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Solid Phase Extraction as a Simple Method to Measure and Monitor Trihalomethane Precursors in Surface Waters

By

Charles D. Goss

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Department of Civil Engineering

University of Manitoba

Winnipeg, Manitoba, Canada

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

This study investigated the isolation of hydrophobic (HPO) dissolved organic matter (DOM) using solid phase extraction (SPE) as an alternative method to the most commonly applied method using the XAD-8 resin. The objective being SPE is a simple method that could be suitable for monitoring seasonal changes in DOM composition and for evaluating water treatment performance by following the removal of trihalomethane (THM) precursors. This research was conducted in three phases.

In Phase I, seven SPE cartridges: Bond Elute ENV (ENV), Bond Elute Plexa, Bond Elute PPL (PPL), Strata X, C18-E, C18-U, and Oasis HLB were tested against the XAD method for their ability to isolate HPO DOM. Quantitative and qualitative analyses were conducted to determine if the SPEs tested isolated a similar HPO fraction as the XAD method. The results from Phase I found the Strata X, ENV and PPL isolated an HPO fraction comparable to the XAD method. As a result, these three SPEs were considered applicable for field testing of natural surface waters.

Phase II measured the HPO character in three surface waters: Lake Winnipegosis, the Waterhen River and the La Salle River over a 13-month period using ENV, PPL and Strata X. The results found all three SPEs isolated an HPO fraction; however, the Strata X isolated more DOM compared to ENV and PPL. FTIR results indicated that Strata X, and to some extent PPL, isolated some hydrophilic DOM. Phase II measured the THM formation potential (THMFP) and Specific THM formation potential (STHMFP) of fractions isolated using ENV, PPL and Strata X. The results concluded the HPO fraction isolated by each SPE had greater THMFP and STHMFP than the HPI fraction.

Of the three SPE studied in Phase II, ENV was found to be the most appropriate for Phase III, onsite testing by water treatment operators. The results from Phase III indicated that ENV was suitable

for monitoring by operators, and that valuable information was gained from monitoring the removal of THM precursors using ENV.

The objective of this research was attained, demonstrating the value of monitoring THM precursors onsite using SPE, particularly ENV.

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Lastly, and most importantly, I would like to thank my wife, *Ms. Lesley Goss,* and my sons, *Thoren and Caspian*, for supporting me and being my inspiration.

Thank-you.

Dedication

I would like to dedicate this work to those who live their day-to-day life without access to clean water.

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List of Abbreviations

Al^{3+}	Aluminum (III)
°C	Degree Celsius
µg/L	Microgram per litre
µgTHM/mgDOM	Microgram trihalomethane per milligram dissolved organic matter
µm	Micrometer
^{13}C-NMR	Carbon 13 Nuclear Magnetic Resonance
4CL	4-coumarate:COA ligase
Å	Angstrom
AHS	Aquatic Humic Substances
ALT	Alanine aminotransferase
AMF	Arbuscular mycorrhiza fungi
AOM	Aquatic Organic Matter
ATR	Attenuated Total Reflectance
BDCM	Bromodichloromethane
C3H	p-coumarate 3-hydroxylase
C4H	Cinnamate 4-hydroxylase
Ca^{2+}	Calcium ion
$CaCO_3$	Calcium Carbonate
CAD	Cinnamyl alcohol dehydrogenase
CCoAOMT	Caffeoyl-CoA o-methyltransferase
CCR	Cinnamoyl-CoA reductase
CIP	Clean in Place
cm^{-1}	Reciprocal centimeters or wave numbers
CMC	Critical Micelle Concentration
CO_2	Carbon dioxide
COMT	Cafferic acid o-methyltransferase

COOH	Carboxyl
C_{org}	Organic Carbon
Da	Daltons
DAF	Dissolved Air Flotation
DBCM	Dibromochloromethane
DBP	Disinfection By-product
DDT	Dichlorodiphenyltrichloroethane
DI	Deionized
DNA	Deoxyribonucleic acid
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
ECD	Electron Capture Detection
ENV	Bond Elute ENV
F5H	Ferulate 5-hydroxylase
FA	Fulvic Acid
Fe^{3+}	Iron (III)
FPA	Focal Plane Array
FTIR	Fourier Transform Infrared Spectroscopy
G	Guaiacyl
GAC	Granular Activated Carbon
GC	Gas Chromatography
Gt	Gigaton
H	*p*-hydroxyphenyl
HA	Humic Acid
HAAs	Halogenated Acetic Acids
HCl	Hydrochloric Acid
HCT	p-hydroxycinnamoly-CoA:Quinate/Shikimate p-hydroxycinnamoyltransferase

HPI	Hydrophilic
HPIA	Hydrophilic Acid
HPIB	Hydrophilic Base
HPIN	Hydrophilic Neutral
HPO	Hydrophobic
HPOA	Hydrophobic Acid
HPOB	Hydrophobic Base
HPON	Hydrophobic Neutral
HPSEC	High Pressure Size Exclusion Chromatography
IHSS	International Humic Substances Society
IR	Infrared
K_D	Distribution Coefficient
kDa	Kilodaltons
km	Kilometers
L	Litre
LMS	Linear multistage
LOAEL	Lowest-observable-adverse-effect-level
LPSEC	Low Pressure Size Exclusion Chromatography
m	Meter
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
mg	Milligrams
mg/kg	Milligram per kilogram
mg/kg/day	Milligram per kilogram per day
mg/LM	Milligram per litre
Mg^{2+}	Magnesium ion
MIEX	Magnetic Ion Exchange Resin

mL/min	Millilitre per minute
mm	Millimetres
mmHg	Millimetres mercury
MS	Mass Spectrometer
mV	Millivolt
MW	Molecular Weight
MWTP	Macdonald Water Treatment Plant
MX	3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone
N/A	Not Applicable
N_2	Nitrogen
Na^+	Sodium ion
NaOH	Sodium Hydroxide
NCI	National Cancer Institute
NH_2	Amine
NH_4^+	Ammonium
nm	Nanometer
NO_3^-	Nitrate
NOAEL	No-observable-adverse-effect-level
NOEL	No-observable-effect-limit
NOM	Natural Organic Matter
NPV	*N*-vinylpyrrolidone
NR	No Result
NTU	Nephelometric Turbidity Unit
NVP	*N*-vinylpyrrolidone
OCH_3	Methoxy
OH	Hydroxy
OR	Odds Ratio

PACl	Polyaluminum Chloride
PAL	Phenylalanine ammonia-lyase
pKa	Acid dissociation constant
POC	Particulate Organic Carbon
POM	Particulate Organic Matter
PPL	Bond Elute PPL
RfD	Reference Dose
RO	Reverse Osmosis
RR	Risk Ratio
RSC	Relative Source Contribution
S	Syringyl
SDH	Sorbitol dehydrogenase
SDWA	Safe Drinking Water Act
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscope
SOM	Soil Organic Matter
SPE	Solid Phase Extraction
SR	Suwannee River
Stage 1-DBPR	Stage 1 Disinfectants/Disinfection By-product Rule
Stage 2-DBPR	Stage 2 Disinfectants/Disinfection By-product Rule
ST-DVB	Styrene Divinylbenzene
STHMFP	Specific Trihalomethane Formation Potential
SUVA	Specific UV Absorbance
TCU	True Colour Unit
THMFP	Trihalomethane Formation Potential
THMs	Trihalomethanes
TOC	Total Organic Carbon

TOX	Total Organic Halogen
UF	Ultrafiltration
USA	United States of America
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UV_{254}	Ultraviolet light at 254 nm
WHO	World Health Organization
WTP	Water Treatment Plant
WWTP	Waterhen Water Treatment Plant

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Chapter 1: Problem Statement and Research Objectives

