHg(n_g)} \cdot 100 \] ratio. Consequently, it was only possible to test this ratio for the Influent and Concentrate fractions. In the Inlet Influent and Concentrate, approximately 7% of the estimated TotHg (Table 22 in Appendix E.5) was in the form of MeHg. For the Outlet Influent and Concentrate, approximately 2.4% of the estimated TotHg was in the form of MeHg, suggesting that there was a relative net loss of MeHg in the lake.

The estimated MeHg concentrations in the 100 kDa membrane cut-off size fractions for the fall samples, are given in Figure 29 and Table 21 in Appendix E.4. The MeHg values obtained for the Inlet and Outlet Permeate fractions were close to the MLOD, however, they are presented because they were used to calculate the distribution percentage of MeHg in each fraction, and because the relative standard deviation (RSD) did not exceed 20% of uncertainty.

The highest 
\[ \frac{MeHg(n_g)}{TotHg(n_g)} \cdot 100 \] estimated ratio, was found in the LMW DNOM fraction, where 16.3% and 6.1% of the TotHg was in the form of MeHg in the Inlet and Outlet Permeate, respectively (Table 24 Section E.5). This fraction was also characterised as the most bioavailable fraction (Section 4.7.2 biodegradation experiment). A net loss of MeHg from the Inlet to the Outlet of the lake was also observed. The percentage of TotHg in the form of MeHg decreased from 4.6% to 3.0% for the Influent samples, and from 4.5% to 2.9% for the Concentrate samples.

Statistical significant differences (p < 0.01), were found in the concentration of MeHg in the Inlet and Outlet of the lake from January to December in 2016 (Unpublished data: de Wit and Braaten, pers. comm., 2016). Concentrations were found to be lower in the Outlet compared to the Inlet.
Summary

A net loss of MeHg from the Inlet to the Outlet of the lake was observed in all the size fractions in the summer and the fall. A two-samples t-test, assuming equal variance, showed statistical significant differences ($p < 0.05$) in the concentration of MeHg in the Inlet and Outlet of Langtjern from January to December in 2016 (de Wit; Braatan pers. comm; 2016).

The highest estimated $\frac{MeHg(\text{ng}/L)}{TotHg(\text{ng}/L)} \cdot 100$ ratio in the samples fractionated with 100 kDa was found in the LMW Inlet and Outlet size fractions, i.e. $< 100$ kDa, with values of 16% and 6%, respectively. This ratio could not be tested, however, for the LMW Inlet and Outlet Permeate samples due to the concentration of MeHg and DOC being below MLOD and MLOQ, respectively.
4.6  Structural Characterization of DNOM

4.6.1 UV-VIS Absorbance

Absorbance at \( \lambda \) 254 nm (UV) and \( \lambda \) 400 nm (VIS), as well as the spectroscopic calculated proxies sUVa, sVISa and SAR are given in Tables 27 and 28 in Appendix F.2. The UV-VIS spectra for the Inlet and Outlet size fractions are shown in Figures 31-43 in Appendix F.3.

sUVa values for the Inlet and Outlet samples fractionated with 10 kDa and 100 kDa cut-off are depicted in Figures 30 and 31, respectively. sUVa generally decreased with molecular size, suggesting that the Permeate fractions (LMW) are less aromatic than the Influent and Concentrate fractions (HMW). This is in accordance with the general consensus found in the literature (Vogt and Gjessing, 2008; Frimmel and Abbt-Braun, 2009). However, the < 10 kDa Permeate in the Outlet sample (Figure 30), has a slightly higher sUVa compared to the Concentrate. This is likely due to the large uncertainty in the DOC concentration, being below MLOQ, in this fraction.

sVISa values fall (100 kDa)

![sUVa values summer (10 kDa)](image_url)

![sUVa values fall (100 kDa)](image_url)

Figure 30 (left) Values for the Specific UV Absorbance, \((\text{Abs}_{254\text{nm/DOC}})\cdot 100\), of the different fractions in the samples collected from the Inlet and Outlet in the summer of 2016, and fractionated with a 10 kDa cut-off. Figure 31 (right) Values for the Specific UV Absorbance, \((\text{Abs}_{254\text{nm/DOC}})\cdot 100\), of the different fractions in the samples collected from the Inlet and Outlet in the fall of 2016, and fractionated with a 100 kDa cut-off.

sVISa values for the 10 and 100 kDa cut-off size fractions are presented in Figures 32 and 33, respectively. sVISa decreased, as inherently expected, with decreasing molecular
weight. The samples presenting the lowest sVISa were therefore the Permeate samples, especially the Outlet Permeate < 10 kDa. In the summer sample, the Inlet fractions presented a higher sVISa than the Outlet fractions (Figure 32). This implies that the DNOM in the Inlet had a higher amount of HMW chromophores. However, this was not observed for the fall samples, where the sVISa values for the Inlet and Outlet were not significantly different.

The highest SAR values were found in the Permeate size fraction in all the samples (Figure 34 and 35). This confirmed that this fraction is mainly dominated by LMW DNOM, while the Influent and Concentrate are more dominated by HMW aromatic DNOM. The SAR values thus supported previous findings using sUVa and sVISa proxies. The higher the sUVa and sVISa, and the lower the SAR, the more difficult it is for bacteria to biodegrade the organic matter in the sample. This is further explained in Section 4.7.
4.6.2 UV-VIS Fluorescence excitation-emission matrix contour plots

Fluorescence Excitation-Emission Matrix (EEM) contour plots, corrected for absorbance, of the DNOM in the different size fractions are presented in Figures 36 and 37. The peaks observed in these EEM contour plots, A and C, have a broad emission maximum containing many conjugated fluorescence molecules, and are mainly derived from vascular plants (primarily of terrestrial origin). Peaks located in Position C (Figure 36) represent more aromatic and hydrophobic humic acid, whereas peaks located in Position A correspond to more aliphatic and fulvic acids (Section 2.4.2). The location of the two main peaks, A and C, in the EEM contour plots based on their excitation and emission wavelengths ($\lambda_{em}$, $\lambda_{ex}$), are given in Tables 29 and 30 in Appendix G.1.

The fluorescence EEM contour plots for the samples fractionated with 10 kDa are presented in Figure 36. The general picture observed for both the Inlet and the Outlet samples is that with decreasing molecular weight towards the LMW size fraction, i.e. from Concentrate to Permeate, the aromatic acid (Peak C) completely disappeared, and the fulvic acid (Peak A) remained, however, to a very low degree.
Figure 36 Fluorescence EEM spectra contour plots for the different size fractions of the Inlet and Outlet samples collected in the summer of 2016, and fractionated using a 10 kDa cut-off.
The Fluorescence EEM contour plots for the samples fractionated with 100 kDa membrane are presented in Figure 37. The same trend as that observed in the 10 kDa cut-off samples was observed in the 100 kDa cut-off samples. With decreasing molecular weight, the fluorophore moieties also contained less humic and fulvic acids than in the HMW size fractions. The content of fluorophore moieties appeared to be higher in the Outlet Permeate compared to the Inlet Permeate.

Figure 37 Fluorescence EEM spectra contour plots for the different size fractions of the Inlet and Outlet samples, collected in the fall of 2016, and fractionated using a 100 kDa cut-off.
4.7 Biodegradation

4.7.1 DNOM spectroscopic properties before and after biodegradation

UV-VIS measurements (Section 3.4.4.1) were performed before and after biodegradation in the set of samples fractionated with 10 kDa membrane cut-off. This was done in order to see whether there were significant changes in the spectroscopic properties of DNOM caused by bacterial degradation.

sUVa values before (b) and after (a) biodegradation of the DNOM in the different size fractions of the Inlet and the Outlet samples are presented in Figures 38 and 39. The highest sUVa value before biodegradation was found in the Influent fraction (< 0.2 µm) for both the Inlet and the Outlet samples (Section 4.5.1). This indicates that this total dissolved size fraction was more aromatic than the other fractions, and thus more difficult to degrade. The lowest sUVa values were found in the Permeate fraction (< 10 kDa), implying that this LMW fraction contained the most biodegradable DNOM material. This assumption matched with the results obtained by the biodegradation experiment (Section 4.7.2).

sUVa measurements after biodegradation were higher for all the size fractions compared to the measurements made before (Figures 38 and 39). This can be explained by considering that the moieties with the lowest sUVa were what was decomposed by the bacteria, leaving a more refractory material remaining after biodegradation. The change in sUVa after biodegradation was much higher for the LMW size fraction than for the others. This fit with the theory that LMW DNOM is the most bioavailable fraction.
Figure 38 (left) Specific UV Absorbance, \((s\text{UVa} = \text{Abs 254nm}/\text{DOC}) \cdot 100\), in the different size fractions of the samples collected in the summer of 2016 from the Inlet of Langtjern and fractionated with 10 kDa, before (b) and after (a) biodegradation. Figure 39 (right) Specific UV Absorbance, \((s\text{UVa} = \text{Abs 254nm}/\text{DOC}) \cdot 100\), in the different size fractions of the samples collected in the summer of 2016 from the Outlet of Langtjern and fractionated with 10 kDa, before (b) and after (a) biodegradation.

\(s\text{VISa}\) data for the set of water samples fractionated with 10 kDa before (b) and after (a) biodegradation are shown in Figures 40 and 41.

As described in Section 4.6.1, the highest \(s\text{VISa}\) values were found in the Influent and Concentrate size fraction (< 0.2 µm), and the lowest in the Permeate size fraction (< 10 kDa). This is in accordance with the theory, describing that high \(s\text{VISa}\) indicates HMW chromophores (Section 2.4.1).

Higher \(s\text{VISa}\) values after biodegradation indicate that the HMW chromophores remained after the more bioavailable LMW DNOM moieties were depleted. Moreover, the larger differences before and after biodegradation were found in the most bioavailable LMW DNOM fraction, i.e. the Permeate. This decrease in \(s\text{VISa}\) after biodegradation can be explained by the fact that bacteria easily degrade components with more simple structures, leaving behind those with more complex structures, which are more difficult to degrade.
Figure 40 (left) Specific Visible Absorbance, \( (\text{Abs}400\text{nm}/\text{DOC}) \times 1000 \), in the different size fractions of the samples collected in the summer of 2016 from the Inlet and fractionated with a membrane cut-off of 10 kDa, before (b) and after (a) biodegradation. Figure 41 (right) Specific Visible Absorbance (sVISa= (Abs400nm/DOC)-1000) in the different size fractions of the samples collected in the summer of 2016 from the Outlet and fractionated with a membrane cut-off of 100 kDa, before (b) and after (a) biodegradation.

SAR in the different size fractions for the Inlet and Outlet samples before (b) and after (a) biodegradation are presented in Figures 42 and 43, respectively.

The highest SAR values were, as expected, found in the LMW DNOM fraction, i.e. the Permeate, before biodegradation (Section 4.6.1). After biodegradation, a decrease in SAR in all the size fractions was observed, being especially significant in the LMW DNOM fraction in the Outlet sample. This decrease in SAR values after biodegradation can be explained by bacteria’s preference for LMW organic compounds.
4.7.2 DNOM biodegradation measurements

Respiration rates (mmol O₂/L·h) relative to the DOC concentration (mg C/L) for the Inlet and Outlet samples, fractionated with 10 and 100 kDa, are presented in Figures 44 and 45.

The calculation of the respiration rate is explained in Figure 45 in Appendix H.1. Biodegradation graphs for each size fraction fractionated with 10 and 100 kDa, obtained by monitoring the oxygen consumption, are presented in Figures 46 to 57 in Appendix H.3.

As explained in Section 2.5, the more hydrophobic DNOM compounds with more condensed aromatic structures, more conjugated fluorescence molecules, and higher molecular size are commonly found to be more refractory in the environment as they are less biodegradable (Marschner and Kalbitz, 2003). This is because microorganisms have limited ability to degrade aromatic compounds and large molecules (Marschner and Kalbitz, 2003). Thus, it is reasonable that the Permeate size fraction of DNOM is the most biodegradable as this is inherently the fraction with the lowest molecular weight and aromaticity, i.e. with the highest SAR and the lowest sUVa.
4.7.2.1 Biodegradation of DNOM sizes fractionated with a membrane cut-off of 10 kDa.

Biodegradability of the DNOM presented as Respiration Rate/DOC (mmol O\textsubscript{2}/L·h), for the Inlet and Outlet samples are shown in Figure 44. The Permeate size fraction had by far the highest biodegradability for both the Inlet and the Outlet samples, followed by the Influent and the Concentrate, which had similar values. This confirms that the LMW DNOM is the preferable size fraction for bacteria to biodegrade.

