Effect of Photo-Oxidation on Size, Structure and Biodegradability of Dissolved Natural Organic Matter

Janaki Rajakumar

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Janaki Rajakumar

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Abstract

The concentration of dissolved natural organic matter (DNOM) in freshwater systems in the Nordic countries, Scotland and North-Eastern America have increased over the last decades due to the combined effects of climate change, decreased acid rain loading, and increased biomass. DNOM absorbs light, resulting in the yellow to brown colour of water. The DNOM in surface waters partly photo-degrades when exposed to sunlight. This breaks down the larger DNOM compounds into molecules with lower molecular weights, which become food for aquatic organisms.

The character of DNOM in surface waters is influenced by the catchment characteristics, and therefore vary between different locations. DNOM from a large lake (Päijänne in Finland), studied in the NOMiNOR project, along with reverse osmosis isolated DNOM samples from peat water (Hellerudmyra) and a mineral soil dominated watershed (Trehøningen) from the NOM-typing project (Gjessing et al., 1999) were therefore investigated. These samples are referred to as Lake, Peat and Soil, respectively.

The main aim of this study is to assess the changes in the structural characteristics of DNOM and its bioavailability between the size fractions and before and after photo-oxidation.

All samples were filtered at 0.2 um. Half of the sample volumes were photo-oxidised for 53 hours using artificial sunlight. Half of the photo-oxidised and non-photo-oxidised samples were then size fractionated at 10 kDa. This resulted in four different samples from each location. A series of measurements and experiments were then done on the samples, including analysis of DOC, fluorescence, UV-Vis, and biodegradation.

The low molecular weight (LMW) DNOM fraction (i.e. < 10 kDa) in Peat water constituted the least amount of total DNOM, due to a larger fraction of more aromatic and longer conjugated double bonds. Photo-oxidation caused a reduction in DNOM concentration. The reduction is stronger in high molecular weight (HMW) than in the LMW fractions. The spectroscopic proxies of DNOM confirmed that photo-oxidation alters the DNOM structurally by mineralisation and transformation, causing the DNOM to be less coloured and thus less aromatic and with lower Mw.
The more aromatic, hydrophobic, and HMW DNOM was refractory compared to the other samples and its biodegradability was higher after photo-oxidation. This implies that refractory DNOM are partly decomposed to more bioavailable compounds. The biodegradability of the DNOM from the Lake sample was relatively low, even though it has the lowest molecular weight. This is likely due to the long residence time in the large lake, allowing for an advanced bio-degradation of the non-refractory moieties of the material.

The biodegradability experiment showed that the relatively more aliphatic and LMW (i.e. 10 kDa) DNOM fraction samples were the most bioavailable. These results confirmed that the microorganisms prefer to consume smaller M₇ DNOM with relatively more aliphatic structures. This supports previous studies by Marschner et al. (2003) and is in accordance with the hypothesis that LMW DNOM are more bioavailable than HMW DNOM.

Surface waters are commonly used as raw water sources by waterworks and they act as photo-reactive tanks. Moreover, in most waterworks, after the removal of most of the DNOM, the raw water is irradiated with a high dose of UV light to kill pathogenic microorganisms. Photo-oxidation alters the remaining DNOM into less aromatic and lower M₇ moieties. These LMW moieties are more bioavailable for microorganisms, allowing them to grow. This can cause fouling in water distribution networks.
Preface

The research studies presented in this thesis were conducted as an integral part of NOMiNOR [Natural Organic Matter (NOM) in Nordic drinking waters] project. This master thesis has been carried out at the Department of Chemistry, University of Oslo (UiO). Part of the study was carried out at the Department of Biology, UiO and at the Norwegian University of Life Sciences (NMBU).

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1 Introduction

All surface waters contain a certain amount of dissolved natural organic matter (DNOM) (Gjessing et al., 1999). Moreover, the DNOM in surface waters have increased significantly in Scotland, southern Norway and southern Sweden over the last decades. This increase is likely due to the combined effects of reduced acid rain and climate change (Eikebrokk et al., 2017).

DNOM originate from a great number of different sources and is a heterogeneous mixture of organic molecules. In headwater catchments, lake water DNOM is typically derived primarily from allochthonous sources such as organic soil horizons (Schiff et al., 1997). DNOM is mainly formed through biochemical decomposition reactions of the soil organic matter. Most of the organic matter is converted back to carbon dioxide through heterotrophic activity as well as photochemical reactions in the surface waters. A very small percentage (< 1%) of the organic carbon (refractory) accumulates in soils, sediments and natural waters for long time (Perdue, 2009). Each water sample is thus a mixture of organic molecules of different form, size and functional groups composition (Tipping, 2002).

DNOM contains a variety of weak acid functional sites such as carboxylic, alcoholic and phenolic and sulfhydryl groups. The presence of DNOM therefore lower the pH in poorly buffered waters, typically found in the Boreal domain. The functional groups also cause the DNOM to be a ubiquitous complexing agent of metals such as iron (Fe), aluminium (Al), and other Type II (soft or heavy) metals, while its lipophilic moieties absorb organic contaminants (Tipping, 2002, Al-Reasi et al., 2011). DNOM increases thus the mobility of these metal ions and persistent organic pollutants (POPs), and thereby increase the loading of these pollutants from soil to surface waters. Moreover, allochthonous DNOM is also an important source of nutrients and energy for heterotrophic aquatic organisms. Noteworthy, the DNOM play an important role in the transport of mercury (Hg) and methylmercury (MeHg) from large pools in the forest floor into the waters in regions of Fennoscandia exposed to long-range transported air pollutants. High Hg levels in fish are therefore usually associated with lakes with high concentrations of DNOM (Braaten et al., 2017). This is of major concern since MeHg is a compound that is highly bioactive and strongly neurotoxic. These factors affect freshwater sport fishing and thereby its tourism.
DNOM contains chromophores through which DNOM absorbs photons from UV/VIS light. The absorbed photons cause the chromophores to be excited to a higher electronic energy state. This absorbed energy can be utilized in photo-oxidation of DNOM, or released to the environment as heat or longer wavelength radiation (fluorescence). Photo-oxidation can thus mineralize the DNOM completely into inorganic carbon dioxide, or moreover just partly cleave the DNOM into a variety of photoproducts with reduced average molecular weight ($M_W$), which are more bioavailable.

DNOM affects water's physical properties due to its chromatographic moieties that absorb sunlight radiation. Waters containing high concentration of DNOM are thus usually yellow to brown in colour. This characteristic is also associated to a higher concentration of Fe. The chromophores of both DNOM and Fe are thus important sources of the colour of fresh water (Kritzberg et al., 2012). An increase in the concentrations of Fe in our surface water has therefore contributed to the increase in the apparent colour of DNOM (Xiao et al., 2015).

Surface waters are commonly used as raw water sources by waterworks for tap water production in many countries. Increasing concentrations of DNOM in water may affect colour, taste and odour in drinking water if not treated properly. Moreover, DNOM that is not removed can react with the disinfection chemicals, such as hypochlorite, to form a number of disinfection by-products (DBPs), which can cause health problems (Sharma et al., 2014). Depending on the removal procedure in the waterworks the increased DNOM levels causes either increased fouling of ultra-filtration membranes or demand for DNOM coagulants and disinfectants (Eikebrokk et al., 2017). An alternative disinfection method commonly used in waterworks is UV irradiation, in which the remains of DNOM are photodegraded to smaller and conceivably more bioavailable compounds. In the house plumbing, where the water can be standing still for some time, there are also problems with biofilm formation of amoebae-protected Legionella bacteria. The increasing concentration of DNOM constitutes thus a severe challenge for process control systems and for operation performance of water treatment and distribution systems (Eikebrokk et al., 2017).

The research study presented in this thesis is partly conducted as an integral part of the NOMiNOR [Natural Organic Matter (NOM) in Nordic drinking waters] project. NOMiNOR was an end-user oriented project that ran from 2013 to 2017 and was governed by Norwegian Water (Norsk Vann) and Swedish Water Works Association (Svenskt Vatten). The strategy of the NOMiNOR-project was to use the same set of DNOM characterisations method to different
water treatment works in Scotland, Finland, Sweden and Norway. From this approach, the results will be more directly comparable, thus facilitating the exchange of knowledge between the water works. The characterisation of the DNOM have been related to reduction in acid rain, climate change, and weather conditions to assess the role of different factors governing the DNOM in space and time (Vogt, 2016, Haaland et al., 2017, Háland, 2017, Vogt et al., 2018). This knowledge is prerequisite to predict future trends in DNOM amount and quality (Haaland et al., 2018). The project has thereby provided valuable information on optimum future design and operations to water treatment works.

1.1 Aim of the Study

The aim of this study has been to assess what effect photo-oxidation has on the size distribution and bioavailability of DNOM with different characters. The photochemical and biological reactivity of DNOM depend on its source and hence its chemical composition. Thus understanding the chemical composition of DNOM is prerequisite for understanding the changes of DNOM absorption properties and bioavailability. Photo-oxidation alters the characteristics of the DNOM, having effect on its aromaticity, size, colour and bioavailability. The changes in DNOM quality and explanatory factors for the effects of photo-oxidation on size distribution are assessed by measuring changes in relative absorbencies, fluorescence indexes, bioavailability, and Fe and Al fractions.

Moreover, the results from the bioavailability of DNOM from this study is compared to the DNOM from Háland (2017).

The character of DNOM in surface waters is influence by the catchment characteristics and differ therefore between different locations. A water sample from a large lake (Päijänne in Finland), studied in the NOMiNOR project, was therefore investigated along with reverse osmosis isolated DNOM samples from a peat water (Hellerudmyra) and a mineral soil dominated watershed (Trehøningen) from the NOM-typing project (Gjessing et al., 1999). These samples are referred to as Lake, Peat and Soil, respectively. The water in the large lake has had long residence time allowing the lake to act as a natural reactor tank. This DNOM from is therefore not expected to respond strongly to the additional photo-oxidation. Moreover, the labile part of the DNOM is expected to have been consumed by microorganisms, leaving only the recalcitrant part that is relative resistant to microbial decomposition. Peat water typically
has DNOM that is more aromatic, has higher $M_W$ and is darker in colour. On the other side, the mineral soil water represents the water draining through the forest with mineral soils. This typically allows only the DNOM that has LMW and is less aromatic to pass through.