1.1 Problem Statement

In the last two decades there has been interest in the removal of dissolved organic matter (DOM) fractions by water treatment processes to better control the formation of disinfection by-products (DBPs), as well as, understanding the impact of specific DOM fractions on treatment processes. There have been studies investigating the removal, or effect, of DOM fractions by treatment processes such as coagulation (Musikavong et al., 2005), ozonation ((Marhaba & Van, 2000), filtration (Schafer et al., 1998; Collins et al., 1996) and chlorination (Singer, 1999; Reckhow et al., 1990). However, the composition of DOM is strongly influenced by the local water conditions such as pH, alkalinity, temperature, salinity, microbial presence, as well as precipitation and runoff which alter the physical-chemical character of DOM (Chow, 2005). Therefore, a logical statement is that the removal of DOM by water treatment processes, and the formation of DBPs, are dependent on the chemical characteristics of the local DOM. Consequently, understanding the changes in the DOM composition in a surface water source could improve the operation of a water treatment facility and the quality of finished water. However, the problems associated with the most common DOM fractionation method (i.e., the XAD method) is that the XAD method is labor intensive and requires specialized equipment and training limiting its use to research settings (APHA, 2017). As a result, most water treatment plant (WTP) operators only test for the total organic carbon (TOC) or dissolved organic carbon (DOC) concentration and know little of the chemical characteristics of local organic matter. In turn, the approach to control DBPs by most WTPs is through process optimization to remove total DOM and is not typically designed to target specific fractions of DOM found to have a greater potential to form DBPs, such as trihalomethanes (THMs). If a simple and rapid fractionation method was made available, WTP operators could apply the method to measure the composition of

organic matter in their source water allowing for optimization of treatment processes that target the removal of problematic DOM fractions. Furthermore, if the DOM composition was measured over a long period of time the results could be used to identify trends in seasonal changes in the DOM composition providing insight as to when problematic fractions are in greater concentration. The information could also be used to guide the design of a new WTP to specifically target DOM fractions which negatively impact treated water quality.

There is a need for a simple method to quantitatively isolate the hydrophobic DOM fraction from natural waters that would be suitable as a simple test for WTP operators to monitor the seasonal changes in DOM composition as well as the reduction of THM precursors during treatment.

1.2 Research Objectives

The objective of this research is to identify a simple and rapid solid phase extraction (SPE) method to extract the hydrophobic (HPO) fraction of DOM. The goal of this research is that the SPE method would be applied over the common HPO fractionation method (i.e., the XAD-8 Method), and that the method would provide WTP operators and engineers with a simple tool to measure and monitor the composition of DOM in a source water.

DOM characterization could be measured throughout the year gaining seasonal information of the changes in DOM character. The information gained could be used by engineers to guide the design, optimization, or upgrade, of systems in a WTP system treating surface waters high in DOM (i.e., coagulation or membrane filtration). For a rapid fractionation method to be applicable in a WTP scenario the following criteria should be met:

1. Short experimental run times, 2-4 hours total would be ideal.
2. Minimal sample preparation. This should be limited to filtration and pH adjustment.

3. Limited specialized equipment. Ideally one SPE cartridge, two flasks (sample and receiving), pH meter, Teflon® tubing, and a pump would be the only equipment required. A TOC analyser would be beneficial, but this can be substituted by preserving the sample and sending it to an accredited chemical analysis company (i.e., ALS Laboratories).
4. Limited solvents required. Ideally one for SPE conditioning, and one for extraction (although not necessarily required for HPO concentration determination) as well as distilled water with DOC concentration <10 $\mu g\ L^{-1}$.
5. Is simple to conduct and does not require the need to extensive specialized training.

In order to meet the objective, this research study was separated into three phases. The following sections will present the major goals of each phase of the research.

1.2.1 Phase I

The goal of Phase I is to determine a suitable SPE, or SPEs, that isolates the HPO fraction of DOM that is comparable, quantitatively and qualitatively, to the XAD Method. Suitable SPE candidates would also be evaluated for their application as a monitoring method for WTP operators by comparing sample testing time and materials required to conduct the test. Suitable SPE candidates identified in Phase I would be carried forward into Phase II of the study.

1.2.2 Phase II

Phase II was designed to evaluate the ability of the SPE candidates identified in Phase I to monitor seasonal changes in the composition of DOM in natural surface waters, as well as the changes in DOM composition following potable water treatment processes (e.g., coagulation or filtration). SPE candidates would also be evaluated for their ability to isolate the fraction of DOM most prone to form THMs. The goal of Phase II was to identify the most suitable SPE candidate, based on the results from Phase I and II, to further undergo onsite testing by water treatment plant operators.

1.2.3 Phase III

The final phase of this research was to have water treatment plant operators conduct the SPE testing onsite. The goal of this was to evaluate if the SPE method was simple enough for operators to conduct, and that the test did not interfere with general day-to-day operations. Additionally, Phase III assessed if the data obtained by the operators was valuable in understanding compositional changes in the DOM character of their source water.

The overall goal of this research was to identify a suitable SPE that would measure HPO DOM, and that water treatment operators could apply the test on a weekly, monthly, or quarterly basis to evaluate the DOM composition of their source water. Furthermore, the intent is that this method could be applied to evaluate treatment processes, gaining a better understanding of which processes are removing HPO organic matter. Lastly, a rapid SPE method for the determination of the concentration of the HPO fraction could be used in conjunction with DBP formation potential testing, allowing operators to gauge the efficiency of the system for reducing THM precursors. Although it is strongly believed that the HPO fraction contain the main precursors to THM formation (Singer, 1999), there is evidence in the literature suggesting other fractions are capable of forming THMs (Goss & Gorczyca, 2013; Hwang *et al.*, 1999). The differences in THM formation seen between the HPO and non-HPO fractions is likely attributed to the composition of DOM which is affected by the local environment. Therefore, if treatment plant operators fractionate local DOM collected from the source water and analyze each fraction for THM formation potential (THMFP) they would know what fraction in their water source forms THMs to the greatest extent. This information, in combination with the understanding of organic matter fraction removal by their treatment train, could be used to better control the formation of THMs. To determine THM concentration requires the use of

specialized equipment (i.e., gas chromatography equipped (GC) with a mass spectrometer (MS) or electron capture detection (ECD)). However, the sample could be fractionated on site, preserved, and sent to an accredited laboratory for THMFP analysis. Developed successfully, a rapid SPE method will allow water treatment plant operators and engineers to make more informed decisions when trying to improve the removal of DOM to control the formation of harmful DBP's, such as THMs.

1.3 Thesis Structure

Chapter 2: Literature Review

Chapter 2 provides an in-depth review of the formation of humic matter in the natural environment (terrestrial and aquatic), including the degradation of plant matter and the subsequent formation of humic and non-humic organic matter fractions. This chapter also presents discussion of the impact of DOM and DOM fractions on water treatment systems and drinking water quality, with focus given to the formation and health implications of THMs. Lastly, this chapter discusses methods for controlling THM formation in potable water systems.

Chapter 3: Experimental Methods

This chapter introduces the experimental methods, design and procedures used to achieve the outlined research objectives and goals of the study.

Chapter 4: Results and Discussion

Chapter 4 presents the experimental results and interpretations of the data. This chapter provides a discussion of the results obtained during this study and how they pertain to the objective and goals of this research.

Chapter 5: Future Work and Engineering Significance

Chapter 5 presents future research directions based on the results and outcomes of the current study. This chapter also outlines the significance this current research brings to the area of civil and environmental engineering.

Chapter 6: Conclusion

The final chapter to this thesis will provide concluding remarks regarding the results and interpretation of the data collected and outcomes regarding the research objectives and goals.

Chapter 2: Literature Review

2.1 Natural Organic Matter in the Environment

Natural Organic Matter (NOM) is a general term to define the complex mixture of live and degraded materials from all living things on Earth (Tan, 2014). NOM is an important parameter to environmental scientists as it plays a critical role in the global carbon cycle and accounts for one of the largest active carbon pools on the planet (624 Gt; in terms of dissolved organic matter), comparable to atmospheric carbon dioxide (CO_2) (750 Gt) (Sandron, et al., 2015). NOM is transported through the environment by the hydrologic cycle where terrestrial soil organic matter (SOM) is added to surface and ground water following precipitation and surface runoff (Baghoth, et al., 2008). Once in the aquatic environment, allochthonous and autochthonous NOM will undergo biological, chemical, and physical transformations making it difficult to measure the composition and model the interactions, reactivity, and fate of NOM in the environment.