The Inlet Permeate had the highest biodegradation rate, followed by the Outlet Permeate, with values of 0.73 ± 0.22 mmol O\textsubscript{2}/mg C·h and 0.60 ± 0.11 mmol O\textsubscript{2}/mg C·h, respectively. Due to the DOC concentration being below LOQ in this size fraction it was impossible to conclude whether there were significant differences in the biodegradability of the Inlet and Outlet Permeate fractions due to results with high uncertainty. For the Influent size fraction, the biodegradability for the Inlet and the Outlet were found to be 0.06 ± 0.02 mmol O\textsubscript{2}/mg C·h and 0.04 ± 0.01 mmol O\textsubscript{2}/mg C·h, respectively. Regarding the Concentrate size fraction, no differences were found between the Inlet and the Outlet samples, presenting values of approximately 0.04 ± 0.01 mmol O\textsubscript{2}/mg C·h.

A t-test to study significant differences in the biodegradability of the DNOM material between the different size fractions in all samples could not be carried out. The reason for this was that some problems were encountered during biodegradation; in some of the fractions, one out of three replicates was lost, either due to a bad sealing of the vial, leading to oxygen leakage, or due to inaccurate results in one replicate (Tables 32 and 33 in Appendix H.2).

Glucose was used as reference material due to its high and predictable bioavailability (Maier, 2010). Glucose was thus chosen to verify the presence of bacteria. Its biodegradability was found to be 12.5% and 70.3% higher than that for the Inlet and Outlet Influent samples, respectively, and 46.5% and 61.5% higher than that for the Inlet and Outlet Concentrate samples. The biodegradability of the Permeate, however, was found to be much higher than that for Glucose for both the Inlet and the Outlet samples. The percentage difference in biodegradability between Glucose and Permeate is not presented. This is because, as previously explained, the Permeate had a DOC concentration below MLOQ, thus affecting the uncertainty of the biodegradation results expressed as mmol O\textsubscript{2}/mg C·h.
These results support literature findings that bacteria preferably degrade LMW compounds of the DNOM material (Lynch, 1982; Qualls and Haines, 1992; Guggenberger et al., 1994; Küsel and Drake, 1998; Kaiser et al., 2001; Koivula and Hänninen, 2001). Somewhat surprising is that the biodegradability of the LMW fraction was greater than for Glucose. This can be explained by the fact that the inoculum used for the biodegradation experiment was indigenous bacteria prepared from a Langtjern sample, and these bacteria are more used to consuming the indigenous DNOM rather than Glucose.

Figure 44 Respiration rate/DOC, Inlet and Outlet, summer samples (10 kDa)

4.7.2.2 Biodegradation of DNOM sizes fractionated with a membrane cut-off of 100 kDa.

Biodegradability of the DNOM presented as Respiration Rate/DOC (mmol O$_2$/mg C·h), for the Inlet and Outlet samples size fractionated with 100 kDa, can be seen in Figure 45 and in Tables 34 and 35 in Appendix H.2.

The highest biodegradation rate was, as for the 10 kDa cut-off fractionation, was obtained in the LMW size fraction, i.e. Permeate < 100 kDa. In this case the Permeate fraction had a DOC concentration above MLOQ, making it possible to confirm that the LMW fraction,
having the lowest sUVa and the highest SAR, was the preferable DNOM fraction for bacterial degradation.

New caps and more parafilm were used in this biodegradation experiment in order to avoid oxygen leakage and thereby loss of sample replicates. As a result, only one replicate (Outlet Permeate) was discarded due to leakage, and another (Outlet Concentrate) due to a value clearly out of range (Table 35 Appendix H.2).

The biodegradation of the Inlet Permeate was substantially less than the Outlet Permeate, with values of 0.22 ± 0.04 and 0.85 ± 0.01 mmol O₂/mg C·h, respectively (Figure 45). A two-sample t-test, assuming unequal variance, for the Influent samples showed significant differences (p < 0.05) in biodegradation between the Inlet and the Outlet samples, being higher in the Inlet sample. For the Concentrate samples, a significant difference of in biodegradability was found between the Inlet and Outlet, with values of 0.031 and 0.051 mmol O₂/mg C·h, respectively.

By comparing these results with the reference material Glucose, it can again be concluded that the bacteria from Langtjern rather degrade the LMW organic compounds in DNOM than the HMW moieties in DNOM, as well as Glucose.

![Figure 45 Respiration rate/DOC, Inlet and Outlet, fall samples (100 kDa)](image)

**Figure 45** Respiration rate/DOC (mmol O₂/mg C·h) of the Inlet and Outlet samples collected in the fall of 2016, and size fractionated with a membrane cut-off of 100 kDa, and Glucose as reference material.
Summary

Based on the scientific literature it was expected that the LMW DNOM, i.e. with the lowest sUVa and the highest SAR, would be the most biodegradable fraction. This was confirmed for the samples collected in the summer and in the fall in 2016 and fractionated with 10 and 100 kDa cut-off, respectively. These results confirmed the hypothesis to be tested in this master thesis.

In the summer samples, the Inlet Influent had higher biodegradability than the Outlet Influent, with values of 0.056 ± 0.02 and 0.037 ± 0.007 mmol O$_2$/mg C·h, respectively. The Concentrate size fraction, presented no significant difference in biodegradability between Inlet and Outlet with values of approximately 0.04 ± 0.01 mmol O$_2$/mg C·h. Due to the uncertainties encountered in the Permeate size fraction (< 10 kDa), where the DOC concentration was below LOQ, it was impossible to conclude whether the Inlet or the Outlet Permeate samples were significantly different regarding biodegradability.

For the fall samples, a statistical significantly (p < 0.05) higher biodegradability was found for the DNOM in the Inlet compared to the Outlet Influent samples. For the Concentrate size fraction, a higher biodegradability was found in the Outlet compared to the Inlet with values of 0.051 ± 0.002 and 0.031 ± 0.03 mmol O$_2$/mg C·h, respectively. The Permeate size fraction, having a DOC concentration above MLOQ, was the fraction with the highest biodegradation rate with values of 0.22 ± 0.04 and 0.85 ± 0.01 mmol O$_2$/mg C·h for the Inlet and the Outlet, respectively.

4.8 Reverse Osmosis (RO) reference material results

The physicochemical characteristics of the RO reference material from Langtjern is given in Table 8. This reference material was collected in 2005 downstream of the Outlet of the lake. Only the size fraction < 0.2 µm was studied. Thus, it is interesting to compare the DNOM characteristics of this material with the Influent fraction < 0.2 µm in the Outlet samples collected in the summer and in the fall of 2016. The UV-VIS spectrum for this material can be seen in Figure 46 in Appendix F.3 and the biodegradation graph is presented in Figure 58 in Appendix H.3.

Significant differences (p < 0.001 and p < 0.0001), assuming equal variance, in the DOC concentration of the reference material compared to that measured in the summer and in the fall samples, respectively, were found. The reference material was made with a DOC
concentration of 10 mg C/L. The reason for this was because the Outlet of Langtjern in April normally presents a concentration of ~10 mg C/L (Figure 23). Despite these differences in DOC concentration, the spectroscopic proxies, i.e. sUVa, sVISa and SAR, were not found to be significantly different between these three set of samples. Therefore, no significant differences in the biodegradability of the material were expected to be found. However, this was not the case, and a two-sample t-test revealed statistically significant differences (p < 0.05) in the biodegradability of the reference material compared to the other samples. The concentrations of total and methyl mercury were not found to be significant (p < 0.05) different between the three sets of samples.
Table 8 RO reference material results.

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<th>Parameter</th>
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<th>SUVa</th>
<th>sVISa</th>
<th>SAR</th>
<th>TotHg</th>
<th>MeHg</th>
<th>(MeHg/Total Hg)</th>
<th>Biodegradability</th>
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<td>Units</td>
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<td>$(\frac{400\text{nm}}{\text{DOC}}) \cdot 100$</td>
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<td>ng/L</td>
<td>%</td>
<td>mmolO₂/mgC·h</td>
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5. Conclusion

The Inlet had higher concentration of DOC compared to the Outlet in both the summer and the fall. This decrease in DOC from the Inlet to the Outlet of the lake could be due to partial mineralization of the DNOM by biological and abiotic (photo-oxidation) processes. The DOC concentration increased from the summer to the fall for the Inlet and Outlet, with values of 23% and 36%, respectively. The higher DOC concentrations in the fall samples can be explained by that this sampling was preceded by four moderate precipitation periods, lasting from the end of June towards the end of August. These precipitation periods had led to more water flowing though shallow sub-lateral flow paths, richer in DNOM, bypassing the absorptive capacity of the deeper mineral soil layer. This caused more organic matter to be transported from the soil into the surface water. The summer sampling, on the other hand, was not preceded by such precipitation periods.

The highest TotHg density in the DNOM (i.e. $\frac{\text{TotHg} (\text{ng} \text{ L}^{-1})}{\text{DOC} (\text{mg} \text{ L}^{-1})}$ estimated ratio) was found in the HMW DNOM fraction, in both the Inlet and the Outlet samples fractionated with 100 kDa cut-off and collected in the fall. This is in agreement with previous studies by Hintelmann et al., 2000 and Wu et al., 2004. They explained that the HMW DNOM fraction have better ability to complex Hg than the LMW DNOM fraction, because it has higher density of functional groups. This could, however, not be confirmed in the samples collected the summer and size fractionated with 10 kDa. The reason for this was probably the high uncertainty in the determination of the low DOC concentration (< MLOQ) in the Permeate fraction, affecting the calculation of the $\frac{\text{TotHg} (\text{ng} \text{ L}^{-1})}{\text{DOC} (\text{mg} \text{ L}^{-1})}$ ratio. No statistical significant differences (p < 0.05) in the concentration of TotHg in the Inlet and Outlet were found from January to December in 2016 (Unpublished data; de Wit and Braatan, pers. comm., 2016), suggesting a stable concentration of TotHg in the lake. Furthermore, no temporal correlation between DOC and TotHg was found in the Inlet or the Outlet in 2016. This could be explained by that a temporal increase in DOC does not necessarily result in increasing TotHg levels. In fact, in a study by Eklöf et al., 2012 a stronger temporal correlation was found between the color of organic matter (CDOM), measured as absorbance 420 nm, and TotHg.
The highest relative amounts of methylated Hg (i.e. \( \frac{MeHg(\text{ng L}^{-1})}{TotHg(\text{ng L}^{-1})} \cdot 100 \) estimated ratio) in the samples fractionated with 100 kDa cut-off was found in LMW DNOM fraction, in both the Inlet and Outlet Permeate samples, with values of 16% and 6%, respectively. This ratio could, however, not be confirmed for the LMW Inlet and Outlet Permeate samples fractionated with 10 kDa. The reason for this was that MeHg was below MLOD, and the concentration of DOC below MLOQ. Statistical significant differences (p < 0.05) in the concentration of MeHg in the Inlet and Outlet of Langtjern were found from January to December in 2016 (Unpublished data; de Wit and Braaten., Pers.comm., 2016), showing a net loss of MeHg from the Inlet to the Outlet of the lake.

The biodegradability experiment confirmed that the LMW DNOM, i.e. < 10 and 100 kDa, size fraction, with the highest SAR and the lowest sUVa, was by far the most bioavailable DNOM. On the other hand, the HMW size fraction was practically refractory compared to the LMW. These results confirmed the microorganisms’ preference to LMW DNOM, and are supported by previous studies by Marschner and Kalbitz, (2003). For the 100 kDa Permeate size fractions, the Inlet presented a lower biodegradation rate than the Outlet, with values of 0.22 ± 0.04 and 0.85 ± 0.01 mmol O\(_2\)/mg C·h, respectively. These size fractions also had the highest relative amounts of MeHg, making it more bioavailable for bacteria, which could be of large importance regarding its possible introduction into the food chain. In addition, for the 10 kDa Permeate size fractions, the differences in the biodegradability between the Inlet and Outlet could not be confirmed. The reason for this was that the DOC concentration was below MLOQ, presenting therefore a high uncertainty in the biodegradation rate (mmolO\(_2\)/mgC·h).
5. Future work

In the LMW Permeate fraction the DOC, TotHg and MeHg concentrations were only above LOD in the samples that had been fractionated using a membrane cut-off of 100 kDa. It is therefore recommended to continue applying this fractionation method to study if comparable results are obtained with regards to TotHg, MeHg and biodegradability of the material.

It would be interesting to study why the concentration of TotHg did not follow the temporal pattern of the concentration of DOC in 2016, but remained relatively stable in the lake from the Inlet to the Outlet. One reason for this lack of correlation could be that the colour of the organic matter (CDOM), measured as absorbance at 420 nm, also remained stable, and that sVISa did not change much, and even decreased slightly, during runoff peaks where the DOC increased (Håland., 2017). To investigate further, a correlation study between TotHg and CDOM, and TotHg and sVISa should be conducted.