The following hypotheses have been tested:

- Large differences in degree of photo-bleaching of DNOM is mainly governed by its differences in aromaticity given by sUVa and size given by SAR, as well as Fe content.

- Photo-oxidation of refractory DNOM partly decomposes them to more bioavailable compounds.

- Increased total Fe concentrations increases the visual colour of DNOM.

- LMW DNOM are more bioavailable than HMW DNOM.
2 Theory

2.1 Natural Organic Matter (NOM)

The term natural organic matter (NOM) refers to the organic substances in soil, waters and sediments that are formed by humification, which is biological decay and inorganic oxidation of dead plant or animal tissues and waste products from organisms. Some of the organic carbon is mineralized completely and recirculated to the atmosphere as CO$_2$, some are assimilated into microbial tissues, and some are converted in to stable organic compounds referred to as humus, the dark colour organic matter found in soil. Humus includes a broad spectrum of organic constituents. Humus components are commonly distinguished as humic substances and non-humic substances. The non-humic fraction consists of more simple and thus identifiable LMW compounds like carbohydrates, proteins, peptides, amino acids, fats, resins and other low-molecular weight organic substances (Choudry, 1984, Stevenson, 1994).

Humic substances are found in practically all soils, natural waters and sediments, and constitute by far the main part of NOM (Choudry, 1984). These are relatively more degradation-resistant polyelectrolytic high molecular weight (HMW) macromolecules of undefinable structure with a characteristic dark colour (Manahan, 2001).

Soil organic matter (SOM) refers to all the organic matter in soils, including litter, humic fraction, microbial biomass, water soluble organics and stabilized organic matter (humus) (Stevenson, 1994). The microbial products are the largest contributor to stable SOM (Knicker, 2011). NOM is thus a heterogeneous mixture of a continuum of organic biomolecules with a wide range of different chemical compositions, sizes, and functional properties. DNOM refers to the dissolved fraction of NOM, defined as the fraction that passes through a 0.45 µm filter. It is commonly subdivided into fractions according to its physical state (dissolved molecules or colloids), its point of origin (autochthonous or allochthonous), its polarity (hydrophobic or hydrophilic), and its acidity (acidic, neutral or basic) (Perdue, 2009).

Humic substances are composed of a skeleton of alkyl/aromatic units on which the following major functional groups are attached: carboxylic acid, phenolic and alcoholic hydroxyls, ketone and quinone. The humic substances are commonly fractionated into a humic, fulvic and humin fraction. Structurally, these three humic fractions are similar to each other, but that they differ
in molecular weight, and functional group content (Choudry, 1984). Humic substances have molecular weights ranging from a few hundreds to hundreds of thousands of Daltons. Of these, fulvic acids are smaller in size, with molecular weights in the 600 to 5 kDa range, and have a higher functional group content (including carboxylic, phenolic, and ketonic moieties). Humic acids are larger, with molecular weights ranging from 1.5–500 kDa in streams and soils, and have a lower functional group density (Malcolm, 1990, McDonald et al., 2004).

Two general humification processes are biopolymer degradation and abiotic condensation. The degradation mechanism assume selective preservation of recalcitrant plant and microbial macromolecules, as humus, and mineralisation of labile components. In the condensation process some of the LMW substances, derived from the biological degradation of plant and microbial residues, abiotically condense into polymers, forming humus (Cotrufo et al., 2013).

Stevenson (1982) proposed a generic model of HA which is most commonly cited in modern literature (Figure 2.1). This model has an open structure associated with carboxyl and phenolic functional groups. The most commonly accepted generic model for FA was proposed by Buffle (1977) (Figure 2.1).

![Model structure of humic acid (Stevenson 1982)](image1)

![Model structure of fulvic acid](image2)

Figure 2.1. Generic molecular structure of humic acid (HA, left) (Stevenson, 1982) and fulvic acid (FA, right) (Buffle, 1977).

### 2.1.1 Dissolved Natural Organic Matter (DNOM)

NOM in freshwaters exists as dissolved molecules, colloids and particles. The NOM is formed in the water (autochthonous) or it is formed in the soil and transferred to water body via leaching and runoff from land (allochthonous). The term DNOM is operationally defined as the fraction of NOM in the water sample that passes through a 0.45 µm membrane filter (Perdue, 2009). DNOM thus generally refers to the dissolved and colloidal forms of humic substance in solution. In most fresh waters the NOM is similar to the DNOM as there is very little particulate NOM. DNOM behaves as a weakly acidic polyelectrolyte and exhibits buffering capacity in
the entire pH range commonly encountered in nature. It is also known to form stable complexes with polyvalent and Type II cations through chelation (Stevenson, 1994, Palmer et al., 2001). DNOM absorbs light over a wide range of wavelengths due to the chromophores consisting of condensed aromatic structures and conjugated double bonds on aliphatic structural units. DNOM gives the brownish-yellow colour to the natural waters. Since carbon constitutes approximately 50% of the mass of DNOM the TOC concentrations is commonly used as a proxy for DNOM concentration. The TOC concentration is found to vary between less than 0.5 mg C·L⁻¹ in groundwater to more than 40 mg C·L⁻¹ in wetlands (Thurman, 1985). Their blend of hydrophilic and hydrophobic moieties allows the humic materials to function as surfactants, with the ability to bind both hydrophobic and hydrophilic compounds. This is why humic and fulvic acids are effective transporting agents for both organic and inorganic contaminants in the environment (Palmer et al., 2001).

The terms humic acids (HA) and fulvic acids (FA) are commonly used to describe fractions of DNOM in inland waters. Both are classified as hydrophobic acids, though they differ in the solubility at low pH. HA and FA are absorbed on XAD-8 resin at pH 2 and eluted from the resin at high pH. HA can be precipitated at pH 1 while FA is not precipitated (Perdue, 2009).

2.2 Size Fractionation

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 describe the physio-chemical properties and composition of HS.

Molecular weight (Mₜ) is a key control on the physical, chemical, and biological characteristics of dissolved organic matter (DOM). The photo-reactivity and bioavailability of DOM vary widely among different Mₜ fractions (Gao et al., 1998, Edith et al., 2004). HMW, humic-like substances contain more aromatic and large Mₜ DOM than the LMW fulvic-like substances. LMW moieties are relatively more biodegradable than the more HMW fraction. HMW fractions are more photo-reactive than LMW fractions due the higher amounts of conjugated double bonds. In this study, ultrafiltration at a 10 kDa size fraction was used to separate the more fulvic acid like organic matter from the humic acid like organic matter.
2.3 Photo-oxidation of DNOM

When the water with DNOM is in a lake the upper part of the water column is exposed to sunlight causing the organic material to continuously undergo various photochemical and biological transformations. Photo-oxidation causes degradation of DNOM that contain chromophoric moieties. For DNOM to have chromophoric properties it must contain conjugated unsaturated double bonds. CDOM is the fraction of DNOM capable of adsorbing visible light radiation energy. As it is the chromophoric moieties that absorb the light energy, they are also the part of the DNOM that are decomposed by photo-oxidation. Because the coloured chromophores of the DNOM are reduced by the sun light, the photo-oxidation is also referred to as photo-bleaching, as the absorptivity of the DNOM in the visible part of the spectral region is decreased.

Solar radiation, particularly in the ultraviolet (UV-A to UV-B) range, therefore promotes transformation of both the structure, molecular weight, and optical properties of humic substances. When DNOM is exposed to sunlight the energy of the adsorbed radiation causes cleavage of conjugated double bonds so that the average molecular weight ($M_W$) is reduced and a variety of photo-oxidation products are formed (Zepp et al., 1995). Oxygen is consumed in the photochemical reaction and hydrogen peroxide radical are produced.

Some of the DNOM is mineralized completely into inorganic compounds such as CO, CO$_2$, CO$_3^{2-}$ (i.e. DIC), NO$_3^-$, SO$_4^{2-}$ and PO$_4^{3-}$ as well as Al$^{3+}$ and Fe$^{3+}$, with their hydrated and protonates species. Most of photoproducts are nevertheless partly decomposed DNOM which are smaller than the parent molecules. They are more UV-transparent compounds and are decreased in both the absorptivity and fluorescence efficiency (Kieber et al., 1990). Photochemical processes thus transform refractory DNOM into more labile substrates that are readily utilized by heterotrophic bacteria.

2.4 Biodegradation

Bioavailability of DNOM describes the potential of heterotrophic microorganisms to utilize the DNOM as a substrate for energy and nutrients. The DNOM which is consumed by microorganism may be aerobically mineralized completely with oxygen to mainly CO$_2$ and H$_2$O. Biodegradation of DNOM may also be incomplete, leading to partial oxidation and
fragmentation. In addition to the availability of oxygen the extent of biodegradation depends on the size and structure of DNOM (Maier, 2009).

Since the DNOM is a heterogeneous mixture of organic compounds, which consist of broad range of different organic molecules, it is commonly categorized into three fractions in regards to its biodegradability:

1) A labile pool, which is rapidly degradable. This pool consists of LMW DNOM such as simple carbohydrates, fulvic acids, amino acids, amino sugars and low molecular weight proteins.

2) Less degradable pool, which consists of polysaccharides and other slowly degradable products.

3) The recalcitrant pool, which consists of highly aromatic and complex compounds, such as lipids and lignin (Marschner et al., 2003).

The bioavailability of DNOM govern the microbial respiration of DNOM with production of CO\textsubscript{2} and consumption of O\textsubscript{2} (Stefan et al., 2000). Biodegradation can thus be quantified by the removal of either DNOM or O\textsubscript{2}, or the production of CO\textsubscript{2}.