The research presented in this thesis focuses on measuring the composition of dissolved NOM in surface waters to show the benefit this knowledge could have on drinking water treatment and the quality of treated water. The composition of NOM in aquatic environments is heavily influenced by allochthonous NOM from the surrounding terrestrial environment (Tan, 2014). Therefore, it is important to understand the chemistry involved in the formation of soil humic matter following the degradation of living plant matter, and the transport of allochthonous humic and non-humic matter to the aquatic environment, to adequately address the concerns regarding NOM in water treatment (Leenheer & Croué, 2003). Given the connection between terrestrially derived NOM and the composition of NOM in the aquatic environment, the focus of the following sections will be to review

the mechanisms involved in the formation of humic matter in the terrestrial environment (i.e., humification) in order to better understand the composition of NOM in the aquatic environment.

2.1.1 Soil Organic Matter

For more than 200 years, agricultural scientists have studied the occurrence of natural organic compounds in soils (Wershaw, 2000). It was recognised that SOM improved the fertility of agricultural soils, as well as increased the soil's adsorption capacity and retention of water (Tan, 2014; Wershaw, 2000). SOM has been shown to improve overall soil structure by lowering bulk density values and increasing cation-exchange capacity, while also providing an essential nutrient, nitrogen (N_2), to growing plants (Tan, 2014).

SOM is largely composed of dead biomass along with living plant roots and microbial biomass; although there is some disagreement on the inclusion of living root systems and microbial biomass as part of true SOM (Tan, 2014). While animal remains are present in SOM, this fraction is largely ignored due to its overall minimal contribution to SOM (Wershaw, 2000). Therefore, SOM is mainly composed of degraded plant material which can be divided into two groups: (1) partially decomposed plant material and (2) completely decomposed material. Organic matter in Group (1) would be at various degrees of decomposition; however, the overall plant morphology would still be visible. This would include plant litter, which is the most undecomposed fraction of SOM consisting of recently fallen plant materials (e.g., branches or leaves). Litter is an important component to SOM as it plays a role in nutrient cycling and the physical properties in forest and agricultural soils (Tan, 2014; Stevenson, 1994). Although there is disagreement with considering plant litter as a component of SOM (Tan, 2014); the present author tends to side with those of the opinion that because plant litter is the primary source of SOM it should be included as a component.

In U.S. Soil Taxonomy, plant litter is not included in SOM; however, it is described as part of the soil profile and is included in the O horizon (Tan, 2014). The O horizon is subdivided into O, Oi, Oe, and Oa sub horizons (Figure 1), which is a very simple way to begin to view the decomposition of plant material in the terrestrial environment. The O sub horizon is composed of the fresh fallen plant material. The next sub horizons, the Oi and Oe sub horizons, are composed of organic matter at various stages of decomposition from slightly decomposed to intermediately decomposed organic matter (Tan, 2014). The first three sub horizons have also been given the term the light fraction and is known for having rapid turnover and serves as a main nutrient source for plants (Stevenson, 1994). The organic matter in Group (2) is that of fully decomposed organic material and would no longer resemble anatomical structures of the original plant matter. This group or fraction is commonly referred to as humus, or humic matter, and is found in the Oa sub horizon (Tan, 2014). The humic fraction of SOM is formed through a process known as humification and will be discussed in greater detail in the following sections.

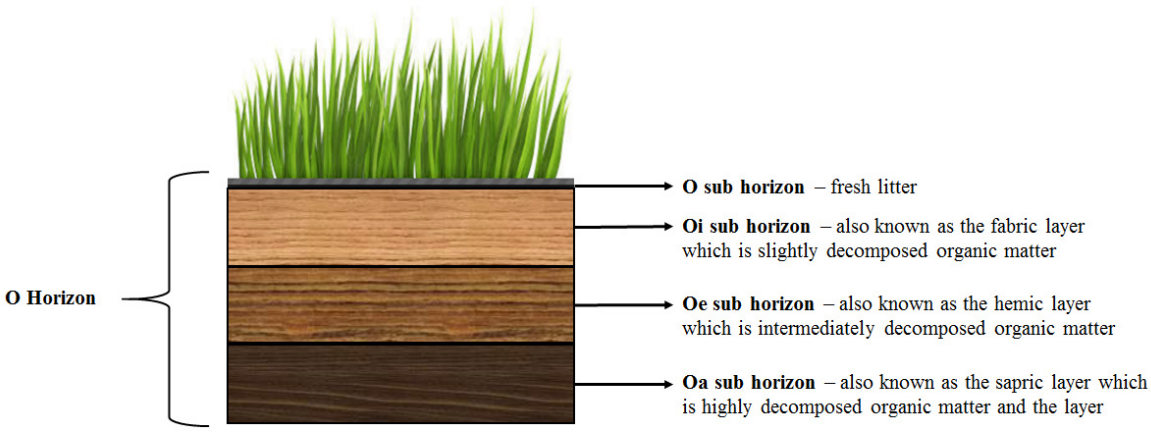


Figure 1: The soil O horizon and sub horizons O, Oi, Oe, and Oa.

2.1.2 Humic Matter

The concept of what constitutes soil humic matter is one of long-standing controversy likely due to the fact that its study spans several fields (i.e., agricultural and soil science, biology, ecology, environmental chemistry, limnology, hydrology, and geology) (Tan, 2014). Scientists across many of these fields have attempted to define humus or humic matter which has caused confusion and controversy to the subject of what actually constitutes humic matter (Tan, 2014). Currently, there is no universally accepted definition for humus or humic matter with the two terms often being used interchangeably (Tan, 2014; Wershaw, 2000). However, there are some that maintain the two should be viewed differently, where humus is thought of as a mixture of both a humic, or humified, and a non-humified fraction (Tan, 2014; Stevenson, 1994). The present author tends to agree with the latter view, that soil humus is a mixture of humic and non-humic matter.

The non-humic fraction contains all the residues of plants and other organisms which are released by decomposition while maintaining distinguishable chemical structures, along with definite chemical and physical properties. Common compounds released during decomposition include carbohydrates, amino acids, lipids, waxes, proteins, nucleic acids and lignin (Tan, 2014; Stevenson, 1994). These compounds undergo further degradation in soil and serve as the main precursors to the formation of the humic fraction, via humification (Tan, 2014). Therefore, to understand the process of humification one must first review the formation of the non-humic fraction of humus mainly resulting from the degradation of living plant material.

2.1.3 Non-humic Fraction

The non-humic fraction of SOM includes decomposition products released from the residues of plants and other organisms. It is hypothesised that these compounds serve as precursors to the humic fraction

through the process of humification. The following section will review the degradation of plant matter forming the major components of the non-humic fraction in order to better review the proposed humification processes currently presented in literature.

2.1.3.1 Carbohydrates

Carbohydrates are produced in plants through photosynthesis and are the main building blocks to the structural components of plant tissues, such as cellulose, hemicellulose, and pectin (Tan, 2014; Martens & Loeffelmann, 2002; Stevenson, 1994). Carbohydrates are compounds that form polyhydroxy aldehydes or ketones following hydrolysis (Tan, 2014; Stevenson, 1994; Thurman, 1985). Carbohydrates, also known as saccharides, can be classified into three groups: monosaccharides, oligosaccharides, and polysaccharides (Tan, 2014). Monosaccharides, or simple sugars such as glucose and fructose, are the monomeric units for oligosaccharides (between 2-10 monomeric units) and polysaccharides (>10 monomeric units) (Figure 2). Carbohydrates are the first component of plant material that will be degraded in the environment mainly by soil microorganisms (Tan, 2014; Stevenson, 1994). Mono and disaccharides are the primary food source for microbes which breakdown these sugars through aerobic and anaerobic reactions (Reactions 1 & 2) (Tan, 2014). Another possible microbial degradation product is ethanol (C_2H_5OH) via fermentation (Reaction 3) (Tan, 2014).