An improved method for the biodegradation experiment is needed. One of the main problems encountered was that sometimes one out of three replicates had to be discarded due to a bad sealing of the vial, producing oxygen leakage, or due to values out of range. It is therefore recommended to ensure better sealing of the vials, and to make at least four replicates instead of three. A clear improvement would be to be able to count the number of cells added to each replicate, to ensure that all the replicates get the same number of cells. NO₃ could also be added to the nutrient solution to stimulate better bacterial growth.
References


EPA 1630., 1998. Methyl mercury in water by distillation, aqueous ethylation purge and trap, and cold vapor atomic fluorescence spectrometry.


Vogt, R.D., Gjessing, E.T., 2008. Correlation between optical and chemical properties of DNOM. From molecular understanding to innovative applications of humic substances, 337.


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A) pH conductivity and temperature

Table 1 and 2 show the pH and conductivity values with their corresponding temperature for the Inlet and Outlet samples size fractionated with 10 kDa and 100 kDa membrane cut-off, respectively. Results from the Raw water analyses are also reported.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw water</td>
<td>5.17</td>
<td>12.51</td>
<td>20.2</td>
</tr>
<tr>
<td>Influent</td>
<td>4.79</td>
<td>12.51</td>
<td>20.1</td>
</tr>
<tr>
<td>Concentrate</td>
<td>4.79</td>
<td>12.49</td>
<td>20.1</td>
</tr>
<tr>
<td>Permeate</td>
<td>5.54</td>
<td>3.56</td>
<td>20.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outlet</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw water</td>
<td>5.09</td>
<td>9.52</td>
<td>20.2</td>
</tr>
<tr>
<td>Influent</td>
<td>5.14</td>
<td>9.52</td>
<td>20.1</td>
</tr>
<tr>
<td>Concentrate</td>
<td>5.25</td>
<td>10.04</td>
<td>20.3</td>
</tr>
<tr>
<td>Permeate</td>
<td>6.02</td>
<td>3.31</td>
<td>20.3</td>
</tr>
</tbody>
</table>

Table 2 pH, conductivity and temperature for the Inlet and Outlet samples size fractionated with a membrane cut-off of 100 kDa.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw water</td>
<td>5.18</td>
<td>12.00</td>
<td>17.6</td>
</tr>
<tr>
<td>Influent</td>
<td>5.30</td>
<td>8.90</td>
<td>23.2</td>
</tr>
<tr>
<td>Concentrate</td>
<td>5.07</td>
<td>17.46</td>
<td>22.3</td>
</tr>
<tr>
<td>Permeate</td>
<td>5.05</td>
<td>14.55</td>
<td>22.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outlet</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw water</td>
<td>5.33</td>
<td>7.32</td>
<td>17.5</td>
</tr>
<tr>
<td>Influent</td>
<td>5.49</td>
<td>9.00</td>
<td>22.0</td>
</tr>
<tr>
<td>Concentrate</td>
<td>5.23</td>
<td>13.12</td>
<td>22.5</td>
</tr>
<tr>
<td>Permeate</td>
<td>5.22</td>
<td>10.96</td>
<td>22.2</td>
</tr>
</tbody>
</table>

B) DOC

B.1 Preparation of the standard solutions

A 1000 mg C/L stock solution was prepared by dissolving 2.125 g of potassium hydrogen phthalate (KH-C₈H₅KO₄), which was previously dried for 1 h at 100°C, in 1000 mL volumetric flask with Milli-Q type I water. A diluted working solution of 100 mg C/L was used to make standards with concentration: 0, 2, 5, 10, 15 and 20 mg C/L.

The instrument measures the DOC concentration in each sample 3-5 times until the RSD is less than 2%. Figure 1 presents the DOC calibration curve with its equation (y) and strength (R²).
B.2 Distribution percent (%) and DOC concentration (mg/L)

Tables 3 and 4 present the measured and estimated DOC concentration of the Inlet and Outlet samples size fractionated with 10 and 100 kDa membrane cut-off, respectively.

Table 3 Measured DOC concentration (mg C/L), and estimated DOC concentration (mg C/L) based on the % distribution of DOC in the different size fractions in the Inlet and Outlet samples size fractionated with a membrane cut-off of 10 kDa. The RSD is approximately 2%.

<table>
<thead>
<tr>
<th>DOC</th>
<th>INLET</th>
<th></th>
<th>OUTLET</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured</td>
<td>%</td>
<td>Estimated</td>
<td>Measured</td>
</tr>
<tr>
<td></td>
<td>DOC (mg C/L)</td>
<td>Distrib.</td>
<td>DOC (mg C/L)</td>
<td>DOC (mg C/L)</td>
</tr>
<tr>
<td>Influent</td>
<td>12.55</td>
<td>100</td>
<td>12.55</td>
<td>8.78</td>
</tr>
<tr>
<td>Concentrate</td>
<td>12.99</td>
<td>98.60</td>
<td>12.37</td>
<td>12.89</td>
</tr>
<tr>
<td>Permeate</td>
<td>0.55</td>
<td>1.39</td>
<td>0.17</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Figure 1 DOC calibration curve.

\[ y = 3.176x + 0.2464 \]

\[ R^2 = 0.9999 \]
Table 4 Measured DOC concentration (mg C/L), and estimated DOC concentration (mg C/L) based on the % distribution of DOC in the different size fractions in the Inlet and Outlet samples size fractionated with a membrane cut-off of 100 kDa. The RSD is approximately 2%.

<table>
<thead>
<tr>
<th>DOC</th>
<th>INLET</th>
<th>OUTLET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured DOC (mg C/L)</td>
<td>% Distrib.</td>
</tr>
<tr>
<td>Influent</td>
<td>15.47</td>
<td>100</td>
</tr>
<tr>
<td>Concentrate</td>
<td>18.76</td>
<td>94.79</td>
</tr>
<tr>
<td>Permeate</td>
<td>3.65</td>
<td>5.51</td>
</tr>
</tbody>
</table>

B.3 Instrument settings
Table 5 shows the instrument settings for the TOC-analyser.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
<td>5 bar</td>
</tr>
<tr>
<td>Flow rate</td>
<td>150 mL/min</td>
</tr>
<tr>
<td>Number of injections</td>
<td>3</td>
</tr>
<tr>
<td>Maxim number of injections</td>
<td>5</td>
</tr>
<tr>
<td>Minim number of injections</td>
<td>3</td>
</tr>
<tr>
<td>Number of washes</td>
<td>6</td>
</tr>
<tr>
<td>Detector</td>
<td>NDIR</td>
</tr>
</tbody>
</table>

C) IC

C.1 Preparation of the standard solutions
A Dionex Seven Anion multi element standard solution from Thermo Fisher Scientific was used to make the standard solutions. The elemental composition of this Dionex Seven Anion Standard is presented in Table 6.

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride (F⁻)</td>
<td>20</td>
</tr>
<tr>
<td>Chloride (Cl⁻)</td>
<td>30</td>
</tr>
<tr>
<td>Nitrite (NO₂⁻)</td>
<td>100</td>
</tr>
<tr>
<td>Bromide (Br⁻)</td>
<td>100</td>
</tr>
<tr>
<td>Nitrate (NO₃⁻)</td>
<td>100</td>
</tr>
<tr>
<td>Phosphate (PO₄³⁻)</td>
<td>150</td>
</tr>
<tr>
<td>Sulphate (SO₄²⁻)</td>
<td>150</td>
</tr>
</tbody>
</table>
Figures 2 to 5 present the calibration curve of each measured ion with its correspondent equation \((y)\) and strength \((R^2)\). These calibration curves were made by plotting the area \((\text{mS-min})\) VS concentration \((\text{mg/L})\) obtained by integrating the peaks corresponding to each anion in the standard solutions (Figures 6-8).

![Calibration curves](image)

Figure 2 (right) Calibration curve for Fluoride \((F^-)\). Figure 3 (left) Calibration curve for Chloride \((Cl^-)\).

![Calibration curves](image)

Figure 4 (right) Calibration curve for sulphate \((SO_4^{2-})\). Figure 5 (left) Calibration curve for nitrate \((NO_3^-)\).
C.2 Instrument settings

Table 7 presents the instrument settings for the IC instrument.

Table 7 Instrument settings for the IC instrument.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC column</td>
<td>AS 4µm anion</td>
</tr>
<tr>
<td>Run time per sample</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Type of flow</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.25 mL/min</td>
</tr>
<tr>
<td>Pump pressure</td>
<td>200 psi</td>
</tr>
<tr>
<td>Pump column, and compartment temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Electrolytic suppressor type</td>
<td>AERS_2mm</td>
</tr>
<tr>
<td>Electrolytics suppressor hydroxide</td>
<td>23 mM</td>
</tr>
<tr>
<td>Electrolytics eluent generator concentration</td>
<td>23 M</td>
</tr>
</tbody>
</table>

C.3 Integration area graphs

Figures 6-20 present the chromatograms and the integration results from the calibration solutions and the samples analysed with the IC instrument.
a) Calibration standards

### Chromatogram and Results

<table>
<thead>
<tr>
<th>Injection Details</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection Name:</td>
<td>std-1</td>
</tr>
<tr>
<td>Vial Number:</td>
<td>4</td>
</tr>
<tr>
<td>Injection Type:</td>
<td>Calibration Standard</td>
</tr>
<tr>
<td>Calibration Level:</td>
<td>1</td>
</tr>
<tr>
<td>Instrument Method:</td>
<td>A818 4um anion</td>
</tr>
<tr>
<td>Processing Method:</td>
<td>Anion A818 4um</td>
</tr>
<tr>
<td>Injection Date/Time:</td>
<td>10.nov.16 16:10</td>
</tr>
<tr>
<td>Run Time (min):</td>
<td>10.00</td>
</tr>
<tr>
<td>Injection Volume:</td>
<td>1666.00</td>
</tr>
<tr>
<td>Channel:</td>
<td>CD</td>
</tr>
<tr>
<td>Wavelength:</td>
<td>n.a.</td>
</tr>
<tr>
<td>Bandwidth:</td>
<td>n.a.</td>
</tr>
<tr>
<td>Dilution Factor:</td>
<td>1.0000</td>
</tr>
<tr>
<td>Sample Weight:</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

### Chromatogram

![Chromatogram](image)

### Integration Results

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time min</th>
<th>Area µS*min</th>
<th>Height µS</th>
<th>Relative Area %</th>
<th>Relative Height %</th>
<th>Amount mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoride</td>
<td>2.880</td>
<td>0.640</td>
<td>7.360</td>
<td>9.70</td>
<td>14.88</td>
<td>0.2520</td>
</tr>
<tr>
<td>2</td>
<td>Chloride</td>
<td>4.137</td>
<td>0.555</td>
<td>5.728</td>
<td>8.42</td>
<td>11.58</td>
<td>0.3803</td>
</tr>
<tr>
<td>3</td>
<td>Nitrite</td>
<td>5.033</td>
<td>1.546</td>
<td>10.852</td>
<td>23.44</td>
<td>21.95</td>
<td>1.2503</td>
</tr>
<tr>
<td>4</td>
<td>Bromide</td>
<td>6.173</td>
<td>2.061</td>
<td>14.766</td>
<td>31.25</td>
<td>29.86</td>
<td>1.2754</td>
</tr>
<tr>
<td>5</td>
<td>Sulfate</td>
<td>7.167</td>
<td>0.791</td>
<td>5.335</td>
<td>12.00</td>
<td>10.79</td>
<td>1.8995</td>
</tr>
<tr>
<td>6</td>
<td>Nitrate</td>
<td>8.803</td>
<td>1.081</td>
<td>5.408</td>
<td>15.18</td>
<td>10.94</td>
<td>1.2719</td>
</tr>
</tbody>
</table>

Total: 6,593 49,449 100.00 100.00

Figure 6 Chromatogram and integration results for the standard solution 1.
Figure 7 Chromatogram and integration results for the standard solution 2.
Figure 8 Chromatogram and integration results for the standard solution 3.
b) Inlet sample with its respective size fractions, summer 2016.