2.5 Structural Characterisation of DNOM by Spectroscopy

2.5.1 Chromophoric Dissolved Organic Matter (CDNOM)

CDOM is found in all natural waters and may constitute a significant fraction of the DNOM pool in natural waters (Stedmon et al., 2000) and influences the photo-processes in the upper part of the water column. Organic compounds with conjugated double bonds absorb radiation in the UV (\(\lambda = 200-400\)) range. Organic compounds with long chains of conjugated double bonds absorb radiation in the visual (Vis) (\(\lambda = 400-800\)) regions of the electromagnetic spectrum. Energy absorbed from the radiation will excite electrons from their ground state orbitals to higher energy, excited state orbital. Electronic transitions can occur at the following energy levels: \(\sigma\) to \(\sigma^*\), \(n\) to \(\sigma^*\), \(n\) to \(\pi^*\) and \(\pi\) to \(\pi^*\). These specific groups of bonds, which absorb radiation, are termed chromophores (Pavia, 2009). CDNOM consists of a varied mixture
of aliphatic and aromatic polymers and is one of the major light absorbing constituents in natural waters. Its absorption is strongest in the ultraviolet region and diminishes to near zero in the red region.

UV-Vis absorption spectroscopy measures excitation transitions from the ground state to the excited state, while Fluorescence spectroscopy measures relaxation transitions from the excited state to ground state by emissions of photons.

### 2.5.2 UV-Vis Absorbency

A set of readily available proxies is used to describe the physiochemical character of DNOM. Structural properties of DNOM may be approximated by absorbency in the UV or Vis range based on its content, length and conjugated double bonds.

The UV absorption of DNOM is conceptually linked to its amount of aromatic carboxylic (peak around 205 nm) and phenolic groups (peak around 270 nm) and to C=C bonds (peak around 180 nm). The specific UV radiation (sUVa) is the UV absorbency relative to the amount of DOC ($\lambda_{254nm}$/mg C·L$^{-1}$). The sUVa varies considerably due to varying degree of unsaturated double bonds, thus especially the aromaticity of the DNOM. The sUVa is thus conceived to describe the relative amount of aromatic moieties in the DNOM.

The sVISa is the VIS absorbency relative to the amount of DOC ($\lambda_{400nm}$/mg C·L$^{-1}$). The sVISa reflects the relative amounts of CDOM that absorbs light in the coloured spectra (at 400 nm) (Vogt et al., 2008). DNOM with a high sUVa and sVISa is therefore relatively aromatic and of high molecular weight and coloured. This material is typically mainly of terrestrial origin (allochthonous). DNOM with low sUVa and sVISa is typically produced in the lake (autochthonous).

SAR is the UV absorbency relative to the visible absorbency of DNOM ($\lambda_{254nm}$/$\lambda_{400nm}$). Low molecular weight (LMW) organic compounds absorb at a lower wavelength. A relatively higher absorbency in the lower wavelength (i.e. blue shift) indicates thus a relative smaller size of the CDOM compounds. Similarly, relative absorbency at longer wavelength (red shift) increase with increased length of the conjugated double bonds. The SAR is therefore considered to reflect the size of the DNOM, with low values indicating high M$_W$, and high values suggesting more low M$_W$ compounds.
2.5.3 Fluorescence Spectroscopy

When a molecule absorbs photon energy from the electromagnetic radiation, a loosely held electron in an atom or molecule is exited to higher energy level state. This excited electron may emit energy in the form of fluorescent light when it returns to its ground state. Some energy is always ‘lost’ from the excited electron by collision, non-radiative decay and other processes, prior to emission, so the energy of the emitted photon is lower than the excitation energy. Organic compounds that absorb electromagnetic radiation and emit fluorescence are fluorophores. The fluorescence of DNOM is attributed to C=O, phenolic, aromatic bonds and quinone moieties (Miller et al., 2009). Compounds such as lignin, tannins, polyphenols, and melanins, which are important moieties of humic DNOM, are thus likely responsible for the bulk of the DNOM fluorescence (Green et al., 1994).

A 3-D fluorescence excitation-emission matrix (EEM) spectrum is obtained by recording multiple emission spectra at increasing excitation wavelengths. The results of these spectra can be presented in a 3-dimensional manner with excitation and emission wavelength on the x-axis and y-axis, and fluorescence intensity on the z-axis (Chen et al., 2003).

Fluorescence spectroscopy is a reliable optical technique for monitoring DNOM quality. The excitation and emission wavelength at which fluorescence occurs are characteristic to specific molecular structures of DNOM. In general, peaks that exhibit emission at long wavelengths referred to as red shifted. The peak such as humic acid like exhibit excitation at long wavelengths are more aromatic compounds, highly conjugated, and likely represent HMW fraction of DNOM pool (Coble, 1996). Such compounds are mainly derived from vascular plants, i.e. mainly of terrestrial origin. In contrast, peaks such as fulvic acid-like exhibit excitation at short wavelengths are referred to as blue shifted. These compounds are less aromatic and of lower molecular weight than humic acid like compounds (Chen et al., 2003). Figure 2.2 illustrates the four distinct types of DNOM fluorescence groups at the different Ex/Em wavelength.
2.6 Iron Chemistry and Fe–DNOM Complex

Iron exists in to oxidation steps in the environment, either as Fe$^{2+}$ or Fe$^{3+}$. In water the ions show increasing hydrolysis with increasing pH. As the pH increases, the Fe$^{2+}$ becomes more readily oxidised to Fe$^{3+}$ (Figure 2.3).
Aqueous Fe$^{2+}$ in drainage water from reducing environments is upon exposure to aeration oxidised to Fe$^{3+}$ and subsequently precipitated as iron oxide-hydroxide at the pH of natural waters (i.e. between 4 and 8). Dissolved iron in waters containing fulvic and humic acids are not primarily present as free, aqueous tri- or divalent ions. The presence of carboxyl, phenol, and carbonyl groups in the fulvic and humic acids are good electron pair donors to metal ions. The iron ions in natural water are thus mainly in the form of metal chelates formed between DNOM and the central metal ions by the complexation of the iron ions by the fulvic and humic acids functional groups (Weber et al., 1975). Especially in waters with pH > 5, the concentration aqueous Fe$^{3+}$ is very low, and the dissolved Fe$^{3+}$ is primarily associated with the DNOM.

The iron (oxy)hydroxides are more likely to aggregate and settle than Fe-DNOM complexes and Fe-DNOM complexes are therefore considered to be transported over longer distances. Changes in pH affects the mobilisation of Fe and DNOM and Fe speciation. Fe-DNOM complexes from different sources and different catchment area can exhibit different metal binding properties (Neubauer et al., 2013).

The contribution of dissolved Fe to colour (Fe %) ranges from 0.2% to 53% (Xiao et al., 2013). The chromophores of Fe is arising from the d-orbitals of Fe (Sherman et al., 1985), while the chromophore of the DNOM and Fe-DNOM complexes are arising from conjugated double bonds and intramolecular charge-transfer reactions between organic chromophores (Del Vecchio et al., 2004), respectively.
3 Materials and Methods

Surface water samples from lake Päijänne in Finland and two reference samples of previously thoroughly characterised reverse osmosis (RO) isolated DNOM were included in this study. A fresh water sample from the large lake Päijänne was collected as part of the NOMiNOR project (Eikebrokk et al., 2017). Päijänne is the raw water source for the Helsinki water treatment plant. The reference samples Trehøningen and Hellerudmyra are from the NOM Typing project (Gjessing et al., 1999). These are previously reverse osmosis ultra-filtrated and freeze-dried DNOM material.

3.1 Sample Sites

3.1.1 Fresh Water Sample from Lake Päijänne

Lake Päijänne is one of the largest lakes in Finland (1 116 km²) (Figure 3.1) with a water retention time of 2.2 yrs. The lake catchment covers an area of 25 400 km². The deepest part of the lake is 95 m and the mean depth is 16 m. The lake has a very long (120 km) and complex shoreline. The basin of the lake is lies in the Precambrian primary rock consisting mainly of granite and schist. The lake is situated 140 km from the sea and is hence not strongly affected by sea salt aerosols. Over the past few decades chloride and sulphate concentrations have increased in the raw water, probably due to anthropogenic pollution, such as from the paper industry. The area surrounding the lake is largely covered by forests, the proportion of farm land being only 10%, and forested peatlands account for 25%. From the peatland humic substances are discharged to the lake. The forests consists mainly pine and spruce (International Lake Environment Committee Foundation, 2018).
The DNOM in lake Päijänne is dominated by the hydrophobic fractions. This fraction constitutes 84% of the DOC while the hydrophilic fraction accounts for the remaining 16%. The sUVa value is 3.2, indicating moderate aromaticity. The TOC level is in the range of 6.7–7.3 mg C·L⁻¹. Most of the organic matter is dissolved, with DOC levels in the range of 6.6–7.1 mg C·L⁻¹ (Eikebrokk et al., 2017).

The biodegradable organic matter is on average only 1.2% of the total DOC. The levels of biodegradable organic matter are significantly increased due to the oxidising effects of ozone on the remaining DNOM. The ozone transforms the hydrophobic DNOM to more biodegradable charged fractions. The biodegradable DNOM formed in the ozonation process is however reduced again during the GAC filtration process. A small increase in ATP is detected in the distribution system samples despite the low biodegradable values, and this may be due to the low doses of chlorine and the corresponding low chlorine residuals in the distribution system.
Colour, as a proxy for DNOM, was modelled very well using temperature, precipitation and conductivity as illustrated in Figure 3.3 (Eikebrokk et al., 2017). This implies that the important drivers for DNOM at Lake Päijänne are temperature, precipitation, and conductivity; dominated by chlorides and sulphate. Carbonate from the watershed is also important. Iron is presumably not an important factor for colour at this site.

![Graph showing colour in Lake Päijänne](image)

Figure 3.2. The colour in Lake Päijänne may be modelled using only precipitation amounts, temperature and conductivity as inputs (Eikebrokk et al., 2017).
3.1.2 RO Samples from Trehørningen (Mineral Soil) and Hellerudmyra (Peat)

Figure 3.3. The border of the drainage basin (black line) of Trehørningen (Soil) (red cross) and the location of Hellerudmyra (red cross).

The RO samples are isolated DNOM from the surface waters in South-Eastern Norway. These samples are two of nine RO samples isolated and characterised in the NOM-Typing project (Gjessing et al., 1999). The main aim of this project was to develop a protocol for the typing of DOM that could assist in the choice and optimisation of water treatment processes. The sites were selected to represent catchments that differed in properties such as climate, vegetation, retention time etc., while at the same time not being under influence of neither agricultural nor industrial activities. All the samples were isolated with exactly the same methods and by same team. In spite of the uniformity of the isolation technique used for the samples, the recoveries of the material differ from sample to sample. This is probably due to the differences in the
nature of DNOM and differences in the composition of water. As this was a project establishing a collaboration for the typing of DNOM, the samples have been distributed to different laboratories. The data generated included results from a large number of methods for analysing and characterising DNOM properties.