Aerobic: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$ (Reaction 1)

Anaerobic: $C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2$ (Reaction 2)

Fermentation: $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 3CO_2$ (Reaction 3)

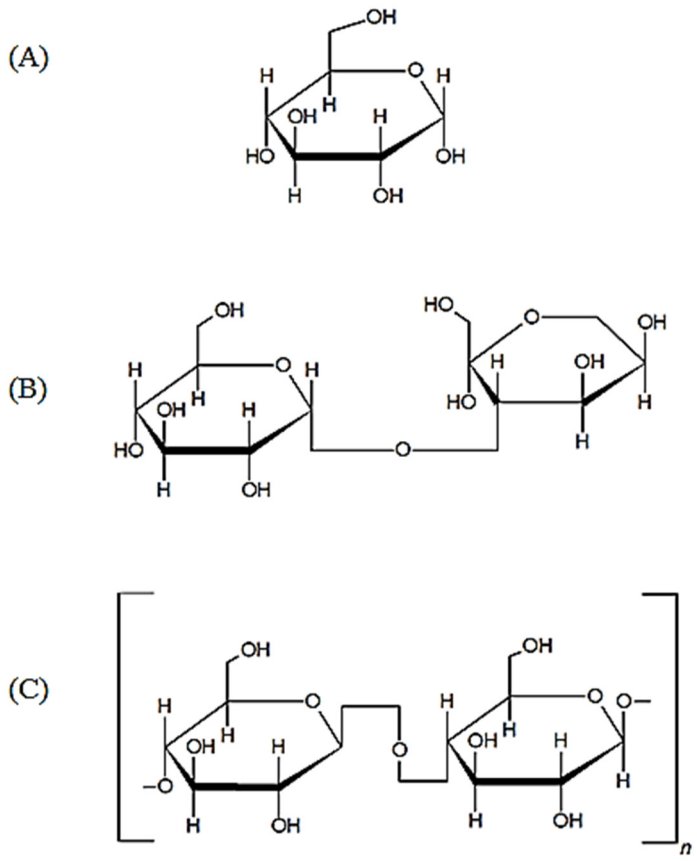


Figure 2: Examples of (A) monosaccharide (glucose), (B) oligosaccharide (disaccharide; sucrose) and (C) polysaccharide (cellulose). Figures recreated from (Tan, 2014; Stevenson, 1994; Thurman, 1985). Permission was obtained by the Copyright Clearance Center Inc. See Appendix C for copyright permission.

Due to preferential attack and degradation of mono and oligosaccharides by soil microbes it is believed that simple sugars are less likely to be found in soils long enough to be incorporated into the humic molecule. In contrast, polysaccharides are found to accumulate in soils due to greater resistance to enzymatic attack resulting from greater branching in the molecular structure, which increases the likelihood these compounds will undergo humification, becoming part of the humic fraction. However, Tan (2014) points out that mono and oligosaccharides could escape microbial degradation and accumulate in soils. Mono and oligosaccharides could become trapped in intermicellar spaces of expanding clay minerals or could become complexed with toxic metals in the soil, making them

inaccessible to microbial enzymatic attack. Furthermore, incomplete degradation of polysaccharides would lead to the formation of mono and oligosaccharides allowing for the possibility of their inclusion into the humic fraction (Tan, 2014). Tan also notes some disagreement as to whether saccharides are core molecules in the formation of humic compounds, or if they are only attached as side chains to the humic molecule. Tan points out that the developments in the early 20th century by Maillard (1916), who was able to synthesize humic matter from simple sugars, along with the more recent advances in understanding the formation of aquatic humic substances (AHS), suggest that saccharides are major precursors in forming humic matter (Tan, 2014).

Amino sugars are also considered to be important precursors to humic matter. Amino sugars are compounds that consist of a simple sugar with substituted amino groups (e.g., glucosamine; Figure 3). Amino sugars are found in mucoproteins and mucopolysaccharides in eggs and saliva, as well as in the mucous layer surrounding bacterial cell walls (Tan, 2014; Stevenson, 1994). Fungi mycelia also contain amino sugars in the form of chitin (N-acetyl-D-glucosamine; Figure 3) which strengthens the structure similar to cellulose in higher plants (Stevenson, 1994). In soil, they serve as sources of nitrogen for both plants and microbes, as well as play a role in stabilizing soils. Amino sugars can react with phenols and quinones forming what would be considered a basic humic molecule (Tan, 2014). The inclusion of amino sugars into the humic molecule will be further examined in *Section 2.1.4.1 Formation of Humic Matter in Soils: Humification.*

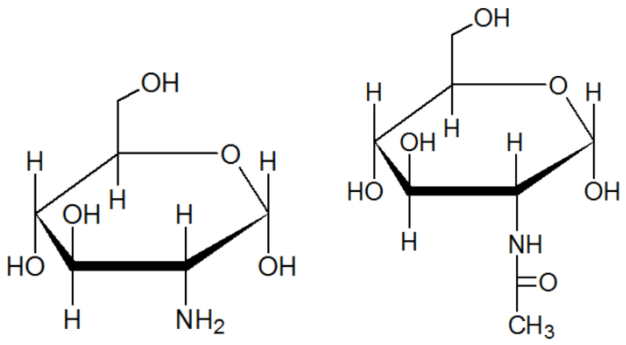


Figure 3**:** Example of an amino sugar (glucosamine; left) and chitin monomer (right). Figure recreated from (Stevenson, 1994). Permission to use this figure was obtained from John Wiley and Sons via the Copyright Clearance Center Inc. See Appendix C for copyright permission.

2.1.3.2 Lignin

Lignin is a general term for a group heterogeneous highly aromatic polymers of phenyl propane formed from the oxidative coupling of 4-hydroxyphenylpropanoids (Tan, 2014; Vanholme, et al., 2010; Whetten & Sederoff, 1995). Lignin, associated with cellulose and hemicellulose, in green vascular plants serves to increase the strength and stability of plant cell walls, allowing plants to stand upright (Tan, 2014; Li & Chapple, 2010). Lignin increases the hydrophobicity of plant cell walls preventing the intrusion of external water, while allowing xylems to transport water within the plant (Li & Chapple, 2010). Along with strengthening cell walls, lignin also protects plant cell wall polysaccharides from microbial attack and degradation (Tan, 2014). Due to the large presence of green vascular plant life on Earth; lignin, along with cellulose and chitin, are among the most abundant natural polymers on this planet (Alfaro, et al., 2014; Whetten & Sederoff, 1995).

The degradation of lignin in the natural environment is of considerable interest to the field of soil science, as lignin and its degradative products are the most important source to the formation of humic matter (Tan, 2014). Therefore, special attention will be given to the formation and degradation of lignin in the terrestrial environment.

Lignin is derived from the polymerization of coniferyl alcohol, para-coumaryl alcohol, and sinapyl alcohol, termed monolignols (Figure 4) (Whetten & Sederoff, 1995). The first step in forming monolignols is the aromatization of nonaromatic carbohydrates to phenylalanine via the Shikimate Biosynthetic Pathway, also known as the Shikimic Acid Pathway (Figure 5) (Tzin & Galili, 2010; Herrmann, 1995; Botting, 1993). Phenylalanine then undergoes a deamination reaction, catalyzed by phenylalanine ammonia-lyase, forming cinnamate as the first step in the synthesis of monolignols along the Monolignol Biosynthetic Pathway. The remaining steps in the Monolignol Biosynthetic Pathway involve a series of enzymatic reactions which ultimately yield the three monolignols (Figure 6) (Vanholme, et al., 2010; Whetten & Sederoff, 1995).

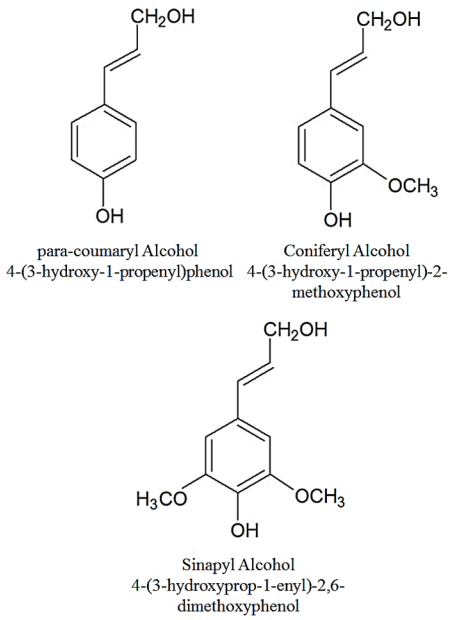


Figure 4: Lignin monomers, also known as monolignols. Figure reproduced from (Whetten and Sederoff, 1995) with permission from the American Society of Plant Physiologists. Permission conveyed through the Copyright Clearance Center Inc. See Appendix C for copyright permission.