### Chromatogram and Results

**Injection Details**

<table>
<thead>
<tr>
<th>Details</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection Name</td>
<td>7</td>
</tr>
<tr>
<td>Vial Number</td>
<td>13</td>
</tr>
<tr>
<td>Injection Type</td>
<td>Unknown</td>
</tr>
<tr>
<td>Calibration Level</td>
<td></td>
</tr>
<tr>
<td>Instrument Method</td>
<td>A818 4um anion</td>
</tr>
<tr>
<td>Processing Method</td>
<td>Anion A818 4um</td>
</tr>
<tr>
<td>Injection Date/Time</td>
<td>10.nov.16 17:58</td>
</tr>
<tr>
<td>Run Time (min)</td>
<td>10.00</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>1666.00</td>
</tr>
<tr>
<td>Channel</td>
<td>CD</td>
</tr>
<tr>
<td>Wavelength</td>
<td>n.a.</td>
</tr>
<tr>
<td>Bandwidth</td>
<td>n.a.</td>
</tr>
<tr>
<td>Dilution Factor</td>
<td>1.0000</td>
</tr>
<tr>
<td>Sample Weight</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

**Chromatogram**

![Chromatogram](image)

**Integration Results**

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time (min)</th>
<th>Area (µS/min)</th>
<th>Height (µS)</th>
<th>Relative Area (%)</th>
<th>Relative Height (%)</th>
<th>Amount (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoride</td>
<td>2.883</td>
<td>0.986</td>
<td>1.123</td>
<td>7.27</td>
<td>10.55</td>
<td>0.0233</td>
</tr>
<tr>
<td>2</td>
<td>Chloride</td>
<td>4.143</td>
<td>0.566</td>
<td>5.666</td>
<td>46.72</td>
<td>54.99</td>
<td>0.3889</td>
</tr>
<tr>
<td>3</td>
<td>Nitrite</td>
<td>5.147</td>
<td>0.147</td>
<td>0.604</td>
<td>12.05</td>
<td>5.68</td>
<td>n.a.</td>
</tr>
<tr>
<td>4</td>
<td>Bromide</td>
<td>6.167</td>
<td>0.339</td>
<td>2.611</td>
<td>27.68</td>
<td>24.52</td>
<td>0.2518</td>
</tr>
<tr>
<td>n.a.</td>
<td>Sulfate</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>5</td>
<td>Nitrate</td>
<td>8.673</td>
<td>0.074</td>
<td>0.455</td>
<td>6.09</td>
<td>4.28</td>
<td>0.1879</td>
</tr>
</tbody>
</table>

**Total:**

|               | 1.217 | 10.650 | 100.00 | 100.00 | 0.1879 |

Figure 9 Chromatogram and integration results for the Inlet Influent sample, summer 2016.
**Injection Details**

- **Injection Name:** 8
- **Vial Number:** 14
- **Injection Type:** Unknown
- **Calibration Level:** n.a.
- **Instrument Method:** AS18 4um anion
- **Processing Method:** Anion AS18 4um
- **Injection Date/Time:** 10.nov.16 18:10
- **Run Time (min):** 10.00
- **Injection Volume:** 1666.00 µL
- **Channel:** CD
- **Wavelength:** n.a.
- **Bandwidth:** n.a.
- **Dilution Factor:** 1,0000
- **Sample Weight:** 1,0000

---

**Chromatogram**

![Chromatogram Image]

**Integration Results**

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time (min)</th>
<th>Area (µS*min)</th>
<th>Height (µS)</th>
<th>Relative Area (%)</th>
<th>Relative Height (%)</th>
<th>Amount (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoride</td>
<td>2,883</td>
<td>0,093</td>
<td>1,107</td>
<td>7,21</td>
<td>9,78</td>
<td>0.0252</td>
</tr>
<tr>
<td>2</td>
<td>Chloride</td>
<td>4,143</td>
<td>0,639</td>
<td>6,578</td>
<td>49,52</td>
<td>58,13</td>
<td>0.4349</td>
</tr>
<tr>
<td>3</td>
<td>Nitrile</td>
<td>5,147</td>
<td>0,148</td>
<td>0,616</td>
<td>11,44</td>
<td>5,45</td>
<td>n.a.</td>
</tr>
<tr>
<td>4</td>
<td>Bromide</td>
<td>6,163</td>
<td>0,341</td>
<td>2,568</td>
<td>26,42</td>
<td>22,87</td>
<td>0.2528</td>
</tr>
<tr>
<td>n.a.</td>
<td>Sulfate</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>5</td>
<td>Nitrato</td>
<td>8,673</td>
<td>0,070</td>
<td>0,426</td>
<td>5,41</td>
<td>3,77</td>
<td>0.1829</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td></td>
<td><strong>1,290</strong></td>
<td><strong>11,315</strong></td>
<td><strong>100.00</strong></td>
<td><strong>100.00</strong></td>
<td><strong>100.00</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

---

Figure 10 Chromatogram and integration results for the Inlet Concentrate sample, summer 2016.
Figure 11 Chromatogram and integration results for the Inlet Permeate sample, summer 2016.
c) Outlet sample with its respective size fractions, summer 2016.

Figure 12 Chromatogram and integration results for the Outlet Influent sample, summer 2016.
Figure 13 Chromatogram and integration results for the Outlet Concentrate sample, summer 2016.
Figure 14 Chromatogram and integration results for the Outlet Permeate sample, summer 2016.
d) Inlet sample with its respective size fractions, fall 2016.

**Chromatogram and Results**

<table>
<thead>
<tr>
<th>Injection Details</th>
<th>Inlet sample with its respective size fractions, fall 2016.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection Name:</td>
<td>3</td>
</tr>
<tr>
<td>Vial Number:</td>
<td>9</td>
</tr>
<tr>
<td>Injection Type:</td>
<td>Unknown</td>
</tr>
<tr>
<td>Calibration Level:</td>
<td>n.a.</td>
</tr>
<tr>
<td>Instrument Method:</td>
<td>Anion AS18 4um anion</td>
</tr>
<tr>
<td>Processing Method:</td>
<td>Anion AS18 4um</td>
</tr>
<tr>
<td>Injection Date/Time:</td>
<td>10.nov.16 17:10</td>
</tr>
</tbody>
</table>

**Integration Results**

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>Height</th>
<th>Relative Area</th>
<th>Relative Height</th>
<th>Amount</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoride</td>
<td>2.880</td>
<td>0,114</td>
<td>1,443</td>
<td>6,12</td>
<td>8,45</td>
<td>0,0338</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Chloride</td>
<td>4,143</td>
<td>1,200</td>
<td>12,274</td>
<td>64,92</td>
<td>71,88</td>
<td>0,8043</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Nitrite</td>
<td>5,160</td>
<td>0,158</td>
<td>0,633</td>
<td>3,71</td>
<td>n.a.</td>
<td>0,2129</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Bromide</td>
<td>6,187</td>
<td>0,274</td>
<td>2,098</td>
<td>14,74</td>
<td>12,29</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>Sulfate</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Nitrate</td>
<td>8,077</td>
<td>0,106</td>
<td>0,628</td>
<td>3,99</td>
<td>3,69</td>
<td>0,2249</td>
<td></td>
</tr>
</tbody>
</table>

Total: 1,887 17,076 100,00 100,00

Figure 15 Chromatogram and integration results for the Inlet Influent sample, fall 2016.
**Chromatogram and Results**

<table>
<thead>
<tr>
<th>Injection Details</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection Name:</td>
<td>4</td>
<td>Run Time (min): 10.00</td>
</tr>
<tr>
<td>Vial Number:</td>
<td>10</td>
<td>Injection Volume: 1666.00</td>
</tr>
<tr>
<td>Injection Type:</td>
<td>Unknown</td>
<td>Channel: CD</td>
</tr>
<tr>
<td>Calibration Level:</td>
<td></td>
<td>Wavelength: n.a.</td>
</tr>
<tr>
<td>Processing Method:</td>
<td>Anion A518 4um</td>
<td>Dilution Factor: 1.0000</td>
</tr>
<tr>
<td>Injection Date/Time</td>
<td>10.nov.16 17:22</td>
<td>Sample Weight: 1.0000</td>
</tr>
</tbody>
</table>

**Chromatogram**

![Chromatogram Image]

**Integration Results**

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time (min)</th>
<th>Area (µS/min)</th>
<th>Height (µS)</th>
<th>Relative Area (%)</th>
<th>Relative Height (%)</th>
<th>Amount (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoride</td>
<td>2.883</td>
<td>0.119</td>
<td>1.476</td>
<td>5.42</td>
<td>7.32</td>
<td>0.0359</td>
</tr>
<tr>
<td>2</td>
<td>Chloride</td>
<td>4.143</td>
<td>1.460</td>
<td>14.841</td>
<td>66.63</td>
<td>73.53</td>
<td>0.9700</td>
</tr>
<tr>
<td>3</td>
<td>Nitrite</td>
<td>5.153</td>
<td>0.143</td>
<td>0.599</td>
<td>6.53</td>
<td>2.97</td>
<td>n.a.</td>
</tr>
<tr>
<td>4</td>
<td>Bromide</td>
<td>6.177</td>
<td>0.329</td>
<td>2.465</td>
<td>15.03</td>
<td>12.21</td>
<td>0.2460</td>
</tr>
<tr>
<td>n.a.</td>
<td>Sulfate</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>5</td>
<td>Nitrate</td>
<td>8.670</td>
<td>0.140</td>
<td>0.802</td>
<td>6.39</td>
<td>3.97</td>
<td>0.2651</td>
</tr>
</tbody>
</table>

**Total:** 2,191 20,183 100.00 100.00

Figure 16 Chromatogram and integration results for the Inlet Concentrate sample, fall 2016.
Chromatogram and Results

Injection Details

<table>
<thead>
<tr>
<th>Injection Name:</th>
<th>2</th>
<th>Run Time (min):</th>
<th>10.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vial Number:</td>
<td>8</td>
<td>Injection Volume:</td>
<td>1666.00</td>
</tr>
<tr>
<td>Injection Type:</td>
<td>Unknown</td>
<td>Channel:</td>
<td>CD</td>
</tr>
<tr>
<td>Calibration Level:</td>
<td></td>
<td>Wavelength:</td>
<td>n.a.</td>
</tr>
<tr>
<td>Processing Method:</td>
<td>Anion AS18 4um</td>
<td>Dilution Factor:</td>
<td>1,0000</td>
</tr>
<tr>
<td>Injection Date/Time:</td>
<td>10.nov.16 16:58</td>
<td>Sample Weight:</td>
<td>1,0000</td>
</tr>
</tbody>
</table>

Chromatogram

Integration Results

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>Height</th>
<th>Relative Area</th>
<th>Relative Height</th>
<th>Amount [mg/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoride</td>
<td>2.677</td>
<td>0.079</td>
<td>1.044</td>
<td>4.17</td>
<td>5.87</td>
<td>0.0193</td>
</tr>
<tr>
<td>2</td>
<td>Chloride</td>
<td>4.140</td>
<td>1.436</td>
<td>14.619</td>
<td>76.07</td>
<td>82.20</td>
<td>0.9542</td>
</tr>
<tr>
<td>3</td>
<td>Nitrite</td>
<td>5.157</td>
<td>0.146</td>
<td>0.589</td>
<td>7.73</td>
<td>3.31</td>
<td>n.a.</td>
</tr>
<tr>
<td>4</td>
<td>Bromide</td>
<td>6.193</td>
<td>0.109</td>
<td>0.851</td>
<td>5.76</td>
<td>4.78</td>
<td>0.1148</td>
</tr>
<tr>
<td>n.a.</td>
<td>Sulfate</td>
<td></td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>5</td>
<td>Nitrate</td>
<td>8.670</td>
<td>0.118</td>
<td>0.683</td>
<td>6.26</td>
<td>3.84</td>
<td>0.2396</td>
</tr>
</tbody>
</table>

Total: 1,888 17,785 100.00 100.00

Figure 17 Chromatogram and integration results for the inlet Permeate sample, fall 2016.
e) Outlet sample with its respective size fractions, fall 2016.