Hellerudmyra is most strongly influenced by bog water and has a small catchment area (0.08 km\(^2\)) within the watercourse of Trehørningen. The colour of the water from which the DNOM was isolated was about 166 mg Pt·L\(^{-1}\) (Gjessing et al., 1999). The surface area of this bog is 50 m\(^2\) with a water retention time of 10 days. The DNOM is therefore considered to be relatively young in the water phase.

Lake Trehørning is the headwater lake of the water course of Trehørningen. It represents water draining a forest with mineral soils. The colour of the water is about 33 mg Pt·L\(^{-1}\) and the catchment area is 6 km\(^2\). The surface area of the lake is 0.33 km\(^2\) with a water retention time of 9.4 months (Gjessing et al., 1999). This lake contains high concentrations of Ca and Mn, which may be related to the bedrock mineralogy.

The Trehøningen and Hellerudmyra samples were from two sites along a watercourse feeding into the raw water reservoir for the waterworks of Bærum municipality, close to Oslo. These two materials were selected as reference samples because the environmental conditions differ between the sites causing them to comprise different characteristics of the DNOM material. The original data of the RO samples for water character and DNOM characteristics are available in the NOM-typing projects results. Some important data are shown in Table 3.1. The samples will be further named as “Peat” for Hellerudmyra and “Soil” for Trehørning according to their DNOM character.
Table 3.1. Some of the important data for both Hellerudmyra (Peat) and Trehørning (Soil) from the NOM-typing original data.

<table>
<thead>
<tr>
<th></th>
<th>Peat</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC (mg C·L⁻¹)</td>
<td>18</td>
<td>4.8</td>
</tr>
<tr>
<td>pH</td>
<td>5.2</td>
<td>6.6</td>
</tr>
<tr>
<td>Conductivity (µS·m⁻¹)</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>sUVa (λ₂₅₄nm/mg C·L⁻¹)</td>
<td>4.6</td>
<td>3.9</td>
</tr>
<tr>
<td>λ₂₅₄nm/λ₄₃₅nm</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>λ₄₃₅nm/mg C·L⁻¹</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Aromatic C/Aliphatic C</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Hydrophilic/Hydrophobic</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Ca²⁺ (µeq·L⁻¹)</td>
<td>60</td>
<td>34</td>
</tr>
<tr>
<td>Mg²⁺ (µeq·L⁻¹)</td>
<td>36</td>
<td>23</td>
</tr>
<tr>
<td>Na⁺ (µeq·L⁻¹)</td>
<td>104</td>
<td>130</td>
</tr>
<tr>
<td>K⁺ (µeq·L⁻¹)</td>
<td>8.4</td>
<td>10</td>
</tr>
<tr>
<td>Cl⁻ (µeq·L⁻¹)</td>
<td>28</td>
<td>31</td>
</tr>
<tr>
<td>Sulphate (SO₄²⁻) (µeq·L⁻¹)</td>
<td>71</td>
<td>65</td>
</tr>
<tr>
<td>Nitrate (NO₃⁻) (µeq·L⁻¹)</td>
<td>0.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

3.1.3 Reference Sample Isolation by Reverse Osmosis (RO)

Both reference materials were isolated from surface waters using the RO method in accordance with Serkiz et al. (1990). Through this method, nearly all of the dissolved organic and inorganic material from 1500 L of Peat and 2000 L of Soil sample water is concentrated. The recovery of the Peat and Soil RO isolates are 85.8 g and 48.7 g respectively. The method has been proven to be efficient for the isolation and preservation of DNOM with regard to its colour, UV absorbance and concentration of both inorganic and organic matter. About 10–15% of organic matter was lost due to that the low molecular size DNOM pass through the RO membrane with a 15 nm cut-off and due to the precipitation of matter during the filtration step (at 0.45 µm), prior to freeze drying. Free aqueous cations were exchanged by Na⁺ in the cation exchanger at the inlet to the RO-unit. This step was performed to prevent the precipitation of insoluble salts such as CaCO₃(s) and CaSO₄(s). None of the cations is removed 100% during the cation exchange process. Calcium as an example removed only 60–95%. This may suggest that some of these elements are partly associated with the DNOM. The raw water from Hellerudmyra (Peat) contains high concentration of DOC, total Al and Fe. The Al and Fe are associated with DOC (Gjessing et al., 1999, Vogt et al., 2001).
3.2 Sample Preparation

The sample from Päijänne was collected during the NOMiNOR meeting at the Helsinki waterwork on the 12th of April, 2016. The sample was transported as carry on hand luggage to Oslo. Upon arrival the sample was covered with aluminium foil and stored at 4 °C. The sample was filtered through a 0.2 µm filter (cellulose acetate, Sartorius) by using a water vacuum pump by Shafia Iftekhar (guest researcher, UiO) on the 6th of June 2016. This was done to filter out any undissolved particulate matter and the bacteria from the samples (Madigan et al., 2006, Maier, 2009) in order to sterilize the sample. The filters were pre-rinsed with 150 mL Type I water (ultrapure, de-ionized water, Milli-Q) to remove any traces of carbon on the filters (Khan et al., 2006). After filtrations the samples was covered with aluminium foil to avoid photo-oxidation and stored at 4 °C.

The frozen RO isolates were thawed carefully by first placing them at 7 °C for 3 h, followed by heating at room temperature (22 °C). The RO isolates were subsequently re-dissolved using 2 L of Type I water to an estimated final concentration of 10 mg C·L⁻¹ based on their known carbon contents. To ensure complete dissolution, the RO samples were stirred, using magnetic stirrers for three days with the samples kept in the dark. After dissolution, the samples were filtered through 0.2 µm membrane filters (cellulose acetate, Sartorius). This pore-size was selected as this was the same as used for the Lake Päijänne sample. Although the microbial activity of these reference samples was assumed to be negligible, the same pore-size was used in order to compare the results from the Lake Päijänne. After filtrations all samples were covered with aluminium foil to avoid photo-oxidation and stored at 4 °C in a cold room until analysis.

Part of the 0.2 µm filtrated sample was photo-oxidised for 53 hours by artificial sunlight using a Q-SUN (see Chapter 3.3). The photo-oxidised and the non-photo-oxidised samples were size fractionated by ultrafiltration through a 10 kDa centrifugal filter (Amicon regenerated cellulose, Millipore). The fractionation was done at 4000 rpm centrifugal force. The filter was rinsed before use with Milli-Q Type I water to remove any traces of carbon on the filters. This cut-off was selected in order to fractionate the HMW HA from the LMW FA in the samples since most of the HA compounds are conceived to have a higher molecular weight (> 10 000 Da) and FA compounds have a lower molecular weight.

The samples were named based on their DNOM character. Each water sample was subdivided into four samples based on its size fraction and photo-oxidation as presented in Figure 3.4. All
the measurements of physicochemical properties of the DNOM were conducted on both filtered samples (0.2 µm and 10 kDa) and before and after exposure (53 hour) to artificial sunlight in Q-SUN (see Chapter 3.3).

![Diagram](image)

Figure 3.4. Sub divisions are made based on size fractions and photo-oxidation of each sample. The “0.2 µm sample” was not fractionated at 10 kDa and Photo-oxidised, the “10 kDa samples” was only fractionated at 10 kDa, the “0.2 µm & photo sample” is only Photo-oxidised and the “10 kDa & Photo sample” was both fractionated at 10 kDa and photo-oxidised.

### 3.3 Photo-oxidation

The photo-oxidation was conducted on a Q-sun Xenon test chamber (Q-Panel Lab Products, Cleveland, OH) (Figure 3.6) at the Bioscience Department, UiO. This is a laboratory simulator of the damaging effects of sunlight. The Figure 3.6 illustrates the correlation between the filtered Xenon arc light with the full spectrum sunlight. Sunlight, and especially ultraviolet (UV) radiation ($\lambda < 400$ nm) is the primary cause for the degradation of DNOM. Irradiance was set to 0.55 W·m$^{-2}$ at 340 nm, as this was the most common irradiance setting used. The irradiance at 0.55 W·m$^{-2}$ is comparable to summer sunlight.

The irradiance was controlled with a CR 20 calibration radiometer (Q-Panel Lab Products) as described by the instrument’s manual. The samples were in 110 mL quartz bottles that were placed in a cooling tank. Tap water was used to cool the samples.
3.4 Water Matrix Characterisation

3.4.1 Conductivity and pH

Conductivity and pH measurements were conducted according to International Standard ISO 7888 (1985) and ISO 10523 (2008). First conductivity was measured in a 15 mL sample aliquot by using Mettler-Toledo FiveGo™ electrode, calibrated with a standard solution of
84 \mu\text{S}\cdot\text{cm}^{-1}, followed by pH measurement using an Orion Dual Star pH-meter with a combined Ross electrode, calibrated with buffer solution with pH 4.01 and pH 7.00.

### 3.4.2 Dissolved Organic Carbon Analysis

Total organic carbon was measured at the Department of Bioscience, UiO, according to ISO 8245 (1999) method by using a TOC-VCPH analyser (Shimadzu) with an ASI-V auto sampler. Calibration standards were made using potassium hydrogen phthalate.

The instrument was set to determine Non-Purgeable Organic Carbon (NPOC), which removes inorganic carbon by acidifying the samples (pH < 3) by adding 1.5 M of HCl and purging with N\textsubscript{2}. The remaining DNOM was decomposed to CO\textsubscript{2} with the aid of catalyst at 6800 °C. The evolved CO\textsubscript{2} is determined by a nondispersive infrared sensor detector (NDIR).