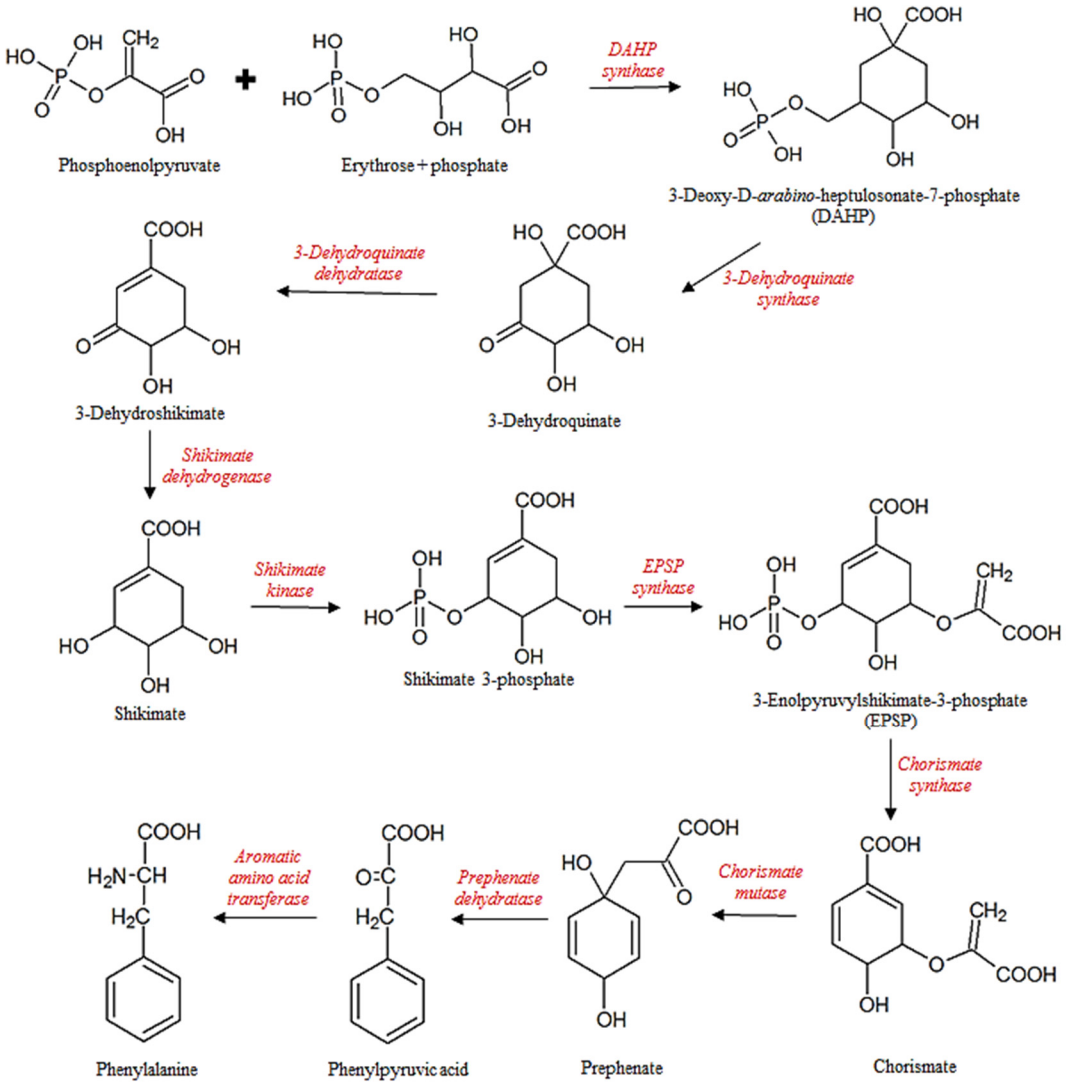


Figure 5: The Shikimic Biosynthetic Pathway. Red text indicates the enzymes mediating the reaction. Figure adapted from (Tzin and Galili, 2010; Herrmann, 1995; Botting, 1993).

The monolignols undergo polymerization through an oxidative mechanism involving phenols with free radical intermediates, which is followed by end-wise coupling of the monolignols to the growing lignin polymer (Vanholme, et al., 2010). The mechanisms governing the abundance of monolignols in the lignin polymer is still unclear. The enzymatic processes which affect lignin composition are also not well understood. Furthermore, lignin composition is suspected to differ not only between plant species, but within different regions of the plant cell wall (Yin, et al., 2013;

Whetten & Sederoff, 1995). The monolignols are termed guiaicyl (G), syringyl (S), and *p*-hydroxyphenyl (H) (Figure 6) as they are incorporated into the growing lignin polymer (Yin, et al., 2013; Vanholme, et al., 2010). The lignin in gymnosperms is largely composed of G-units. On the other hand, the lignin in dicots is made up of G- and S-units (Figure 7). H-units are rich in softwood and are found to be slightly higher in grasses (Yin, et al., 2013; Vanholme, et al., 2010).

The degradation of lignin containing plants in the terrestrial environment in believed to provide the major building blocks for the formation of humic matter. Given the differences in the lignin content found in different plant species; the degradation of these plant species is likely to produce humic matter that is of a unique composition, and likely unique chemical characteristics, lending to the need to understand the formation and degradation of lignin in different plants species to better understand the formation of humic matter within a particular region.