**Chromatogram and Results**

<table>
<thead>
<tr>
<th>Injection Details</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection Name:</td>
<td>10</td>
</tr>
<tr>
<td>Vial Number:</td>
<td>16</td>
</tr>
<tr>
<td>Injection Type:</td>
<td>Unknown</td>
</tr>
<tr>
<td>Calibration Level:</td>
<td>n.a.</td>
</tr>
<tr>
<td>Instrument Method:</td>
<td>A518 4um anion</td>
</tr>
<tr>
<td>Processing Method:</td>
<td>Anion A518 4um</td>
</tr>
<tr>
<td>Injection Date/Time:</td>
<td>10.00 nov.16 18:59</td>
</tr>
<tr>
<td>Run Time (min):</td>
<td>10.00</td>
</tr>
<tr>
<td>Injection Volume:</td>
<td>1666.00</td>
</tr>
<tr>
<td>Channel:</td>
<td>CD</td>
</tr>
<tr>
<td>Wavelength:</td>
<td>n.a.</td>
</tr>
<tr>
<td>Bandwidth:</td>
<td>n.a.</td>
</tr>
<tr>
<td>Dilution Factor:</td>
<td>1.0000</td>
</tr>
<tr>
<td>Sample Weight:</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

**Chromatogram**

Figure 18 Chromatogram and integration results for the Outlet Influent sample, fall 2016.
Figure 19 Chromatogram and integration results for the Outlet Concentrate sample, fall 2016.
Chromatogram and Results

Injection Details

<table>
<thead>
<tr>
<th>Injection Name</th>
<th>9</th>
<th>Run Time (min): 10.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vial Number</td>
<td>15</td>
<td>Injection Volume: 1666.00</td>
</tr>
<tr>
<td>Injection Type</td>
<td>Unknown</td>
<td>Channel: CD</td>
</tr>
<tr>
<td>Calibration Level</td>
<td></td>
<td>Wavelength: n.a.</td>
</tr>
<tr>
<td>Instrument Method</td>
<td>A518 4um anion</td>
<td>Bandwidth: n.a.</td>
</tr>
<tr>
<td>Processing Method</td>
<td>Anion A518 4um</td>
<td>Dilution Factor: 1.0000</td>
</tr>
<tr>
<td>Injection Date/Time</td>
<td>10.nov.16 18:47</td>
<td>Sample Weight: 1.0000</td>
</tr>
</tbody>
</table>

Chromatogram

Integration Results

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time min</th>
<th>Area µS·min</th>
<th>Height µS</th>
<th>Relative Area %</th>
<th>Relative Height %</th>
<th>Amount mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoride</td>
<td>0.523</td>
<td>0.858</td>
<td>2.570</td>
<td>38.46</td>
<td>16.97</td>
<td>n.a.</td>
</tr>
<tr>
<td>2</td>
<td>Chloride</td>
<td>2.880</td>
<td>0.070</td>
<td>0.918</td>
<td>3.15</td>
<td>6.06</td>
<td>0.0157</td>
</tr>
<tr>
<td>3</td>
<td>Nitrate</td>
<td>4.140</td>
<td>0.132</td>
<td>8.714</td>
<td>37.83</td>
<td>57.55</td>
<td>0.5679</td>
</tr>
<tr>
<td>4</td>
<td>Nitrile</td>
<td>5.150</td>
<td>0.030</td>
<td>0.555</td>
<td>5.03</td>
<td>3.67</td>
<td>n.a.</td>
</tr>
<tr>
<td>5</td>
<td>Bromide</td>
<td>6.173</td>
<td>0.247</td>
<td>1.903</td>
<td>11.09</td>
<td>12.56</td>
<td>0.1971</td>
</tr>
<tr>
<td>n.a.</td>
<td>Sulfate</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>6</td>
<td>Nitrate</td>
<td>7.403</td>
<td>0.010</td>
<td>0.066</td>
<td>0.45</td>
<td>0.44</td>
<td>n.a.</td>
</tr>
<tr>
<td>7</td>
<td>Nitrile</td>
<td>8.667</td>
<td>0.069</td>
<td>0.417</td>
<td>3.08</td>
<td>2.75</td>
<td>0.1816</td>
</tr>
</tbody>
</table>

Total: 2,229 15,143 100.00 100.00

Figure 20 Chromatogram and integration results for the Outlet Permeate sample, fall 2016.
D) ICP-OES

D.1 Preparation of the standard solutions

Seven multi-element standard solutions containing Al$^{3+}$, Ca$^{2+}$, Fe$^{2+}$, K$^+$, Mg$^{2+}$, Mn$^{2+}$, and Na$^+$ were made with increasing concentration.

Figure 21 and Table 8 present the wavelength ($\lambda$) selected for each element. This selection was based on peak height, and peaks presenting less interferences.

![Figure 21 Wavelength used for the characterization of Al$^{3+}$, Ca$^{2+}$, Fe$^{2+}$, K$^+$, Mg$^{2+}$, Mn$^{2+}$, and Na$^+$. The chosen $\lambda$ are highlighted with a pink marker.](image)

Table 8 Chosen $\lambda$ for each cation.

<table>
<thead>
<tr>
<th>Element</th>
<th>$\lambda$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al$^{3+}$</td>
<td>396.152</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>396.847</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>238.204</td>
</tr>
<tr>
<td>K$^+$</td>
<td>766.491</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>285.213</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>257.610</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>589.592</td>
</tr>
</tbody>
</table>
Figures 22-28 present the calibration curves for each cation.

Figure 22 (right) Calibration curve for Aluminium (Al\textsuperscript{3+}). Figure 23 (left) Calibration curve for Calcium (Ca\textsuperscript{2+}).

Figure 24 (right) Calibration curve for iron (Fe\textsuperscript{3+}). Figure 25 (left) Calibration curve for potassium (K\textsuperscript{+}).

Figure 26 (right) Calibration curve for magnesium (Mg\textsuperscript{2+}). Figure 27 (left) Calibration curve for sodium (Na\textsuperscript{+}).
D.2 Instrument settings
The instrument settings for the ICP-OES are represented in Table 9

Table 9 Instrument settings for the ICP-OES instrument.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF power</td>
<td>1.00 Kw</td>
</tr>
<tr>
<td>Plasma Ar flow</td>
<td>15.0 L/min</td>
</tr>
<tr>
<td>Auxiliar Ar flow</td>
<td>1.50 L/min</td>
</tr>
<tr>
<td>Nebulizer Ar flow</td>
<td>0.75 L/min</td>
</tr>
<tr>
<td>Sample flow rate</td>
<td>1.00 mL/min</td>
</tr>
<tr>
<td>Reading time</td>
<td>1.0 min</td>
</tr>
</tbody>
</table>
D.3 Anions and cations calculations

Tables 10 and 11 present the concentration of ions (mg/L) in the Influent size fraction (< 0.2 µm) in the Inlet and Outlet samples collected in the summer and the fall, respectively. The LOD (3·SD of 6 blanks) is also presented. ND stands for not detected.

Table 10 Ion concentration (mg/L) in the Influent size fraction (< 0.2 µm) in the Inlet and Outlet samples, summer of 2016.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Inlet (mg/L)</th>
<th>Outlet (mg/L)</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl⁻</td>
<td>0.389± 0.009</td>
<td>0.435± 0.046</td>
<td>0.115</td>
<td>0.38</td>
</tr>
<tr>
<td>F⁻</td>
<td>0.023± 0.002</td>
<td>0.021± 0.002</td>
<td>0.008</td>
<td>2.6·10⁻²</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.378± 0.039</td>
<td>0.578± 0.006</td>
<td>0.151</td>
<td>0.50</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0.188± 0.008</td>
<td>0.173± 0.005</td>
<td>0.087</td>
<td>0.28</td>
</tr>
<tr>
<td>Al³⁺</td>
<td>0.250± 0.030</td>
<td>0.120± 0.020</td>
<td>0.040</td>
<td>0.13</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.510± 0.121</td>
<td>0.690± 0.026</td>
<td>0.001</td>
<td>3.3·10⁻³</td>
</tr>
<tr>
<td>Fe³⁺</td>
<td>0.340± 0.009</td>
<td>0.060± 0.009</td>
<td>0.048</td>
<td>0.13</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>ND</td>
<td>ND</td>
<td>0.024</td>
<td>7.9·10⁻²</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.280± 0.017</td>
<td>0.400± 0.037</td>
<td>0.001</td>
<td>1.32·10⁻²</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.500± 0.023</td>
<td>0.260± 0.011</td>
<td>0.002</td>
<td>6.6·10⁻³</td>
</tr>
</tbody>
</table>

Table 11 Ion concentration (mg/L) in the Influent size fraction (< 0.2 µm) in the Inlet and Outlet samples, fall of 2016.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Inlet (mg/L)</th>
<th>Outlet (mg/L)</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl⁻</td>
<td>0.400± 0.019</td>
<td>0.454± 0.050</td>
<td>0.115</td>
<td>0.38</td>
</tr>
<tr>
<td>F⁻</td>
<td>0.034± 0.004</td>
<td>0.025± 0.002</td>
<td>0.008</td>
<td>2.6·10⁻²</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.319± 0.018</td>
<td>0.496± 0.012</td>
<td>0.151</td>
<td>0.50</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0.225± 0.013</td>
<td>0.162± 0.005</td>
<td>0.087</td>
<td>0.28</td>
</tr>
<tr>
<td>Al³⁺</td>
<td>0.31± 0.017</td>
<td>0.25± 0.027</td>
<td>0.040</td>
<td>0.13</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.49± 0.010</td>
<td>0.57± 0.020</td>
<td>0.001</td>
<td>3.3·10⁻³</td>
</tr>
<tr>
<td>Fe³⁺</td>
<td>0.68± 0.026</td>
<td>0.28± 0.003</td>
<td>0.048</td>
<td>0.13</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>ND</td>
<td>ND</td>
<td>0.024</td>
<td>7.9·10⁻²</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.11±0.010</td>
<td>0.15± 0.026</td>
<td>0.001</td>
<td>1.32·10⁻²</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.51±0.019</td>
<td>0.34± 0.035</td>
<td>0.002</td>
<td>6.6·10⁻³</td>
</tr>
</tbody>
</table>

Tables 12 and 13 present the conversion from mg/L to µeq/L of the ions in the Inlet and Outlet samples in the summer and the fall in 2016, respectively.
Table 12 Conversion from mg/L to µeq/L for the major anions and cations, summer 2016.

<table>
<thead>
<tr>
<th></th>
<th>INLET</th>
<th></th>
<th>µeq/L</th>
<th></th>
<th>µeq/L</th>
<th></th>
<th>µeq/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>mg/L</td>
<td>Z</td>
<td>meq/L</td>
<td></td>
<td>Z</td>
<td>meq/L</td>
<td></td>
</tr>
<tr>
<td>F⁻</td>
<td>0.02</td>
<td>1</td>
<td>1.05·10⁻³</td>
<td>1.05</td>
<td>F⁻</td>
<td>0.021</td>
<td>1.11·10⁻³</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>0.39</td>
<td>1</td>
<td>0.011</td>
<td>11.00</td>
<td>Cl⁻</td>
<td>0.435</td>
<td>0.012</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.38</td>
<td>2</td>
<td>7.91·10⁻³</td>
<td>7.91</td>
<td>SO₄²⁻</td>
<td>0.578</td>
<td>0.012</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0.19</td>
<td>1</td>
<td>3.06·10⁻³</td>
<td>3.06</td>
<td>NO₃⁻</td>
<td>0.173</td>
<td>2.79·10⁻³</td>
</tr>
<tr>
<td>DNOM-A⁻</td>
<td>------</td>
<td></td>
<td>0.12</td>
<td>119.50</td>
<td>DNOM-A⁻</td>
<td>------</td>
<td>0.09</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.51</td>
<td>2</td>
<td>0.025</td>
<td>25.45</td>
<td>Ca²⁺</td>
<td>0.690</td>
<td>3.45·10⁻²</td>
</tr>
<tr>
<td>K⁺</td>
<td>ND</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>K⁺</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>ND</td>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>Mn²⁺</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.28</td>
<td>2</td>
<td>2.34·10⁻²</td>
<td>23.04</td>
<td>Mg²⁺</td>
<td>0.400</td>
<td>3.29·10⁻²</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.50</td>
<td>1</td>
<td>2.17·10⁻²</td>
<td>21.75</td>
<td>Na⁺</td>
<td>0.26</td>
<td>1.13·10⁻²</td>
</tr>
<tr>
<td>H⁺</td>
<td>4.79</td>
<td>1</td>
<td>1.62·10⁻²</td>
<td>16.22</td>
<td>H⁺</td>
<td>5.140</td>
<td>7.24·10⁻³</td>
</tr>
</tbody>
</table>


Table 13 Conversion from mg/L to µeq/L for the major anions and cation, fall 2016.

<table>
<thead>
<tr>
<th>Element</th>
<th>mg/L</th>
<th>Z</th>
<th>meq/L</th>
<th>µeq/L</th>
<th>Element</th>
<th>mg/L</th>
<th>meq/L</th>
<th>µeq/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>F⁻</td>
<td>0.03</td>
<td>1</td>
<td>1.58·10⁻³</td>
<td>1.58</td>
<td>F⁻</td>
<td>0.025</td>
<td>1.32·10⁻³</td>
<td>1.32</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>0.40</td>
<td>1</td>
<td>1.21·10⁻²</td>
<td>11.28</td>
<td>Cl⁻</td>
<td>0.454</td>
<td>1.28·10⁻²</td>
<td>12.82</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.32</td>
<td>2</td>
<td>6.66·10⁻³</td>
<td>6.66</td>
<td>SO₄²⁻</td>
<td>0.496</td>
<td>1.03·10⁻²</td>
<td>10.32</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0.22</td>
<td>1</td>
<td>3.55·10⁻³</td>
<td>3.55</td>
<td>NO₃⁻</td>
<td>0.162</td>
<td>2.61·10⁻³</td>
<td>2.61</td>
</tr>
<tr>
<td>DNOM-A⁻</td>
<td>-----</td>
<td></td>
<td>0.15</td>
<td>155.00</td>
<td>DOC-A⁻</td>
<td>------</td>
<td>0.12</td>
<td>120.00</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.49</td>
<td>2</td>
<td>2.45·10⁻²</td>
<td>24.45</td>
<td>Ca²⁺</td>
<td>0.57</td>
<td>2.84·10⁻²</td>
<td>28.44</td>
</tr>
<tr>
<td>K⁺</td>
<td>ND</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>K⁺</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>ND</td>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>Mn²⁺</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.11</td>
<td>2</td>
<td>9.05·10⁻³</td>
<td>9.05</td>
<td>Mg²⁺</td>
<td>0.15</td>
<td>1.23·10⁻²</td>
<td>12.34</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.51</td>
<td>1</td>
<td>2.22·10⁻²</td>
<td>22.17</td>
<td>Na⁺</td>
<td>0.34</td>
<td>1.48·10⁻²</td>
<td>14.79</td>
</tr>
<tr>
<td>H⁺</td>
<td>6.14</td>
<td>1</td>
<td>7.24·10⁻⁴</td>
<td>0.72</td>
<td>H⁺</td>
<td>5.49</td>
<td>3.24·10⁻³</td>
<td>3.24</td>
</tr>
</tbody>
</table>
Tables 14 and 15 present the ion concentrations in the different size fractions of the Inlet and Outlet samples size fractionated with 10 and 100 kDa cut-off, respectively.