### 3.4.3 Cation Analysis

Analyses of total concentration of major cations [sodium (Na\textsuperscript{+}), potassium (K\textsuperscript{+}), calcium (Ca\textsuperscript{2+}) and magnesium (Mg\textsuperscript{2+})] along with total aluminium (Al\textsuperscript{3+}) and iron (Fe) were determined in accordance with ISO 22036 (2008) using a Varian VISTA AX CCD simultaneous axial view Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) with a V-groove nebulizer with Sturman Master spray chamber. It measured emissions at specific wavelengths from the analyte atoms and ions that are excited by the plasma at 6000-10000 K. The emission was measured axially as this allows for a longer viewing path length, and thus better sensitivity and lower detection limit. All standard solutions and samples were digested and matrix matched by adding 1% (v/v) of 65% HNO\textsubscript{3} to a concentration of 0.15 M prior to analysis. Samples were introduced to the nebulizer through a peristatic pump, were it is transformed to an aerosol, which is carried to the plasma by a flow of Ar gas.

### 3.4.4 Anion Analysis

The concentrations of chloride (Cl\textsuperscript{−}), fluoride (F\textsuperscript{−}), nitrite (NO\textsubscript{2}−), bromide (Br\textsuperscript{−}), sulphate (SO\textsubscript{4}^{2−}), and nitrate (NO\textsubscript{3}−) in the samples were determined according to ISO 10304-1 (2007) at the Department of Chemistry, UiO, by using a ThermoFisher Scientific Dionex Integrion HPIC System with a Dionex AS-DV Autosampler.
A 4 μm AS anion analytical column, along with an anion electrolytically regenerated suppressor (Dionex™ AERSTM 500, 2 mm) and a conductivity detector (Dionex™ Integrion™ Conductivity Detector) were used for the analysis. Potassium hydroxide was used as the eluent. For the operation of the instrument and for preliminary data analysis, the software Chromeleon 7, from Thermo Scientific, was used.

3.5 Al/Fe Fractionation

Determination of monomeric organic aluminium and iron (Alo and Feo) and labile inorganic Al and Fe (Ali and Fei) fractions was conducted following the procedures developed by Barnes (1975) and Driscoll (1984) (Rudberg, 1995).

Monomeric organic forms are separated from polymeric forms of aluminium and iron by complexation with 8-hydroxyquinoline at pH 8.3 with subsequent extraction into MIBK organic phase during 20 seconds. The analysis is done at pH 8.3 to avoid most interferences that may occur at other pH conditions. Further fraction of organic bound monomeric aluminium (Alo) and iron (Feo) from inorganic aluminium (Ali) and iron (Fei) was achieved by trapping the Ali and Fei fraction on to an Amberlit IR-120 ion exchange column. Concentrations of aluminium and iron in the samples were determined by absorbance of radiation at λ = 395 and 600 nm in the MIBK phase using a Shimadzu UV-1800 UV-Visible Spectrophotometer, using 1 cm glass cuvettes. The absorbance at λ = 395 nm corresponds to both Al and Fe, while the absorbance at λ = 600 nm corresponds to Fe in the sample and act as a correction factor. The readings at λ = 600 nm pertain only to Fe, they can also be used to measure both originally bound and inorganic Fe.

3.6 DNOM Characterisation

Spectroscopic analysis is used to assess the structure, composition and relative size of DNOM.

3.6.1 UV-Vis Absorbency

Each sample was analysed by full spectrum absorbency at wavelengths from 200 nm to 800 nm. The spectrum was recorded on a Shimadzu UV-1800 UV-Visible Spectrophotometer, using 1 cm quartz cuvettes. The spectrophotometer was background corrected prior to analysis using both cuvettes containing Type I water. During the scan a cuvette with Type I water was kept as
a reference. Values of absorbency at $\lambda = 254$ and 400 nm were used to calculate specific UV absorbency, $s_{\text{UV}}$ ($\lambda_{254\text{nm}}$/mg C·L$^{-1}$), $s_{\text{VIS}}$ ($\lambda_{400\text{nm}}$/mg C·L$^{-1}$) and specific absorbance ratio, SAR ($\lambda_{254\text{nm}} / \lambda_{400\text{nm}}$).

### 3.6.2 Fluorescence Spectroscopy

Fluorescence Excitation-Emission Matrix (EEM) was measured by using Varian Carry Eclipse Fluorescence Spectrophotometer using clear faced 1 cm quartz cuvette at the Norwegian University of Life Sciences (NMBU). The EEM spectra were generated by subsequently scanning the emission from 300 to 600 nm by incrementing the excitation wavelength by 30 nm from 240 to 450 nm. Data were processed using Varian Cary Eclipse software.

### 3.7 Biodegradation Experiment

Biodegradation experiment was conducted by using SensorDish® Reader (SDR), which monitors oxygen levels in liquids. The system consists of three main components:

1) the Sensor Vials containing the samples;

2) the Sensor dish readers; and

3) a computer with designated software for data processing and operating the systems.

The Sensor vials consist of 5 mL wells made of borosilicate glass. The vials are closed with screwcaps and a rubberized septum to ensure they are properly sealed from the outside air. At the bottom of each vial is a 5 mm sensor spot. This spot contains a luminescent dye that becomes exited by the SDR. Once exited the sensor spot will emit phosphorescence light. This emission is recorded non-invasively through the transparent bottom of the vials by the SDR placed below them. Oxygen quenches the phosphorescence. The speed of which the light degreases is thus a measure of the level of oxygen in the sample.
3.7.1 Inoculum

Raw water sample from Langtjern was used to prepare the inoculum since similar biodegradation experiments at Langtjern water was conducted by Martínez Francés (2017) and Håland (2017) and the results are then more comparable with their results, ensuring precision and comparing the results from similar reference samples. More than 100 mL of raw water from Langtjern was filtered through 2.0 µm membrane filter (polycarbonate, Isopore) to allow for bacteria to pass through the filter (Maier et al., 2009) while removing predatory organisms larger than 2.0 µm that could feed on the bacteria. This is to ensure that bacteria are the dominant organism in the inoculum. Then 100 mL of filtrate was transferred to an Erlenmeyer flask. 1 mL of nutrients stock solutions (10 mM of phosphate and 10 mM of nitrate) were added to the inoculum to get a final concentration of 0.1 mM. The Erlenmeyer flask was covered with aluminium foil to remove light and thus avoid growth of photoautotrophic algae. The inoculum was incubated at room temperature for a minimum of two days to grow microorganism, on a shaking table at medium shaking speed (250 rpm), ensuring that the inoculum is kept properly saturated with oxygen.

3.7.2 Sample Preparation

Small aliquots of sample were poured into 25 mL volumetric flasks and added 250 µL of nutrient stock solution (10 mM of phosphate and 10 mM of nitrate) and 250 µL of inoculum. Then samples were added to the 25 mL mark and shaken by hand for 30 seconds to ensure proper mixing. Samples from each flask were filled into 5 mL sensor vials, ensuring that no
headspace was left inside the vials during sealing. The vials were then sealed using designated screw caps and Parafilm. The vials were placed on the SDR system and kept inside the incubator for three days for the consumption of O$_2$ at 20 °C.

Blank sample was prepared from 25 mL Type 1 water with 250 µL nutrient and 250 µm inoculum. A reference sample was made of 25 mL Glucose solution with 250 µL nutrient and 250 µm inoculum.

The results from the SDR instrument were used to calculate the respiration rate by using R-Studio. The start of exponential O$_2$ consumption is defined as the point at which the measured values are more than three times standard deviation lower than the initial measurements during lag time. This is to avoid any fluctuations that occur in the beginning of the measurements due to temperature fluctuation. The program then calculate the respiration rate and subtracts the blank.
4 Results and Discussion

The chapter starts with a presentation of the water chemistry of the samples in order to characterise the matrix in which the DNOM is studied. Following this, the structural character of DNOM, the effect of photo-oxidation, and size fraction are described. Finally, the results of the biodegradation experiments of the samples are presented and discussed in light of the matrix composition and the quality of DNOM in the different size fractions.

4.1 Sample Matrix Characterisation

Water chemistry in the two size fractions (i.e. < 2 µm and < 10 kDa) of Peat, Lake and Soil water samples, before and after exposure (53 hour) to artificial sunlight in Q-SUN, are presented here. The chemistry of the water samples provides information regarding the chemical environment of the DNOM.

4.1.1 Electrical Conductivity and pH

The pH and conductivity of the samples are presented in Table 4.1. The two RO samples are more acidic than the Lake samples. These differences are mainly due to that the RO samples are mainly from head water lakes with generally high concentration of DNOM while the Lake sample is from high Strahler order lakes.

The conductivity of the Lake water sample is high, reflecting the high ionic strength found in the sample (Chapter 4.2.2). Neither filtration nor oxidation have any significant (p > 0.05) effect on conductivity.

The photo-oxidation caused a slight increase in pH in the poorly buffered Peat water samples. This could be due to the mineralisation of organic acids (see Chapter 2.2). The Lake sample does not show any changes in pH. This is expected since the water is more strongly buffered and contains low concentrations of DNOM of a more recalcitrant nature. On the other hand, the oxidation of DNOM in the Soil water samples caused a decrease in pH, possibly due to the production of CO₂, and thus protolysation of carbonic acid in the pH neutral water. pH was lowest in the 10 kDa fraction, likely reflecting the more acidic character of the LMW DNOM fraction.
Table 4.1. Conductivities and pH of the Peat, Lake, and Soil water samples, for filtered (at 0.2 µm and 10 kDa), before, and after exposure (53 hour) to artificial sunlight in Q-SUN.

<table>
<thead>
<tr>
<th>Site</th>
<th>Peat water</th>
<th>Lake water</th>
<th>Soil water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut-off</td>
<td>0.2 µm</td>
<td>0.2 µm</td>
<td>0.2 µm</td>
</tr>
<tr>
<td>Oxidised</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Conductivity (µS·cm⁻¹)</td>
<td>15.4</td>
<td>13.5</td>
<td>11.4</td>
</tr>
<tr>
<td>pH</td>
<td>5.76</td>
<td>6.43</td>
<td>5.55</td>
</tr>
</tbody>
</table>

4.2 Elemental Composition and Speciation

4.2.1 Dissolved Organic Carbon (DOC)

DOC is the main component in DNOM. The DOC concentrations were therefore measured as a proxy for DNOM in the water samples.

The concentration of organic carbon in each sample, both filtrated (0.2 µm and 10 kDa) and before and after photo-oxidation (53 hours) is presented in Figure 4.1.