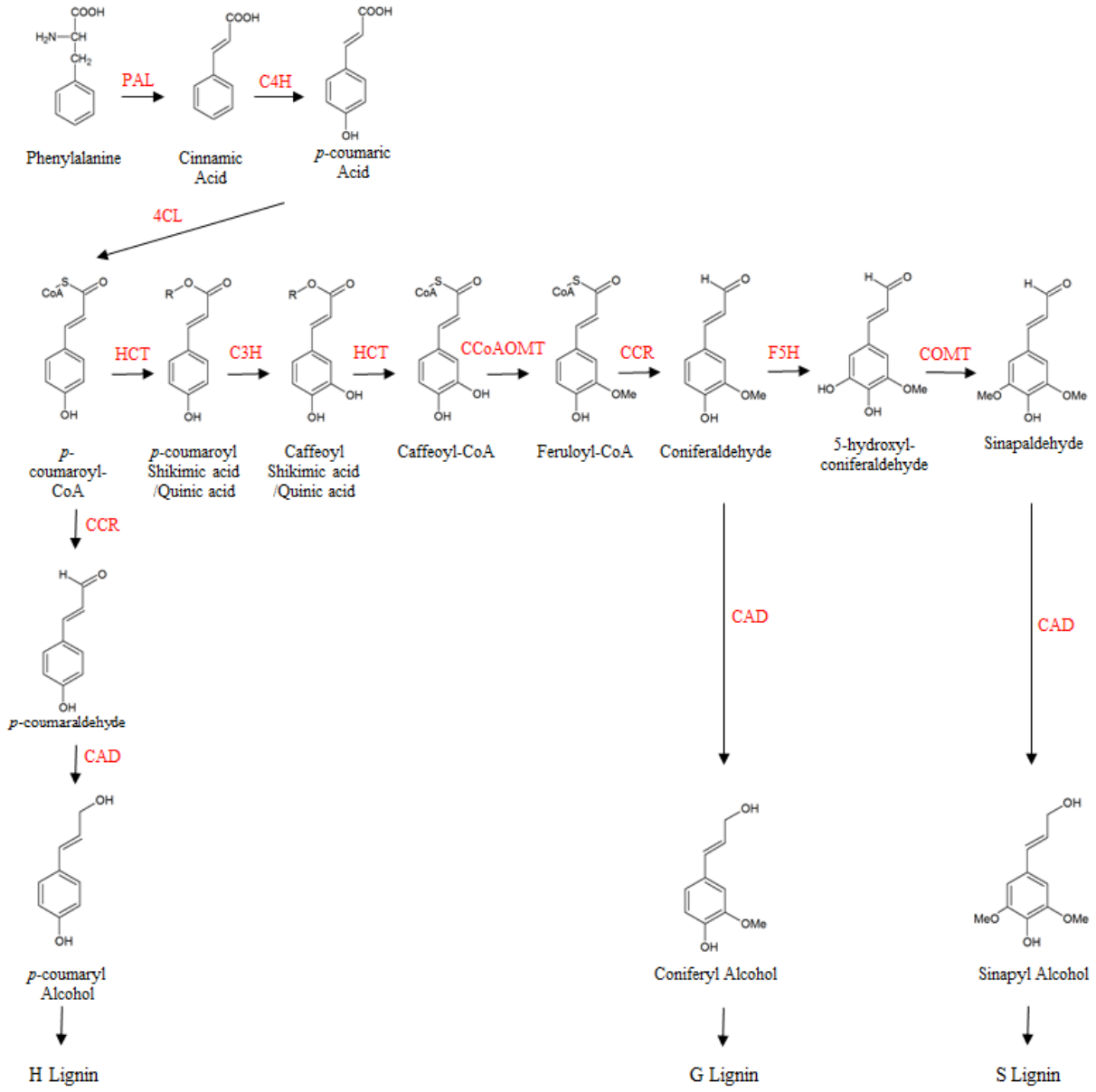


Figure 6: The Monolignol Biosynthetic Pathway. Enzyme (red) abbreviations: PAL, Phenylalanine ammonia-lyase; C4H, Cinnamate 4-hydroxylase; 4CL, 4-coumarate:COA ligase; C3H, *p*-coumarate 3-hydroxylase; HCT, *p*-hydroxycinnamoly-CoA:Quinate/Shikimate *p*-hydroxycinnamoyltransferase; CCoAOMT, Caffeoyl-CoA *o*-methyltransferase; CCR, Cinnamoyl-CoA reductase; F5H, Ferulate 5-hydroxylase; COMT, Cafferic acid *o*-methyltransferase; CAD, Cinnamyl alcohol dehydrogenase. Figure recreated from (Vanholme, et al., 2010) with permission from the American Society of Plant Biologists. Permission conveyed through the Copyright Clearance Center Inc. See Appendix C for copyright permission.

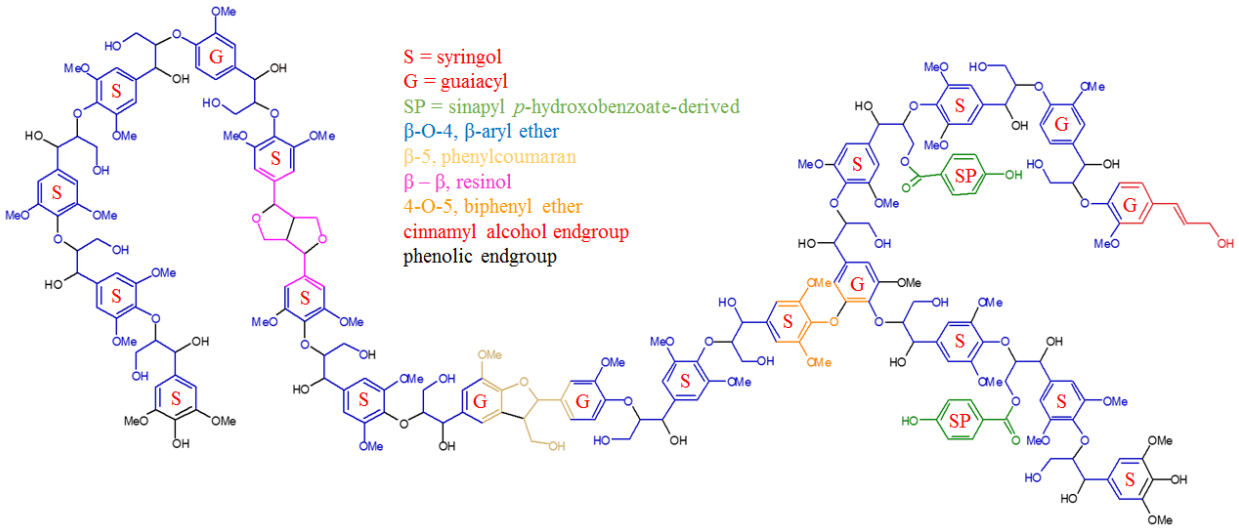


Figure 7**:** Structural representation of lignin in an angiosperm (poplar). Figure recreated from (Vanholme, et al., 2010) with permission from the American Society of Plant Biologists. Permission conveyed through the Copyright Clearance Center Inc. See Appendix C for copyright permission.

Lignin is resistant to microbial degradation; however, there are groups of specialized microbes or fungi that do possess the ability to break down lignin, and more specifically the lignocellulose complex (Alfaro, et al., 2014; Tan, 2014). The degradation of the lignocellulose complex occurs through extracellular oxidative enzymes secreted by basidiomycetes (e.g., white-rot fungi and brown-rot fungi) and ascomycetes (e.g., *Trichoderma reesei*) (Husaini, et al., 2011; Dashtban, et al., 2010). White-rot fungi (e.g., *Phanerochaete chrysosporium)* are the most efficient organism involved in the degradation of wood plant material due to their ability to rapidly degrade lignin, cellulose, and hemicellulose, resulting in a characteristic cellulose-rich white material (Tan, 2014; Bugg, et al., 2011; Husaini, et al., 2011; Dashtban, et al., 2010). Brown-rot fungi (e.g., *Fomitopsis palustris*) are typically unable to degrade lignin and are only able to degrade plant polysaccharides following partial lignin modification (Bugg, et al., 2011; Dashtban, et al., 2010). Similarly, ascomycetes have limited ability to break down lignin and are more adapted to degrade cellulose and hemicellulose (Dashtban, et al.,

2010). The decomposition of lignocellulosic material by white-rot fungi produce hydroxyphenols, which can further be oxidized to form quinones which are believed to be important components to the formation of humic matter (Tan, 2014). Brown-rot fungi are capable of removing methoxyl, -OCH_3, groups from lignin forming a brown residue rich in hydroxyphenols which are noted as an important source of aromatic compounds to humic SOM (Tan, 2014; Bugg, et al., 2011). Figure 8 is a simple representation presented by Tan (2014) showing the formation of a phenol, pyrogallol, following the oxidation and demethylation of a simple lignin monomer, sinapyl alcohol.

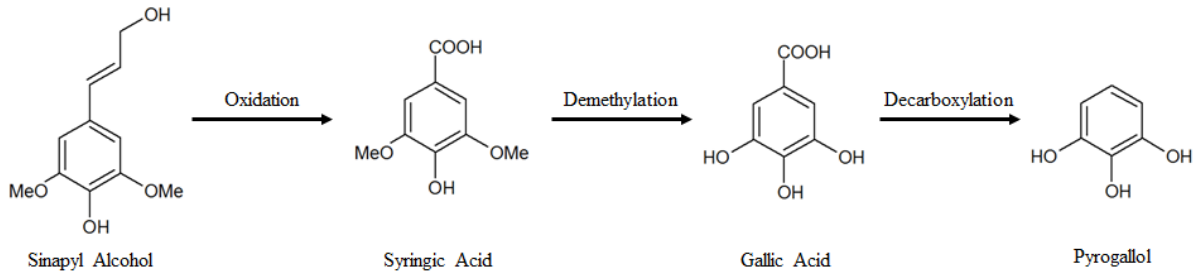


Figure 8: Simplified mechanism for the formation of a phenol (Pyrogallol) from the decomposition of lignin. Figure recreated from (Tan, 2014). Permission requested from the Copyright Clearance Center Inc. See Appendix C for permission request.

The lignin degradation by white-rot fungi occurs as a secondary metabolic process that is initiated when there is a depletion of nutrients (Ward, et al., 2004). Since lignin itself is not used as an energy source; its degradation serves mainly to expose the energy rich cellulose and hemicellulose (Kubicek, 2013) which, in turn, provides glucose as the energy source for fungal growth (Ward, et al., 2004). Phenols are not only formed following microbial degradation of lignocellulosic plant matter but can also be synthesized via primary metabolic processes where glucose is used as an energy source. Two likely pathways for the formation of phenols from the metabolism of glucose by microbes are the acetate-malonate pathway and the Shikimic acid pathway (Figure 9) (Tan, 2014).