Table 14 Ion concentration in the size fractions of the Inlet and Outlet samples fractionated with 10 kDa membrane cut-off. ND stands for not detected.

<table>
<thead>
<tr>
<th></th>
<th>INLET</th>
<th></th>
<th></th>
<th>INLET</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent</td>
<td>Concentrate</td>
<td>Permeate</td>
<td>Influent</td>
<td>Concentrate</td>
<td>Permeate</td>
<td>LOD</td>
</tr>
<tr>
<td>Cl⁻ (mg/L)</td>
<td>0.389</td>
<td>0.435</td>
<td>0.491</td>
<td>0.435</td>
<td>0.502</td>
<td>0.479</td>
<td>0.115</td>
</tr>
<tr>
<td>F⁻ (mg/L)</td>
<td>0.023</td>
<td>0.025</td>
<td>0.007</td>
<td>0.021</td>
<td>0.020</td>
<td>0.002</td>
<td>0.008</td>
</tr>
<tr>
<td>SO₄²⁻ (mg/L)</td>
<td>0.378</td>
<td>0.379</td>
<td>0.097</td>
<td>0.578</td>
<td>0.581</td>
<td>0.109</td>
<td>0.151</td>
</tr>
<tr>
<td>NO₃⁻ (mg/L)</td>
<td>0.188</td>
<td>0.183</td>
<td>0.120</td>
<td>0.173</td>
<td>0.229</td>
<td>ND</td>
<td>0.087</td>
</tr>
<tr>
<td>Al³⁺ (mg/L)</td>
<td>0.25</td>
<td>0.25</td>
<td>-0.01</td>
<td>0.120</td>
<td>0.13</td>
<td>-0.07</td>
<td>0.040</td>
</tr>
<tr>
<td>Ca²⁺ (mg/L)</td>
<td>0.51</td>
<td>0.53</td>
<td>0.05</td>
<td>0.69</td>
<td>0.67</td>
<td>0.27</td>
<td>0.001</td>
</tr>
<tr>
<td>Fe (mg/L)</td>
<td>0.34</td>
<td>0.34</td>
<td>0.01</td>
<td>0.06</td>
<td>0.07</td>
<td>-0.03</td>
<td>0.048</td>
</tr>
<tr>
<td>K⁺(mg/L)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mg²⁺(mg/L)</td>
<td>0.28</td>
<td>0.28</td>
<td>0.19</td>
<td>0.40</td>
<td>0.37</td>
<td>0.43</td>
<td>0.001</td>
</tr>
<tr>
<td>Mn²⁺(mg/L)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.02</td>
</tr>
<tr>
<td>Na⁺ (mg/L)</td>
<td>0.50</td>
<td>0.51</td>
<td>0.20</td>
<td>0.26</td>
<td>0.25</td>
<td>0.08</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Table 15 Ion concentration in the size fractions of the Inlet and Outlet samples size fractionated with 100 kDa membrane cut-off. ND stands for not detected.

<table>
<thead>
<tr>
<th></th>
<th>INLET</th>
<th></th>
<th></th>
<th>INLET</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Concentrate</td>
<td></td>
<td></td>
<td>Concentrate</td>
<td></td>
<td>Permeate</td>
<td></td>
</tr>
<tr>
<td>F^- (mg/L)</td>
<td>0.034</td>
<td>0.036</td>
<td>0.0120</td>
<td>0.025</td>
<td>0.024</td>
<td>0.016</td>
<td>0.115</td>
<td></td>
</tr>
<tr>
<td>Cl^- (mg/L)</td>
<td>0.400</td>
<td>0.970</td>
<td>0.954</td>
<td>0.454</td>
<td>0.613</td>
<td>0.568</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>SO_4^{2-} (mg/L)</td>
<td>0.319</td>
<td>0.369</td>
<td>0.172</td>
<td>0.496</td>
<td>0.597</td>
<td>0.296</td>
<td>0.151</td>
<td></td>
</tr>
<tr>
<td>NO_3^- (mg/L)</td>
<td>0.225</td>
<td>0.265</td>
<td>0.240</td>
<td>0.162</td>
<td>0.205</td>
<td>0.182</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td>Al^3+ (mg/L)</td>
<td>0.31</td>
<td>0.40</td>
<td>0.07</td>
<td>0.25</td>
<td>0.31</td>
<td>0.11</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>Ca^{2+} (mg/L)</td>
<td>0.57</td>
<td>0.74</td>
<td>-0.08</td>
<td>0.49</td>
<td>0.70</td>
<td>0.06</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Fe (mg/L)</td>
<td>0.68</td>
<td>0.81</td>
<td>0.11</td>
<td>0.28</td>
<td>0.33</td>
<td>0.09</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>K^+ (mg/L)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Mg^{2+} (mg/L)</td>
<td>0.15</td>
<td>0.18</td>
<td>0.05</td>
<td>0.11</td>
<td>0.13</td>
<td>0.06</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Mn^{2+} (mg/L)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Na^+ (mg/L)</td>
<td>0.51</td>
<td>0.58</td>
<td>0.35</td>
<td>0.34</td>
<td>0.39</td>
<td>0.27</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>
E) Cold vapour atomic fluorescence spectrometry (CVAFS)

E.1 Instrument settings

Tables 16 and 17 present the instrument settings regarding the CVFS used in the analysis of total and methyl mercury, respectively.

Table 16 Instrument settings CVFS for the analysis of total mercury.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purge gas</td>
<td>$\text{N}_2$</td>
</tr>
<tr>
<td>Light source</td>
<td>253.7 nm</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>High ultra-purity Ar</td>
</tr>
<tr>
<td>Pressure of the gas</td>
<td>17 psi</td>
</tr>
<tr>
<td>Integration type</td>
<td>Peak area</td>
</tr>
<tr>
<td>Run duration</td>
<td>2.5 min</td>
</tr>
<tr>
<td>Heating duration</td>
<td>2.5 min</td>
</tr>
<tr>
<td>Cooling duration</td>
<td>1.0 min</td>
</tr>
<tr>
<td>Retention start time</td>
<td>0.5 min</td>
</tr>
<tr>
<td>Retention stop time</td>
<td>1.1 min</td>
</tr>
<tr>
<td>Purge duration</td>
<td>6.0 min</td>
</tr>
<tr>
<td>Drying duration</td>
<td>3.0 min</td>
</tr>
<tr>
<td>Needle injection depth</td>
<td>155 mm</td>
</tr>
</tbody>
</table>

Table 17 Instrument settings CVFS for the analysis of methyl mercury.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purge gas</td>
<td>$\text{N}_2$</td>
</tr>
<tr>
<td>Light source</td>
<td>253.7 nm</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>High ultra-purity Ar</td>
</tr>
<tr>
<td>Pressure of the gas</td>
<td>17 psi</td>
</tr>
<tr>
<td>Integration type</td>
<td>Peak height</td>
</tr>
<tr>
<td>Run duration</td>
<td>5.0 min</td>
</tr>
<tr>
<td>Heating duration</td>
<td>9.9 seconds</td>
</tr>
<tr>
<td>Cooling duration</td>
<td>3.0 min</td>
</tr>
<tr>
<td>Retention start time</td>
<td>1.0 min</td>
</tr>
<tr>
<td>Retention stop time</td>
<td>1.5 min</td>
</tr>
<tr>
<td>Purge duration</td>
<td>5.0 min</td>
</tr>
<tr>
<td>Drying duration</td>
<td>3.0 min</td>
</tr>
<tr>
<td>Needle injection depth</td>
<td>155 mm</td>
</tr>
</tbody>
</table>
E.2 Calibration curves
Figures 29 and 30 present the calibration curves for total and methyl mercury, respectively.

Figure 29 (right) TotHg calibration curve. Figure 30 (left) MeHg calibration curve.

E.3 Distribution (%) and Total Hg concentrations (ng/L)
Tables 18 and 19 present the measured TotHg concentration, and the estimated TotHg concentration calculated based on the TotHg distribution in the different size fraction for the samples size fractionated with a membrane cut-off of 10 and 100 kDa, respectively.

Table 18 Measured TotHg concentration (ng/L), and estimated TotHg concentration (ng/L) based on the % distribution of TotHg in the different size fractions in the Inlet and Outlet samples size fractionated with 10 kDa. The MRSD is ~ 20%, and the MLOD = 0.1 ng/L.

<table>
<thead>
<tr>
<th>TotHg</th>
<th>Measured TotHg (ng/L)</th>
<th>% Distrib.</th>
<th>Estimated TotHg (ng/L)</th>
<th>Measured TotHg (ng/L)</th>
<th>% Distrib.</th>
<th>Estimated TotHg (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>1.84 ± 20%</td>
<td>100</td>
<td>1.84</td>
<td>2.91 ± 20%</td>
<td>100</td>
<td>2.91</td>
</tr>
<tr>
<td>Concentrate</td>
<td>3.27 ± 20%</td>
<td>96.75</td>
<td>1.78</td>
<td>2.94 ± 20%</td>
<td>97.46</td>
<td>2.84</td>
</tr>
<tr>
<td>Permeate</td>
<td>0.33 ± 20%</td>
<td>3.25</td>
<td>5.98 \cdot 10^{-2}</td>
<td>0.23 ± 20%</td>
<td>2.54</td>
<td>7.39 \cdot 10^{-2}</td>
</tr>
</tbody>
</table>
Table 19 Measured TotHg concentration (ng/L), and estimated TotHg concentration (ng/L) base on the % distribution of TotHg in the different size fractions in the Inlet and Outlet samples size fractionated with 100 kDa. The MLOD= 0.1 ng/L. The RSD was calculated.

<table>
<thead>
<tr>
<th>TotHg</th>
<th>INLET</th>
<th></th>
<th></th>
<th></th>
<th>OUTLET</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured TotHg (ng/L)</td>
<td>% Distrib.</td>
<td>Estimated TotHg (ng/L)</td>
<td>Measured TotHg (ng/L)</td>
<td>% Distrib.</td>
<td>Estimated TotHg (ng/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Influent</strong></td>
<td>2.80± 1.3%</td>
<td>100</td>
<td>2.80</td>
<td>2.97± 3.2%</td>
<td>100</td>
<td>2.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Concentrate</strong></td>
<td>3.23± 1.3%</td>
<td>99.29</td>
<td>2.78</td>
<td>3.66± 2.8%</td>
<td>98.10</td>
<td>2.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Permeate</strong></td>
<td>0.082± 9.1%</td>
<td>0.71</td>
<td>2.00·10^{-2}</td>
<td>0.062±1.4%</td>
<td>1.90</td>
<td>5.6·10^{-2}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E.4 Distribution (%) and MeHg concentrations (ng/L)

Tables 20 and 21 present the measured MeHg concentration, and the estimated MeHg concentration calculated based on MeHg distribution in the different size fractions for the samples size fractionated with 10 and 100 kDa, respectively.

Table 20 Measured MeHg concentration (ng/L), and estimated MeHg concentration (ng/L) base on the % distribution of MeHg in the different size fractions in the Inlet and Outlet samples size fractionated with 10 kDa. The MRSD is ~ 20%, and MLOD= 0.02 ng/L.