The DOC concentration was expected to be lower in the 10 kDa size fraction due to the removal of HMW organic molecules. However, the results show a significant (p < 0.01) DOC increase in the Peat and Lake water samples. This may be due to the contamination of the samples from the filter or from the containers. The 10 kDa filters contain trace amounts of LMW glycerine, and although the filters were rinsed with Type I water before filtering the actual samples, the glycerine may not have been completely removed during the washing process.

Since the glycerine has no absorbency in the UV and visible range, the presence of glycerine in the samples will not affect the spectroscopic measurements. This can be seen in Figures 4.2 and 4.3, where the absorbencies of the different size fractions show expected trends, with a lower absorbency after filtration.

Photo-oxidation is expected to remove some organic carbon from DNOM by photo-mineralisation to CO₂. In the bulk water samples (i.e. only filtered at 0.2 µm), photo-oxidation decreased the DOC concentration significantly (p < 0.02) between 5.5% and 13%. There is a larger decrease in the concentration in the Peat water sample than in the mineral soil water. This is because the peat water contains more HMW DNOM on which the effect of photo-oxidation
is stronger due to the higher content of chromophores. The photo-oxidation of the two reference samples (Peat and Soil water) with DNOM size less than 10kDa shows an increase in the DOC concentrations, but this may be due to contamination from the filtration process or the container, as described above.

The Soil water samples show very high DOC concentration (36.5 mg C·L⁻¹) compared with the inorganic constituents in the sample. This is clear from comparing the ratios of the concentration data for DOC and the ratios for inorganic constituents in the RO isolates from (Gjessing et al., 1999) with the concentrations measured in the prepared samples (Table 4.2). For the Soil water sample the concentration of inorganic constituents are around 1.6 times higher in the water used in this study, while the DOC is 7.6 times higher. Relative to the inorganic constituents in the mineral Soil water sample the DOC concentration should have been (4.8 mg C·L⁻¹ × 1.6 =) 7.68 mg C·L⁻¹. There is no explanation for this, other than that this might be due to contamination during the sample preparation process, such as from the container used.

![Figure 4.1. DOC concentration (in mg C·L⁻¹) of the water size fractionated [0.2 µm (2) and 10 kDa (10)] samples from Peat (P), Lake (L) and mineral Soil (S) before and after exposure (53 hour; P) to artificial sunlight in Q-SUN.](image-url)
Table 4.2. Important data for both Hellerudmyra (Peat) and Trehørning (Soil) from the NOM-typing projects. Original data were used to normalize the new data.

<table>
<thead>
<tr>
<th></th>
<th>Peat</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RO</td>
<td>This study</td>
</tr>
<tr>
<td><strong>DOC (mg C·L⁻¹)</strong></td>
<td>17.7</td>
<td>7.7</td>
</tr>
<tr>
<td><strong>Ca²⁺ (mg·L⁻¹)</strong></td>
<td>1.2</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Mg²⁺ (mg·L⁻¹)</strong></td>
<td>0.44</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Na⁺ (mg·L⁻¹)</strong></td>
<td>2.4</td>
<td>1.13</td>
</tr>
<tr>
<td><strong>Cl⁻ (mg·L⁻¹)</strong></td>
<td>1.0</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>SO₄²⁻ (mg·L⁻¹)</strong></td>
<td>3.4</td>
<td>1.67</td>
</tr>
</tbody>
</table>

4.2.2 UV-Vis Absorbency

Using UV₂₅₄ absorbency as a proxy for DNOM provides more reasonable response patterns. It is necessary to assume that all the samples have a constant relative absorbency between the size fractions before and after photo-oxidation, although this is not conceptually sound (see Chapter 4.3.1). The UV absorbance is lower in the < 10 kDa fraction than in the bulk sample due to the removal of the more aromatic HMW DNOM. The < 10 kDa fraction of Peat, Lake, and Soil bulk water samples account for 66%, 85%, and 73% of the UV₂₅₄ absorption, respectively. The reduction is highest in the Peat bulk sample. This indicates that it has more aromatic and HMW DNOM. The next highest reduction is found in the Soil bulk water sample. This indicates that the Soil water sample has more aromatic and more HMW compared to the Lake sample. This is in accordance with the previous data found in the NOM-typing and NOMiNOR projects (Chapters 3.1.1 and 3.1.2). The 10 kDa filtration of LMW samples constitutes between 84% and 91% UV₂₅₄ absorption. This indicates that the LMW samples contain less aromatic and less HMW DNOM.

The photo-oxidation causes the UV absorbance to decrease. The breaking up of the large DNOM compounds to smaller molecules may cause this during photo-oxidation. The photo-oxidation caused an expected 23–47% reduction in the UV₂₅₄ in the bulk sample (< 0.2 µm) and between 17 and 32% reduction in the fraction with LMW DNOM (i.e. < 10 kDa). This is reasonable as the LMW fraction is conceived to be less photo-oxidisable due to that it has less chromophores. The same relative trends are found using the absorbency at 400 nm as a proxy for DNOM: The photo-oxidation caused a 29–52% reduction of the colour of DNOM in the bulk sample, and between 21 and 38% reduction of colour in the LMW fraction.
Figure 4.2. UV$_{254}$ absorbency of the water size fractionated [0.2 µm (2) and 10 kDa (10)] samples from Peat (P), Lake (L) and mineral Soil (S) before and after exposure (53 hour; P) to artificial sunlight in Q-SUN.

Figure 4.3. UV$_{400}$ absorbency of the water size fractionated [0.2 µm (2) and 10 kDa (10)] samples from Peat (P), Lake (L) and mineral Soil (S) before and after exposure (53 hour; P) to artificial sunlight in Q-SUN.
4.2.3 Major Cations and Anions

The equivalent concentration (µeq·L⁻¹) of major cations and anions for the samples are presented in Figure 4.4. The equivalent concentration of carbonate species (i.e. DIC = HCO₃⁻ + 2 CO₃²⁻) was calculated based on the pH and assuming equilibrium with the pCO₂ in the atmosphere (i.e. = 3.42). Carbonates from the watershed is important for Lake Päijänne (Chapter 3.1.1). The anionic contribution of DNOM as DNOM–A⁻ in the samples was estimated based on data for pH and DOC concentrations using a model developed by Oliver et al. (1983). For lakes located in southern Norway, it was determined that the weak acid contribution of organic carbon is 5.5 µeq/mg C (Henriksen et al., 1980). The highest negative balance is found in the mineral Soil water sample due to the high concentration of DNOM–A⁻. The mineral Soil water samples have an anion surplus of about 150 µeq·L⁻¹. This is likely due to the error in the DOC concentration as discussed in Chapter 4.2.1. The normalized results show that Peat ratio of DOC is close to the ratio of the other ions. However, the DOC of the Soil ratio is about 4.7 times higher than the other ions. This indicates that the large amount of DOC found in this sample is from contamination, and is not from the RO isolates. Changing the DOC concentration to the estimated 7.68 mg C·L⁻¹, gives approximate charge balance also in these samples.

The remaining minor differences between cations and anions is expected due to the large number of analysis and assumptions made to calculate this value. An anion deficiency is commonly found because the cations are measured as total concentrations after acid digestion. Thus, any cations associated with inorganic and organic colloids are included in the measurements. On the other hand, anions are measured as free anions, so any anions bound or complexed are not measured. In addition, Al and Fe were not included in the measurements because they are bound to organic matter, and Fe can take on different oxidation states.

The highest total charge concentration is found in the Lake sample. The lake is situated 140 km from the sea, and is not expected to be strongly affected by sea-salt episodes. The high concentration of chloride and sulphate anions are mainly due to emissions from local anthropogenic pollution sources. Ca²⁺, Mg²⁺ and Na⁺ are the dominant cations in the Lake water sample, and probably mainly originates from the watershed by the weathering and dissolution of carbonate minerals from the basin of the lake.
RO samples contain higher concentrations of sodium (Na\(^+\)) compared to other cations. This is due to the method of isolation by reverse osmosis wherein the sample was pumped through a cation exchanger to replace the other cations with Na\(^+\). This was done in order to prevent the precipitation of insoluble salts such as CaSO\(_4\) and MgSO\(_4\) (Chapter 3.1.3).

Most of the polymeric cations such as Ca\(^{2+}\) and Mg\(^{2+}\) do not pass through the filter due to that they are electrostatically attracted to the negative charged HMW DNOM-A\(^-\), causing lower concentrations of these constituents in the 10kDa filtration compared to the 0.2 µm filtered sample.

![Major Anions and Anions](image)

Figure 4.4. Concentration (in µeq·L\(^{-1}\)) of the major cations [calcium (Ca\(^{2+}\)), magnesium (Mg\(^{2+}\)), sodium (Na\(^+\)), potassium (K\(^+\)), and hydrogen (H\(^+\))] and anions [DNOM–A\(^-\), chloride (Cl\(^-\)), sulphate (SO\(_4^{2-}\)), nitrate (NO\(_3^-\)), nitrite (NO\(_2^-\)), fluoride (F\(^-\)), bromide (Br\(^-\)), and DIC] in the samples.

### 4.2.4 Al/Fe Fractionation

The concentration of total and monomeric Fe and Al fractions in the samples are shown in Figure 4.5. The fractionation into organic and inorganic species using the method of Barnes (1975) and Driscoll (1984) is problematic in samples with pH values above 5.8, as found in the all the studied samples except for the non-photo-oxidised samples from the Peat.
Figure 4.5. Total Fe and Al from ICP-OES, and organic bound and labile monomeric Fe (top) and Al (bottom) fractions in the samples.

The concentration of total Al is larger than the monomeric Al fractions due to polymeric Al compounds in the sample, such as amorphous oxy-hydroxides and clay particles. This is because the concentration of total Al, determined using ICP-OES, is measured after acid digestion. Thus, any cations associated with inorganic and organic colloids are included in the
measurements. The concentration of total Fe lower than the monomeric forms implying some problems with data.