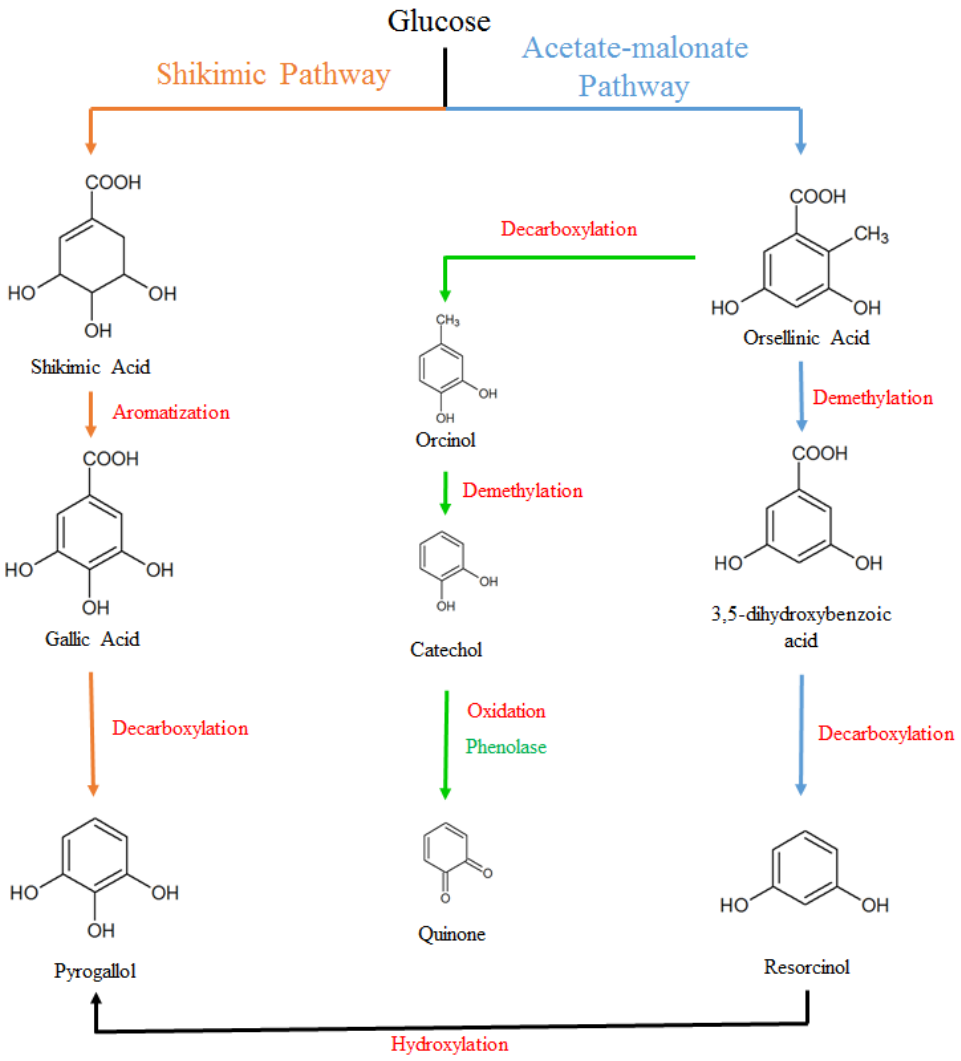


Figure 9: Formation of phenols (pyrogallol and resorcinol) from the metabolism of glucose by fungi by the Shikimic Pathway (orange) and the Acetate-malonate Pathway (blue). The formation of quinones (green) occurs from the decarboxylation of orsellinic acid along the acetate-malonate pathway. Resorcinol can undergo hydroxylation to from pyrogallol (black). Red text indicates the enzymatic mechanism involved. Figure adapted from (Tan, 2014). Permission requested from the Copyright Clearance Center Inc. See Appendix C for permission request details.

The phenols and quinones formed from either the degradation of lignocellulose, or from the metabolism of glucose by microbes, are suspected to serve as the main precursors to humic matter in terrestrial environments (Tan, 2014). The formation of humic matter from these precursors will be further reviewed in *Section 2.1.4.1 Formation of Humic Matter is Soils: Humification.*

2.1.3.3 Proteins and Amino Acids

Proteins are macromolecules composed of complex combinations of amino acids held together by a peptide bond between an amino (NH_2) group of one amino acid and a carboxyl (COOH) group of another (Figure 10) (Tan, 2014). Proteins are composed of anywhere from 50 to as many as 2000 amino acid residues. In nature, proteins often form conjugates with other biomolecules, such as glycogen (i.e., glycoproteins), glucose (i.e., glucoproteins), or lipids (i.e., lipoproteins) (Tan, 2014; Moe, 2013).

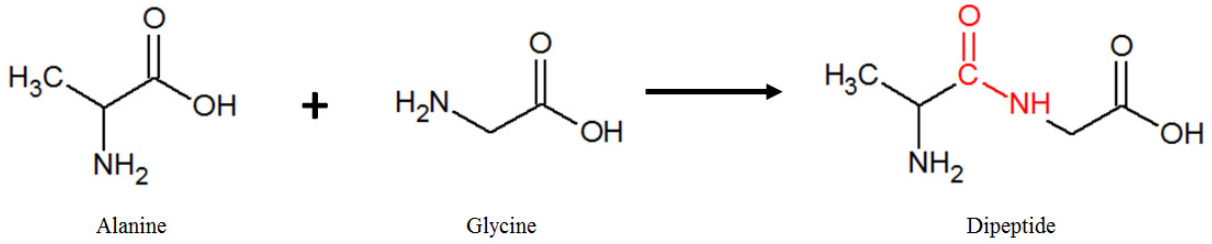


Figure 10: Example of dipeptide formed from two amino acids: alanine and glycine. The peptide bond formed between the amino group and carboxyl group is highlighted in red.

Nitrogen is an essential nutrient require for plant growth. Typically, plants prefer to utilize inorganic nitrogen (e.g., NH_4^+ or NO_3^-); however, proteins and amino acids have been found to be important nitrogen sources for both plants and microorganisms (Tan, 2014; Apostel, et al., 2013; Moe, 2013; Govindarajulu, et al., 2005). Plant roots share in a symbiotic relationship with mycorrhizal fungi which reside in the plant rhizosphere (Moe, 2013). Ectomycorrhiza describes the symbiotic relationship where the fungi remain outside the plant, where as endomycorrhiza, part of the fungal hyphae is situated within the plant root itself, known as the intraradical mycelium (Parniske, 2008; Govindarajulu, et al., 2005). The extraradical mycelium is the part of the fungal body that extends into

the rhizosphere. Arbuscular mycorrhiza fungi (AMF) are the most common endomycorrhiza symbiotic species which are associated with 70-90% of all terrestrial plants (Parniske, 2008).

Plants excrete significant amounts of high molecular weight organic compounds (e.g., proteins and polysaccharides), as well as low molecular weight compounds (e.g., amino acids) that support a large abundance of microbes residing in the rhizosphere (Moe, 2013). Extracellular proteinase enzymes secreted by the fungi hydrolyse proteins into peptides, which are further degraded into amino acids by peptidase enzymes. The amino acids undergo deamination reactions by oxidase or amino acid dehydrogenase enzymes, yielding ammonium (NH_4^+) which can be taken up by the fungi or plants (Moe, 2013; Govindarajulu, et al., 2005). In times where inorganic nitrogen is low, or in areas such as boreal forests where organic nitrogen is in abundance, AMF are able to provide nitrogen to plants by transferring the inorganic nitrogen formed by extracellular deamination reactions from the rhizosphere to the plant root (Parniske, 2008; Moe, 2013). Inorganic nitrogen is taken up by the extraradical mycelium and transported to the intraradical mycelium where it is then transferred to the plant root and can be utilized for plant growth. Since ammonium would be toxic to AMF, the inorganic nitrogen is assimilated into glutamine via nitrate reductase and glutamate synthase. The glutamine is then converted to arginine and transported from the extraradical mycelium to the intraradical mycelium where it is broken down releasing urea or ornithine. Urea is broken down into ammonium and transported from the AMF to the plant root where it can be utilized for amino acid or protein production. Ornithine is broken down into amino acids that can be used by AMF for the synthesis of amino acids in the intraradical mycelium (Govindarajulu, et al., 2005). AMF provide inorganic nitrogen to the host plant root, in turn, the plant provides organic carbon to the AMF in the form of carbohydrates, which can be used to synthesize amino acids, proteins, and other biomolecules (Figure 11) (Parniske, 2008).