<table>
<thead>
<tr>
<th>MeHg</th>
<th>INLET</th>
<th></th>
<th></th>
<th></th>
<th>OUTLET</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured MeHg (ng/L)</td>
<td>% Distrib.</td>
<td>Estimated MeHg (ng/L)</td>
<td>Measured MeHg (ng/L)</td>
<td>% Distrib.</td>
<td>Estimated MeHg (ng/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Influent</strong></td>
<td>0.14±20%</td>
<td>100</td>
<td>0.140</td>
<td>0.07±20%</td>
<td>100</td>
<td>0.070</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Concentrate</strong></td>
<td>0.12±20%</td>
<td>99.17</td>
<td>0.138</td>
<td>0.09±20%</td>
<td>98.90</td>
<td>0.069</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Permeate</strong></td>
<td>3.0·10^{-3}±20%</td>
<td>0.83</td>
<td>1.162·10^{-3}</td>
<td>3.0·10^{-3}±20%</td>
<td>1.10</td>
<td>7.7·10^{-4}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 21 Distribution (%) and TotHg concentrations (mg C/L) of the different size fractions of the Inlet and Outlet samples fractionated with 100 kDa. The RSD was calculated, and MLOD= 0.02 ng/L.

<table>
<thead>
<tr>
<th>MeHg</th>
<th>INLET</th>
<th></th>
<th>OUTLET</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured MeHg (ng/L)</td>
<td>% Distrib.</td>
<td>Estimated MeHg (ng/L)</td>
<td>Measured MeHg (ng/L)</td>
</tr>
<tr>
<td>Influent</td>
<td>0.13±2.2%</td>
<td>100</td>
<td>0.130</td>
<td>0.09±3.2%</td>
</tr>
<tr>
<td>Concentrate</td>
<td>0.11±2.4%</td>
<td>97.50</td>
<td>0.126</td>
<td>0.11±3.9%</td>
</tr>
<tr>
<td>Permeate</td>
<td>0.01± 18%</td>
<td>2.50</td>
<td>3.25·10⁻³</td>
<td>0.01± 12%</td>
</tr>
</tbody>
</table>

E.5 TotHg/DOC ratio, MeHg/DOC ratio and (MeHg/TotHg)100 ratio for the different size fractionation using 10 and 100 kDa cut-off, respectively.

Tables 22 present the estimated TotHg to DOC ratio, the MeHg to DOC ratio and the MeHg to TotHg ratio for the samples size fractionated with 10 kDa cut-off. Table 23 present the measured TotHg to DOC ratio, the MeHg to DOC ratio and the MeHg to TotHg ratio for the samples size fractionated with 100 kDa cut-off.

Table 22 Estimated TotHg/DOC ratio, MeHg/DOC ratio and MeHg/TotHg ratio (%) for the samples size fractionated with 10 kDa membrane. NC stands for not calculated.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>DOC (mgC/L)</th>
<th>TotHg (ng/L)</th>
<th>MeHg (ng/L)</th>
<th>(\frac{\text{TotHg}}{\text{DOC (mg/L)}})</th>
<th>(\frac{\text{MeHg}}{\text{DOC (mg/L)}})</th>
<th>(\frac{\text{MeHg}}{\text{TotHg (mg/L)}}) \times 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>12.55</td>
<td>1.84</td>
<td>0.140</td>
<td>0.147</td>
<td>0.011</td>
<td>7.61%</td>
</tr>
<tr>
<td></td>
<td>12.37</td>
<td>1.78</td>
<td>0.138</td>
<td>0.144</td>
<td>0.011</td>
<td>7.75%</td>
</tr>
<tr>
<td></td>
<td>0.17</td>
<td>5.98·10⁻²</td>
<td>1.16·10⁻³</td>
<td>0.353</td>
<td>6.84·10⁻³</td>
<td>NC</td>
</tr>
<tr>
<td>Concentrate</td>
<td>8.78</td>
<td>2.91</td>
<td>0.070</td>
<td>0.331</td>
<td>7.97·10⁻³</td>
<td>2.41%</td>
</tr>
<tr>
<td>Permeate</td>
<td>8.65</td>
<td>2.84</td>
<td>0.069</td>
<td>0.328</td>
<td>8.0·10⁻³</td>
<td>2.43%</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>7.39·10⁻²</td>
<td>7.7·10⁻⁴</td>
<td>0.538</td>
<td>5.92·10⁻³</td>
<td>NC</td>
</tr>
</tbody>
</table>
Table 23 Measured TotHg/DOC ratio, MeHg/DOC ratio and MeHg/TotHg ratio (%) for the samples size fractionated with 10 kDa membrane.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>DOC (mgC/L)</th>
<th>TotHg (ng/L)</th>
<th>MeHg (ng/L)</th>
<th>TotHg (ng/L)</th>
<th>MeHg (ng/L)</th>
<th>MeHg/TotHg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>12.55</td>
<td>1.84</td>
<td>0.140</td>
<td>0.15</td>
<td>1.12 \cdot 10^{-2}</td>
<td>7.61%</td>
</tr>
<tr>
<td>Concentrate</td>
<td>12.99</td>
<td>3.27</td>
<td>0.12</td>
<td>0.25</td>
<td>9.24 \cdot 10^{-3}</td>
<td>3.67%</td>
</tr>
<tr>
<td>Permeate</td>
<td>0.55</td>
<td>0.33</td>
<td>3.03 \cdot 10^{-3}</td>
<td>0.60</td>
<td>5.51 \cdot 10^{-3}</td>
<td>NC</td>
</tr>
</tbody>
</table>

Table 24 presents the estimated TotHg to DOC ratio, the MeHg to DOC ratio and the MeHg to TotHg ratio for the samples size fractionated with a membrane cut-off of 100 kDa. Table 25 presents the measured TotHg to DOC ratio, the MeHg to DOC ratio and the MeHg to TotHg ratio for the samples size fractionated with a membrane cut-off of 100 kDa.

Table 24 Estimated TotHg/DOC ratio, MeHg/DOC ratio and MeHg/TotHg ratio (%) for the samples size fractionated with 100 kDa membrane.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>DOC (mgC/L)</th>
<th>TotHg (ng/L)</th>
<th>MeHg (ng/L)</th>
<th>TotHg (ng/L)</th>
<th>MeHg (ng/L)</th>
<th>MeHg/TotHg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>15.47</td>
<td>2.80</td>
<td>0.130</td>
<td>0.18</td>
<td>8.40 \cdot 10^{-3}</td>
<td>4.64%</td>
</tr>
<tr>
<td>Concentrate</td>
<td>14.66</td>
<td>2.78</td>
<td>0.126</td>
<td>0.19</td>
<td>8.59 \cdot 10^{-3}</td>
<td>4.53%</td>
</tr>
<tr>
<td>Permeate</td>
<td>0.85</td>
<td>2.00 \cdot 10^{-2}</td>
<td>3.25 \cdot 10^{-3}</td>
<td>2.40 \cdot 10^{-2}</td>
<td>3.82 \cdot 10^{-3}</td>
<td>16.25%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outlet</th>
<th>DOC (mgC/L)</th>
<th>TotHg (ng/L)</th>
<th>MeHg (ng/L)</th>
<th>TotHg (ng/L)</th>
<th>MeHg (ng/L)</th>
<th>MeHg/TotHg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>11.94</td>
<td>2.97</td>
<td>9.0 \cdot 10^{-2}</td>
<td>0.25</td>
<td>7.54 \cdot 10^{-3}</td>
<td>3.03%</td>
</tr>
<tr>
<td>Concentrate</td>
<td>10.23</td>
<td>2.92</td>
<td>8.6 \cdot 10^{-2}</td>
<td>0.28</td>
<td>8.41 \cdot 10^{-3}</td>
<td>2.94%</td>
</tr>
<tr>
<td>Permeate</td>
<td>1.70</td>
<td>5.60 \cdot 10^{-2}</td>
<td>3.42 \cdot 10^{-3}</td>
<td>3.29 \cdot 10^{-2}</td>
<td>2.12 \cdot 10^{-3}</td>
<td>6.11%</td>
</tr>
</tbody>
</table>
Table 25 Measured TotHg/DOC ratio, MeHg/DOC ratio and MeHg/TotHg ratio (%) for the samples size fractionated with 100 kDa membrane.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>DOC (mgC/L)</th>
<th>TotHg (ng/L)</th>
<th>MeHg (ng/L)</th>
<th>TotHg (ng/L)</th>
<th>MeHg (ng/L)</th>
<th>MeHg/TotHg (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>15.47</td>
<td>2.80</td>
<td>0.13</td>
<td>0.18</td>
<td>8.40·10^{-3}</td>
<td>4.64%</td>
</tr>
<tr>
<td>Concentrate</td>
<td>18.76</td>
<td>3.23</td>
<td>0.11</td>
<td>0.17</td>
<td>5.86·10^{-3}</td>
<td>3.41%</td>
</tr>
<tr>
<td>Permeate</td>
<td>3.65</td>
<td>0.082</td>
<td>0.01</td>
<td>0.02</td>
<td>2.74·10^{-3}</td>
<td>12.20%</td>
</tr>
<tr>
<td>Outlet</td>
<td>DOC (mgC/L)</td>
<td>TotHg (ng/L)</td>
<td>MeHg (ng/L)</td>
<td>TotHg (ng/L)</td>
<td>MeHg (ng/L)</td>
<td>MeHg/TotHg (100)</td>
</tr>
<tr>
<td>Influent</td>
<td>11.94</td>
<td>2.97</td>
<td>0.09</td>
<td>0.25</td>
<td>7.54·10^{-3}</td>
<td>3.03%</td>
</tr>
<tr>
<td>Concentrate</td>
<td>14.33</td>
<td>3.66</td>
<td>0.11</td>
<td>0.26</td>
<td>7.68·10^{-3}</td>
<td>3.01%</td>
</tr>
<tr>
<td>Permeate</td>
<td>5.48</td>
<td>0.062</td>
<td>0.01</td>
<td>1.13·10^{-2}</td>
<td>1.82·10^{-3}</td>
<td>16.13%</td>
</tr>
</tbody>
</table>

F) UV-VIS Photometer

F.1 Instrument settings
Table 26 presents the photometer’s instrument settings

Table 26 UV-VIS Photometer settings.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrum</td>
<td>800-200 nm</td>
</tr>
<tr>
<td>Measurement mode</td>
<td>Absorbance</td>
</tr>
<tr>
<td>Rect- range</td>
<td>0-4 A</td>
</tr>
<tr>
<td>Scan speed</td>
<td>Slow</td>
</tr>
<tr>
<td>Scan pitch</td>
<td>1.0 nm</td>
</tr>
<tr>
<td>Number of scans</td>
<td>1</td>
</tr>
<tr>
<td>Display mode</td>
<td>Sequential</td>
</tr>
</tbody>
</table>

F.2 Absorbance proxies within the UV-VIS region.
Tables 27 and 28 present the Absorbance proxies within the UV-VIS region measured on the samples size fractionated with 10 and 100 kDa, respectively.

Table 27 Absorbance proxies within the UV (λ=254nm) and VIS (λ=400 nm) region measured on the samples size fractionated using a 10 kDa cut off.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>INLET</th>
<th>OUTLET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent</td>
<td>Concentrate</td>
</tr>
<tr>
<td>λ 254nm</td>
<td>0.675</td>
<td>0.649</td>
</tr>
<tr>
<td>λ 400nm</td>
<td>0.088</td>
<td>0.086</td>
</tr>
<tr>
<td>sUVa</td>
<td>5.378</td>
<td>5.065</td>
</tr>
<tr>
<td>sVISa</td>
<td>7.012</td>
<td>6.620</td>
</tr>
<tr>
<td>SAR</td>
<td>7.670</td>
<td>7.651</td>
</tr>
</tbody>
</table>
Table 28 Absorbance proxies within the UV (λ=254nm) and VIS (λ=400 nm) region measured on the samples size fractionated using a 100 kDa cut off.

<table>
<thead>
<tr>
<th></th>
<th>INLET</th>
<th></th>
<th>OUTLET</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent</td>
<td>Concentrate</td>
<td>Permeate</td>
<td>Influent</td>
</tr>
<tr>
<td>λ 254nm</td>
<td>0.749</td>
<td>0.823</td>
<td>0.121</td>
<td>0.541</td>
</tr>
<tr>
<td>λ 400nm</td>
<td>0.097</td>
<td>0.115</td>
<td>0.011</td>
<td>0.070</td>
</tr>
<tr>
<td>sUVa</td>
<td>4.842</td>
<td>4.387</td>
<td>3.315</td>
<td>4.531</td>
</tr>
<tr>
<td>sVISa</td>
<td>6.270</td>
<td>6.130</td>
<td>3.014</td>
<td>5.863</td>
</tr>
<tr>
<td>SAR</td>
<td>7.722</td>
<td>7.156</td>
<td>11.00</td>
<td>7.729</td>
</tr>
</tbody>
</table>

F.3 UV-VIS spectra
Figures 31 to 33 show the UV-VIS spectra for the Inlet sample with all its fractions (Influent, Concentrate and Permeate) collected in the summer of 2016.