Peat is bog water, which typically has high content of iron. A bog is a wetland that accumulates peat, a deposit of dead plant material—often sphagnum mosses. The large amounts of Fe typically found in bogs is from the weathering of the surrounding mineral soil draining into the bog where it is strongly bound to the soil organic matter. This is why the DNOM from peat soil contain elevated levels of organic Fe. In the Peat water the total concentration of Fe (total) decreases due to the 10 kDa filtration. This is as expected as some of the Feo and colloidal Fei will be removed by the small cut-off. There is also an apparent decrease in Fe (total) due to photo-oxidation in HMW sample. That must be due to precipitation, causing an inhomogeneous sample. The Feo and Alo decreases in Peat water sample as expected due to 10 kDa filtration and photo-oxidation. Ali increases after photo-oxidation probably due to the formation of labile Al from the colloidal DNOM or clay particles or because of the formation of some Al(OH)₄⁻ caused by the concurrent increase in pH (Chapter 4.11).

There were only very low concentrations of inorganic labile Fe found in the samples. This is because the low solubility of inorganic Fe³⁺ due to hydrolysis at the sample pH. Very low levels of inorganic labile Al can be found in the Peat and Lake as the pH of these samples are higher than 5.5, where the solubility of Al³⁺ very low.

4.3 Structural Characterisation of Dissolved Natural Organic Matter (DNOM)

Photo-oxidation due to adsorption of radiation energy by the double-bond chromophores of the DNOM may influence its structure, composition, and size distribution. Absorption of sunlight induces breaking of bonds, producing LMW organic compounds and inorganic species. These processes may thus typically result in the decrease of aromaticity, weight, and colour of the DNOM.

4.3.1 Proxies for DNOM Characteristics

The character of DNOM is known to differ with respect to the nature of the catchment and changes with its retention time in water. The proxy values for DNOM characteristics (sUVa, sVISa, and SAR) are presented in Figures 4.6, 4.7, and 4.8, respectively. The sUVa and sVISa
values of the soil water samples are somewhat uncertain due to contamination found in the
DOC analysis (see Chapter 4.2.1). However, there are clear trends with respect to the effects of
photo-oxidation and size fractionation of the three water samples regardless of the poor DOC
data.

The sUVa was highest in the bulk Peat water sample, showing that the DNOM from bogs are
more aromatic in nature. A high sUVa was consistent with the data of the RO samples in
(Gjessing et al., 1999) and also found in other peat waters by Vogt et al. (2001). sVISa values
indicate that the bulk DNOM from the Peat water sample is also darker in colour and thus has
higher molecular weight ($M_w$). On the other hand, the DNOM from the large Lake sample has
highest SAR, suggesting that it has lower $M_w$ organic matter than the other samples. The results
from these proxies suggest that bog DNOM (Peat sample) is more aromatic, has higher $M_w$ and
is of darker colour than Lake and Soil water samples. The DNOM from the large Lake sample
has less $M_w$ than both Peat and Soil water samples. The sUVa and sVISa values show that the
Soil sample has the lowest aromaticity and least colour. However, these results may not be
completely accurate, because of the uncertainty in the DOC data. The previous data from the
NOM-Typing project give a sUVa value of 3.92 for the Soil water sample. This is 4.45 times
higher compared to the 0.88 value obtained here, and thus also confirms the error in DOC
discussed in Chapter 4.2.1.
Figure 4.6. Values for the specific UV absorbency, sUVa, obtained for the Peat, Lake, and Soil samples. Open bars show the sUVa values when the error in DOC is corrected by a factor of 4.74.

Figure 4.7. Specific visible absorbency, obtained for the samples from Peat, Lake and Soil. Open bars show the sVISa values when the error in DOC is corrected by a factor of 4.74.
4.3.2 Changes in DNOM Characteristics Due to 10 kDa Fractionation

In all three sets of sample the SAR is higher in the < 10 kDa (i.e. LMW) fraction than in the bulk sample (Figure 4.8) due to a decrease in M_w from the removal of HMW DNOM during the fractionation. This blue-shift reflects the fact that the DNOM in the LMW fraction is of smaller M_w than the DNOM in the bulk solution. The sUVa is lower in the LMW fraction (Figure 4.6). This is accordance to the fact that the more aromatic moieties are removed as they are of more HMW. Also, the sVISa decreases after 10kDa filtration (Figure 4.7). This is because the longer conjugated double bonds are removed during filtration, as they are found mainly in the HMW moieties of the DNOM. The LMW fraction of the Peat sample had sUVa and sVISa values that were only 65% and 55% of the values in the bulk sample, respectively. The largest decrease in sUVa between the bulk and < 10 kDa fraction was found in the Peat water, due to the removal of more aromatic moieties. This implies that the Peat DNOM has longer conjugated double bonds than the Lake and Soil water DNOM.
4.3.3 Changes in DNOM Characteristics Due to Photo-oxidation

Photo-oxidation alters the DNOM structurally by transformation and mineralisation, causing the DNOM to be less coloured (bleached), reflecting that it becomes less aromatic and of lower MW.

The SAR increased consistently due to photo-oxidation in all samples and size fractions (Figure 4.8). This is caused by the fact that the photo-oxidation breaks down the larger organic compounds into smaller molecules with lower MW. It is especially the double bonds in the conjugated systems that are broken. The shift is larger for the bulk samples (9–27%) than for the LMW fractions (2–9%). This is likely due to that the high molecular weight DNOM is more susceptible to photo-oxidation since there are more chromophores (i.e. conjugated double bonds) absorbing the photon energy. The sUVa decreased due to the photo-oxidation. This is because it is the aromatic chromophore moieties that are photo-oxidised (Weishaar et al., 2003). Likewise, the sVISa decreases due to photo-oxidation. This is because it is the longer conjugated double bond chromophore moieties that are photo-oxidised by visible light with longer wavelengths.

The Fluorescence Excitation and Emission (EEM) spectra of the water samples are presented in Appendix 4. All spectra of the studied DNOM materials are mainly dominated by two distinct peaks: a humic acid peak that exhibits excitation at long wavelengths (λex = 300–345 nm, λem = 400–460 nm), and thus contains more conjugated aromatic compounds representing the HMW fraction of DNOM. In contrast, fulvic acid peaks exhibit emission at short excitation wavelengths (λex = 240–270 nm, λem = 390–470 nm), reflecting that this fraction contains shorter conjugated chains of double bonds (Chen et al., 2003).

Both size fractions (0.2 µm and 10 kDa) exhibit a reduction in intensity of both the humic and fulvic acid peaks when exposed to artificial sunlight. The exception is for Peat DNOM with size fraction < 0.2 µm (P.2). The reduction in both fractions is in accordance with that photo-oxidation alter the DNOM structurally into less aromatic compounds or mineralize the DNOM completely. Peat with size fraction <0.2 µm had an increased intensity in the fulvic acid peak after photo-oxidation. This may be caused by the photo-oxidation process, which breaks down the larger humic acid organic compounds into smaller MW fulvic acids. This is because higher molecular weight moieties of DNOM are more easily photo-degraded than the lower molecular weight fraction (Lepane et al., 2003). The peak ratio (A:B) is presented in the Table 4.3,
reflecting the amount of humic acids (A) relative to fulvic acids (B), is lower in the LMW fractions compared to the bulk sample and in the photo-oxidised DNOM material. This is due to a relatively stronger decrease in the more aromatic humic acid compounds.

Table 4.3. Peak intensities and peak ratio with the locations of the peaks in the fluorescence spectra based on the excitation and emission wavelengths.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak A</th>
<th>Peak B</th>
<th>Peak ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{ex}$</td>
<td>$\lambda_{em}$</td>
<td>Intensity</td>
</tr>
<tr>
<td>P.2</td>
<td>330</td>
<td>456</td>
<td>47</td>
</tr>
<tr>
<td>P.2P</td>
<td>330</td>
<td>436</td>
<td>35</td>
</tr>
<tr>
<td>P10</td>
<td>330</td>
<td>450</td>
<td>49.2</td>
</tr>
<tr>
<td>P10P</td>
<td>330</td>
<td>441</td>
<td>33</td>
</tr>
<tr>
<td>L.2</td>
<td>320</td>
<td>431</td>
<td>36</td>
</tr>
<tr>
<td>L.2P</td>
<td>310</td>
<td>433</td>
<td>20</td>
</tr>
<tr>
<td>L10</td>
<td>320</td>
<td>430</td>
<td>32</td>
</tr>
<tr>
<td>L10P</td>
<td>310</td>
<td>423</td>
<td>19</td>
</tr>
</tbody>
</table>

Figure 4.9. Peak ratio (A:B) calculated from the EEM data illustrating decrease of aromatic compound due to photo-oxidation and size fractionation.

The explanatory variable that was found to best explain the variation in the reduction of UV$_{254}$ absorbency by ultrafiltration of DNOM was the fluorescence peak ratio (A:B), reflecting the amount of humic acids relative to fulvic acids ($R^2 = 0.96$) (Figure 4.10). The higher the relative
amount of humic to fulvic acids, the more DNOM was removed by ultra-filtration through a 10 kDa filter.

![Graph showing correlation between 10 kDa filtration and fluorescence A/B](image)

Figure 4.10. Correlation of percentage reduction of UV$_{254}$ after 10 kDa filtration with fluorescence A:B peak ratio for the samples from Peat and Lake, before and after exposure (53 hours) to artificial sunlight in Q-SUN.

The concentration of inorganic labile aluminium (Ali; $R^2 = 0.82$) (Figure 4.11) and SAR ($R^2 = 0.69$) (Figure 4.12) were also explanatory for the variation in the relative decrease in DNOM by ultrafiltration. The highest decrease in DNOM was found in samples with the highest Ali. Moreover, as expected, the DNOM with the lowest SAR was the material that was most removed by the ultrafiltration.
Figure 4.11. Correlation of percentage reduction of UV$_{254}$ after 10 kDa filtration with concentration of inorganic Al for the samples.

Figure 4.12. Correlation of percentage reduction of UV$_{254}$ after 10 kDa filtration with SAR for the samples from Peat, Lake and Soil, before and after exposure (53 hours) to artificial sunlight in Q-SUN.

The explanatory variable found to best explain the variation in the reduction in UV$_{254}$ absorbency by photo-oxidation of DNOM was the fluorescence peak ratio (A:B), reflecting the
amount of humic acids relative to fulvic acids ($R^2 = 0.96$) (Figure 4.13). The higher the relative amount of humic to fulvic acids, the more DNOM was removed by photo-oxidation. The concentration of Fe (total) was also significantly correlated to the decrease in DNOM ($R^2 = 0.88$) (Figure 4.14). As expected, the DNOM with the highest Fe was the material that was most removed by photo-oxidation. Also, the SAR was found to be significantly correlated with the removal of DNOM by photo-oxidation ($R^2 = 0.80$).