Figure 31 UV-VIS spectra Influent Inlet sample.  
Figure 32 UV-VIS spectra Concentrate Inlet sample.  
Figure 33 UV-VIS spectra Permeate Inlet sample.
Figures 34 to 36 show the UV-VIS spectra for the Outlet sample with all its fractions (Influent, Concentrate and Permeate) collected in the summer of 2016.

**Figure 34 UV-VIS spectra Influent Outlet sample.**

**Figure 35 UV-VIS spectra Concentrate Outlet sample.**

**Figure 36 UV-VIS spectra Permeate Outlet sample.**
Figures 37 to 39 show the UV-VIS spectra for the Inlet sample with all its fractions (Influent, Concentrate and Permeate) collected in the fall of 2016.

Figure 37 UV-VIS spectra Influent Inlet sample.

Figure 38 UV-VIS spectra Concentrate Inlet sample.

Figure 39 UV-VIS spectra Permeate Inlet sample.
Figures 40 to 43 show the UV-VIS spectra for the Outlet sample with all its fractions (Influent, Concentrate and Permeate) collected in the fall, and Figure 44 shows the spectrum for RO Langtjern isolate.
G) Fluorescence

G.1 Location ($\lambda_{ex}$ and $\lambda_{em}$) of the two peaks A and C found in the EEM contour plots

Table 29 and 30 show the location of the two fluorescence peaks (A and C) for the samples collected in the summer and the fall, and fractionated with 10 and 100 kDa membrane cut off, respectively.

Table 29 Locations ($\lambda_{ex}$/$\lambda_{em}$) of the two fluorescence peaks (A and C). Peak C represents the more aromatic and hydrophobic humic fraction. Peak A represents the more aliphatic fulvic fraction. The probable source of origin is also presented. NF stands for not found.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>Peak C</th>
<th>Peak A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{ex}$ (nm)</td>
<td>$\lambda_{em}$ (nm)</td>
</tr>
<tr>
<td>Permeate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate</td>
<td>NF 320-360</td>
<td>400-450</td>
</tr>
<tr>
<td>Influent</td>
<td>320-360</td>
<td>400-450</td>
</tr>
<tr>
<td></td>
<td>320-360</td>
<td>400-450</td>
</tr>
</tbody>
</table>

Table 30 Locations ($\lambda_{ex}$/$\lambda_{em}$) of the two fluorescence peaks (A and C). Peak C represents the more aromatic and hydrophobic humic fraction. Peak A represents the more aliphatic fulvic fraction. The probable source of origin is also presented.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>Peak C</th>
<th>Peak A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{ex}$ (nm)</td>
<td>$\lambda_{em}$ (nm)</td>
</tr>
<tr>
<td>Permeate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate</td>
<td>305-360</td>
<td>410-480</td>
</tr>
<tr>
<td>Influent</td>
<td>305-380</td>
<td>410-490</td>
</tr>
<tr>
<td></td>
<td>315-370</td>
<td>430-480</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outlet</th>
<th>Peak C</th>
<th>Peak A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{ex}$ (nm)</td>
<td>$\lambda_{em}$ (nm)</td>
</tr>
<tr>
<td>Permeate</td>
<td>300-350</td>
<td>420-480</td>
</tr>
<tr>
<td>Concentrate</td>
<td>325-350</td>
<td>440-460</td>
</tr>
<tr>
<td>Influent</td>
<td>325-350</td>
<td>440-460</td>
</tr>
</tbody>
</table>
H) Biodegradation experiment

H.1 Instrument settings
Table 31 shows the instrument settings for the Thermax cabinet used during the biodegradation experiment.

<table>
<thead>
<tr>
<th>Element</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>20°C and 18°C</td>
</tr>
<tr>
<td>Sensor Dish</td>
<td>Oxygen (cO₂ (µmol))</td>
</tr>
<tr>
<td>Batch number</td>
<td>PST5-1537-01</td>
</tr>
<tr>
<td>Phase Angle calibration 0</td>
<td>54.32</td>
</tr>
<tr>
<td>Phase Angle calibration 100</td>
<td>47.59</td>
</tr>
<tr>
<td>Temperature for calibration 0</td>
<td>23°C</td>
</tr>
<tr>
<td>Air pressure</td>
<td>959</td>
</tr>
</tbody>
</table>

H.2 Respiration rate calculation

Three replicates per sample were studied to test the biodegradability of the material. Figures 45 explains how the respiration rate was calculated for each replicate.

First of all, samples were corrected for the blank to remove the temperature fluctuations caused for temperature instability in the Thermax cabinet (Figure 44).

![Figure 44 Average of the blank and temperature fluctuations.](image)

Replicates were studied individually, and the respiration rate was calculated according to the following formula.

\[
\text{Respiration rate (µmol O}_2 \text{L}^{-1} \text{h}^{-1}) = \left( \frac{O_{2\ initial} - O_{2\ final}}{\Delta T} \right)
\]
The following tables (Table 32 to 35) show the data for the biodegradation experiment.

Table 32 Respiration rate /DOC calculation for the Inlet sample size fractionated with a membrane cut-off of 10 kDa, summer 2016. NC stands for not considered.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>Glucose</th>
<th>Influent</th>
<th>Concentrate</th>
<th>Permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiration rate /DOC R1</strong> (mol O$_2$/g C·h)</td>
<td>0.043</td>
<td>0.072</td>
<td>1.158 (NC)</td>
<td>1.966 (NC)</td>
</tr>
<tr>
<td><strong>Respiration rate /DOC R2</strong> (mol O$_2$/g C·h)</td>
<td>0.077</td>
<td>0.062</td>
<td>0.046</td>
<td>0.952</td>
</tr>
<tr>
<td><strong>Respiration rate /DOC R3</strong> (mol O$_2$/g C·h)</td>
<td>0.069</td>
<td>0.034</td>
<td>0.040</td>
<td>0.516</td>
</tr>
<tr>
<td>X (mol O$_2$/g C·h)</td>
<td>0.063</td>
<td>0.056</td>
<td>0.043</td>
<td>0.734</td>
</tr>
<tr>
<td>SD/ Man-Min</td>
<td>0.017</td>
<td>0.020</td>
<td>0.004</td>
<td>0.218</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>27.72%</td>
<td>35.33%</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Result ± SD or Max-Min</strong></td>
<td>0.063±0.017</td>
<td>0.056±0.020</td>
<td>0.043±0.004</td>
<td>0.734±0.218</td>
</tr>
</tbody>
</table>
Table 33 Respiration rate /DOC calculation for the Outlet sample size fractionated with a membrane cut-off of 10 kDa, summer 2016.

<table>
<thead>
<tr>
<th>Outlet</th>
<th>Glucose</th>
<th>Influent</th>
<th>Concentrate</th>
<th>Permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration rate/DOC R1 (mol O₂/g C·h)</td>
<td>0.043</td>
<td>discarded</td>
<td>0.046</td>
<td>discarded</td>
</tr>
<tr>
<td>Respiration rate/DOC R2 (mol O₂/g C·h)</td>
<td>0.077</td>
<td>0.038</td>
<td>0.032</td>
<td>0.707</td>
</tr>
<tr>
<td>Respiration rate/DOC R3 (mol O₂/g C·h)</td>
<td>0.069</td>
<td>0.035</td>
<td>1.642 (NC)</td>
<td>0.482</td>
</tr>
<tr>
<td>X (mol O₂/g C·h)</td>
<td>0.063</td>
<td>0.037</td>
<td>0.039</td>
<td>0.595</td>
</tr>
<tr>
<td>SD/Max-Min</td>
<td>0.017</td>
<td>0.007</td>
<td>0.008</td>
<td>0.111</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>27.72%</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Result± SD or Max-Min</td>
<td>0.063±0.017</td>
<td>0.037±0.007</td>
<td>0.039±0.008</td>
<td>0.595±0.111</td>
</tr>
</tbody>
</table>
Table 34: Respiration rate /DOC calculation for the Inlet sample size fractionated with a membrane cut-off of 100 kDa, fall 2016.

<table>
<thead>
<tr>
<th>Inlet</th>
<th>Glucose</th>
<th>Influent</th>
<th>Concentrate</th>
<th>Permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiration rate/DOC R1</strong> (mol O₂/g C·h)</td>
<td>0.057</td>
<td>0.074</td>
<td>0.030</td>
<td>0.178</td>
</tr>
<tr>
<td><strong>Respiration rate/DOC R2</strong> (mol O₂/g C·h)</td>
<td>0.057</td>
<td>0.075</td>
<td>0.034</td>
<td>0.239</td>
</tr>
<tr>
<td><strong>Respiration rate/DOC R3</strong> (mol O₂/g C·h)</td>
<td>0.036</td>
<td>0.083</td>
<td>0.028</td>
<td>0.247</td>
</tr>
<tr>
<td>X (mol O₂/g C·h)</td>
<td>0.050</td>
<td>0.077</td>
<td>0.031</td>
<td>0.221</td>
</tr>
<tr>
<td>SD/ Man-Min</td>
<td>0.012</td>
<td>0.005</td>
<td>0.003</td>
<td>0.038</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>34.37%</td>
<td>6.11%</td>
<td>9.90%</td>
<td>17.05%</td>
</tr>
<tr>
<td><strong>Result ± SD or Max-Min</strong></td>
<td>0.050±0.012</td>
<td>0.056±0.020</td>
<td>0.031±0.003</td>
<td>0.221±0.038</td>
</tr>
</tbody>
</table>

Table 35: Respiration rate /DOC calculation for the Outlet sample size fractionated with a membrane cut-off of 100 kDa, fall 2016.

<table>
<thead>
<tr>
<th>Outlet</th>
<th>Glucose</th>
<th>Influent</th>
<th>Concentrate</th>
<th>Permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiration rate/DOC R1</strong> (mol O₂/g C·h)</td>
<td>0.057</td>
<td>0.034</td>
<td>0.049</td>
<td>discarded</td>
</tr>
<tr>
<td><strong>Respiration rate/DOC R2</strong> (mol O₂/g C·h)</td>
<td>0.057</td>
<td>0.052</td>
<td>0.053</td>
<td>0.867</td>
</tr>
<tr>
<td><strong>Respiration rate/DOC R3</strong> (mol O₂/g C·h)</td>
<td>0.036</td>
<td>0.054</td>
<td>0.471 (NC)</td>
<td>0.839</td>
</tr>
<tr>
<td>X (mol O₂/g C·h)</td>
<td>0.050</td>
<td>0.047</td>
<td>0.051</td>
<td>0.853</td>
</tr>
<tr>
<td>SD/ Man-Min</td>
<td>0.012</td>
<td>0.011</td>
<td>0.002</td>
<td>0.014</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>34.37%</td>
<td>24.55%</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td><strong>Result ± SD or Max-Min</strong></td>
<td>0.050±0.012</td>
<td>0.047±0.011</td>
<td>0.051±0.002</td>
<td>0.853±0.014</td>
</tr>
</tbody>
</table>

H.3 Biodegradation graphs

Each graph represents one of a total of three replicates pr. sample to illustrate the variations in response between the different size fractions in the Inlet and Outlet samples.
Biodegradation graphs for the Inlet samples size fractionated with 10 kDa membrane cut-off.

Figures 46 to 48 show the Influent, Concentrate and Permeate size fractions for the Inlet sample size fractionated with 10 kDa membrane cut-off.

Figure 46 Inlet Influent.  
Figure 47 Inlet Concentrate.  
Figure 48 Inlet Permeate.
Biodegradation graphs for the Outlet samples size fractionated with 10 kDa membrane cut-off.

Figures 49 to 51 show the Influent, Concentrate and Permeate size fractions for the Outlet sample size fractionated with 10 kDa membrane cut-off, respectively.

**Figure 49 Outlet Influent.**

**Figure 50 Outlet Concentrate.**

**Figure 51 Outlet Permeate.**
**Biodegradation graphs for the Inlet samples size fractionated with 100 kDa membrane cut-off.**

Figures 52 to 54 show the Influent, Concentrate and Permeate size fractions for the Inlet sample size fractionated with 100 kDa membrane cut-off, receptively.

**Figure 52 Inlet Influent.**

**Figure 53 Inlet Concentrate.**

**Figure 54 Inlet Permeate.**
Biodegradation graphs for the Outlet samples size fractionated with 100 kDa membrane cut-off.

Figures 55 to 57 show the Influent, Concentrate and Permeate size fractions for the Outlet sample size fractionated with 100 kDa membrane cut-off, receptively.

Figure 55 Outlet Influent.

Figure 56 Outlet Concentrate.

Figure 57 Outlet Permeate.
Figures 58 present the biodegradation graph for the RO Langtjern isolate < 0.2 µm.

![Figure 58 RO Langtjern isolate](image-url)