![Photo-oxidation vs. Fluoresence A:B](image)

Figure 4.13. Correlation of percentage reduction of $UV_{254}$ after photo-oxidation with fluorescence A:B peak ratio for the samples from Peat and Lake.
Figure 4.14. Correlation of percentage reduction of UV$_{254}$ after photo-oxidation with Fe (total) for the samples from Peat, Lake and Soil.

4.3.4 Biodegradation

This experiment was done to assess the effect of size fractionation and photo-oxidation on the biodegradability of DNOM. Microorganisms that consume DNOM as a source of energy and nutrients prefer LMW DNOM. HMW fractions of DNOM tend thus to be more refractory. Likewise, the photo-oxidation is conceived to decrease the molecular weight and thus render the material more bioavailable. On the other hand, the material becomes more oxidised and thus less energy rich for the bacteria.

The biodegradation results were presented as rates relative to the reference sample in Figure 4.15.

The biodegradation of DNOM in Langtjern has been studied in a set of master studies. It was demonstrated that run-off episodes due to heavy precipitation events influence the concentration and size characterisation of DNOM. This area has a mix of mineral soil and bogs. During low flow, the water supplied from the stream originated primarily from the water with saturated peats. The peat contain high molecular weight DNOM with a high degree of aromaticity. Continued precipitation caused an increase in DNOM concentration, but most of
the DNOM introduced had a lower molecular weight character and had less aromatic character (Håland, 2017). The DNOM in the mineral soils has lower molecular weight and is less aromatic than the DNOM coming from the bogs feeding the runoff during baseflow. During a heavy rain, water flushed from the mineral soils and over the peat caused the increased in soil DNOM. This caused a shift in the overall contribution of DNOM with lower aromaticity, less molecular weight and less colour.

Figure 4.15. Biodegradability of the DNOM for the samples presented as relative to the glucose reference.

The relatively more aromatic, hydrophobic and HMW DNOM from the Peat is less bioavailable than the DNOM in the mineral Soil water sample. This is because microorganisms have limited ability to degrade aromatic compounds and HMW DNOM than the relatively more aliphatic, hydrophilic and LMW DNOM (Marschner et al., 2003) found to more prevailing in the mineral Soil water sample. Biodegradability was likewise found to have a strong negative correlation with sUVa values found in the Peat and Lake samples ($R^2 = 0.86$ and $0.94$, respectively). Strong negative correlations were also found between the biodegradability and sVISa values of the Peat and Lake samples ($R^2 = 0.90$ and $0.85$, respectively).

The biodegradability of the DNOM from the Lake sample was relatively low, even though it has the lowest molecular weight, i.e. highest SAR. This is likely due to the long residence time
in the large lake, allowing for an advanced degradation of the non-refractory moieties of the material.

Biodegradability is greater in the 10 kDa fraction than in the bulk sample. This is in accordance with that the more LMW moieties are relatively more biodegradable than the more HMW fraction. In addition this LMW DNOM has low sUVa implying low aromaticity.

The biodegradability of the DNOM from the Peat is higher after photo-oxidation. This is in accordance with the hypothesis that refractory humic compounds are partly converted to more bioavailable compounds. LMW moieties of the DNOM in the mineral Soil water respond less to photo-oxidation because they have lower content of long conjugated double bonds (low sUVa and sVISa).

Due to the long residence time, DNOM found in the Lake sample do not respond strongly to photo-oxidation. The lake functions as a large photo-reaction tank, which means that the DNOM has already been heavily photo-oxidised.

Bioavailability of the DNOM found in the Soil water sample is very high and it does not show much differences between size fractions nor due to photo-oxidation. Rather surprisingly, the biodegradability of Soil DNOM was greater than for the reference (glucose). This can possibly be explained by a process referred to as Priming Effect (PE) (Dijkstra et al., 2007). The effect is seen when the biodegradability of organic matter increases or decreases with the addition of other organic matter. For example, when bacteria are faced with recalcitrant DNOM, the bacteria struggle to grow and subsequently produces a low biodegradation rate. When labile DNOM is added to the recalcitrant DNOM, the bacteria will grow faster with the help of the labile fraction, allowing for a higher number of bacteria. The increased amount of bacteria will then increase the biodegradation of both fractions. The exact mechanism behind PE is not fully understood, but since PE has never been observed in sterile samples, it is believed to be microbial mediated (Catalán et al., 2015).
5 Conclusion

Proxies (sUVa, sVISa and SAR) for DNOM characteristics indicate that DNOM from Peat is more aromatic, has higher $M_W$ and is of darker colour than the DNOM materials from a mineral Soil dominated watershed and a large Lake. Lake DNOM is least aromatic, has lowest $M_W$ and is of lighter colour than the DNOM materials from other sources.

The LMW DNOM fraction (i.e. < 10 kDa) in Peat water constituted least of the total DNOM, implying that it is composed of higher $M_W$. The colour in the LMW fraction was also less than in the bulk sample due to the removal of longer conjugated double bonds. Photo-oxidation caused a reduction in DNOM concentration. The reduction is stronger in HMW than in the LMW fractions. This is because the higher $M_W$ DNOM sample is more susceptible to photo-oxidation, since more chromophores are available to absorb the photon energy.

The humic and fulvic acid peaks in fluorescence EEM spectra of both size fractions (0.2 µm and 10 kDa) exhibit a reduction in intensity when exposed to artificial sunlight. The exception is for Peat DNOM with size fraction < 0.2 µm (P.2). In this sample the peak representing fulvic acid-like compounds increased after being exposed to sunlight, indicating that photo-oxidation transformed the more aromatic and larger $M_W$ humic acids to the less aromatic and smaller fulvic acids.

The spectroscopic proxies of DNOM confirmed that photo-oxidation alters the DNOM structurally by mineralisation and transformation, causing the DNOM to be less coloured and thus less aromatic and with lower $M_W$.

The more aromatic, hydrophobic, and HMW DNOM in the Peat water sample was refractory compared to the other samples. However, the biodegradability of the DNOM from the Peat was higher after photo-oxidation. This is in accordance with the hypothesis that refractory DNOM are partly decomposed to more bioavailable compounds. The biodegradability of the DNOM from the Lake sample was relatively low, even though it has the lowest molecular weight, i.e. highest SAR. This is likely due to the long residence time in the large lake, allowing for an advanced bio-degradation of the non-refractory moieties of the material.

The biodegradability experiment confirmed that the relatively more aliphatic and LMW (i.e. 10 kDa) DNOM fraction samples were the most bioavailable. These results confirmed that the
microorganisms prefer to consume smaller $M_w$ DNOM with relatively more aliphatic structures. This supports previous studies by Marschner et al. (2003) and is in accordance with the hypothesis that LMW DNOM are more bioavailable than HMW DNOM.

It was also found that the higher iron content in the samples correlated with lower biodegradation rates. This may partly be due to that both are co-varied with the influence of bog in the watershed, as DNOM from peat has high content of Fe and has a low biodegradability.
6 Recommended Future Work

Having more refined fractionations and a larger variation in the photo-oxidation experiments would be an interesting study to perform. For example, fractionating the samples more (for example, at less than 100 kDa and less than 10 kDa) may reveal more about the structure and characteristics of DNOM in each fraction. Doing longer photo-oxidation experiments at specific intervals (for example, at 24, 48, 72 hours, etc.) may also show how DNOM reacts to both short- and long-term exposure to sunlight, and changes in the seasons.

Exploring other fractionation methods may also be helpful. The method used in this study allowed for the collection of the LMW DNOM fraction, but not the HMW fraction which remained in the filter. Being able to collect both HMW and LMW fractions may provide a better idea as to the biodegradability and structure of the DNOM in each fraction.

Finally, collecting more samples from different catchment areas and at different periods of the year may provide a better idea as to the spatial and temporal trends in DNOM bioavailability and structure.
References


## Appendix

### A.1 DOC and UV-Vis Results

Table A.1. Sample DOC and UV absorbencies at 254 and 400 nm, with calculated sUVa, sVISa, and SAR values. sUVa* and sVISa* are corrected values for the Soil samples, multiplied by a factor of 4.74. %\textsubscript{254-F} and %\textsubscript{254-P} are the calculated percentage reduction in UV\textsubscript{254} absorbance after fractionation and photo-oxidation, respectively.

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<th>UV\textsubscript{400}</th>
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<th>sUVa*</th>
<th>sVISa</th>
<th>sVISa*</th>
<th>SAR</th>
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### A.2 Major Cations and Anions

Table A.2. Major cations analysed using ICP-OES, with H\textsuperscript{+} calculated from sample pH.

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<th>K\textsuperscript{+} (mg·L\textsuperscript{-1})</th>
<th>Mg\textsuperscript{2+} (mg·L\textsuperscript{-1})</th>
<th>Mg\textsuperscript{2+} (μeq·L\textsuperscript{-1})</th>
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58
Table A.3. Major anions analysed using ion chromatography.

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Table A.4. Major anions (in μeq L⁻¹) with calculated contributions of DOC and DIC.

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### A.3 Al/Fe Fractionation

Table A.5. Results from the Al/Fe fractionation experiments. Al (total)* and Fe (total)* are the results from the ICP-OES analysis.

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<th>Al&lt;sub&gt;i&lt;/sub&gt; µmol·L&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Al (total) µmol·L&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Al (total)* µmol·L&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Fe&lt;sub&gt;0&lt;/sub&gt; µmol·L&lt;sup&gt;-1&lt;/sup&gt;</th>
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<th>Fe (total) µmol·L&lt;sup&gt;-1&lt;/sup&gt;</th>
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A.4. Fluorescence EEM Spectroscopy

Figure A.1. Fluorescence EEM spectra of the Peat samples.
Figure A.2. Fluorescence EEM spectra of Lake samples.

Figure A.3. Fluorescence EEM spectra of Soil samples.
A.5 Biodegradation

Table A.6. Biodegradation rates for the reference glucose and DNOM samples. Biodegradation* is the biodegradation rate of the DNOM sample relative to the reference glucose sample.

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