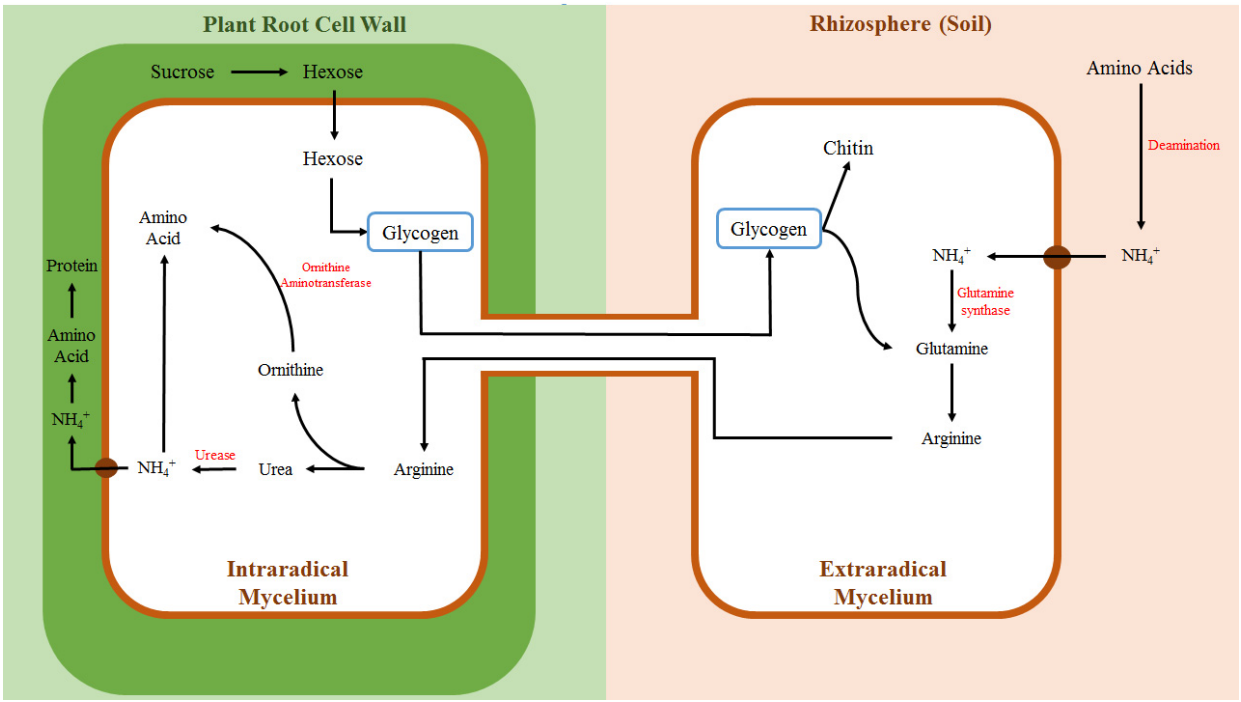


Figure 11: Symbiotic relationship between AFM and plant root. Figure recreated from (Parniske, 2008) with permission from Nature Publishing Group (https://www.nature.com/nrmicro/). See Appendix C for copyright permission.

There are several sources for proteins and amino acids in soil which could become incorporated into the humic molecule following humification. Proteins and amino acids would be released to the soil from the lysis of cells following the death of plants, animals, and other soil microorganisms (Moe, 2013). Likewise, the sloughing off of dead cells from plant roots would also be a source of amino acids or proteins to a humic molecule. As mentioned above, plants excrete a large amount of high and low molecular weight compounds into the rhizosphere where they are degraded by soil microbes. If these compounds are only partially degraded, the remaining portion could accumulate in soil and undergo humification. Oxidative deamination (Reaction 4) of amino acids by microbe's yield NH_4^+ under aerobic conditions; however, under anaerobic conditions non-oxidative decarboxylation reactions only form CO_2, not ammonium, and the amine group remains part of the carbon skeleton (Reaction 5) (Tan, 2014). These compounds could also accumulate in soil providing

another source of amino-precursors to the growing humic molecule. Although the inclusion of proteins and amino acids as precursors to the formation of humic matter has been questioned; the composition of humic matter is said to contain from 1-5% nitrogen content which is thought to come from the amino group of these compounds (Tan, 2014). A more detailed review for the inclusion of proteins and amino acids into the humic molecule will be discussed in *Section 2.1.4.2 Polymer Theory.*

Aerobic Deamination: $R\text{-}CH(NH_2)COOH + O_2 \rightarrow RCOOH + CO_2 + NH_3$ (Reaction 4)

Anaerobic Decarboxylation: $R\text{-}CH(NH_2)COOH \rightarrow R\text{-}CH_2NH_2 + CO_2$ (Reaction 5)

2.1.3.4 Lipids

Lipids are a heterogeneous group of compounds that range from simple fatty acids to complex compounds like sterols, polynuclear aromatic hydrocarbons, waxes, resins, and chlorophyll. Lipids can be either non-polar, or amphiphilic, and have a common property of being soluble in fat solvents such as benzene, methanol, acetone, chloroform, and ether (Tan, 2014; Stevenson, 1994; Braids & Miller, 1975). The presence of these lipids in soils will affect the degree of wetting, due to their hydrophobic nature (Tan, 2014). Lipids are typically found in low concentrations in soils, ranging from 2-6% in most soil (Tan, 2014). Given the low concentrations in soil, lipids have often been overlooked as potential precursors to the humic molecule. However, with the emergence of more modern theories of humification, namely the micellar and nanotube membrane concepts, there has been greater interest in determining what role lipids have in forming the humic molecule (Tan, 2014).

The source of lipids in terrestrial soils is not unlike that of proteins and amino acids, originating from both from both plant and microbial origin. Thus, there are a wide range of lipid components in soil, each with varying degrees of degradation rates, causing some to accumulate in

soils more readily than others (Stevenson, 1994; Braids & Miller, 1975). For example, fatty acids and phospholipids are more easily degraded by microorganisms where as the waxes found in plant cuticle (i.e., the protective layer surrounding the surfaces of leaves, needles and fruits) are more resistant to decomposition and would be expected to accumulate in soils over long periods of times increasing their potential for inclusion into the humic fraction following humification (Tan, 2014; Braids & Miller, 1975). Microorganisms, such as bacteria and fungi, also contribute to the pool of soil lipids since bacteria cells contain between 5-10% lipids, while fungi can have as much as 25% lipid character (Braids & Miller, 1975). These lipid components are released to the soil following death and cell lysis and may be available to undergo humification, becoming part of humic matter.

2.1.4 Humic Fraction

The concept of humic matter, or humus, is one of long-standing controversy, and although the study of organic matter in soils and waters has spanned nearly two centuries there remains no universally accepted definition for humus or humic matter (Wershaw, 2000). Stevenson (1994) defines humic substances as "*a series of relatively high-molecular-weight, yellow to black colored substances formed by secondary synthesis reactions … these materials are distinctive in the soil or sediment environment in that they are dissimilar to the biopolymers of microorganisms and higher plants (including lignin)"* (Tan, 2014; Stevenson, 1994). Although the definition presented by Stevenson seems to describe the concept of humified organic matter in soils, Tan points out that Stevenson's definition of humus, being *"the total of the organic compounds in soil exclusive of undecayed plant and animal tissue, their partial decomposition products, and soil biomass",* introduces some confusion (Tan, 2014; Stevenson, 1994). The definition of humus presented by Stevenson seems to also describe humic substances; however, Stevenson maintains the two are distinctly different.

Furthermore, Stevenson views soil organic matter to be the same as humus, which greatly confuses the matter as one would assume SOM to include non-humic organic compounds. In literature, the terms humus and humic matter are often used interchangeably, which is likely why Stevenson attempted to provide distinction between the two groups (Tan, 2014). To further complicate this, some have used the terms humic substances, humified organic matter, and even humic acids, to describe what is believed to be humic matter or humus, making it difficult to assess what a particular work is actually describing when using these terms (Tan, 2014).

To provide an answer to the question, what is humic matter, researchers at the United States Geological Survey (USGS) introduced an operational concept based on isolation techniques to describe humic substances. For example, Thurman (1985) operationally defines soil humic substances as colored, polyelectrolytic acids that are isolated from soil using 0.1N NaOH. On the other hand, aquatic humic substances are operationally defined as colored, polyelectrolytic acids isolated based on sorption to the XAD-8 (polymethyl methacrylate) resin (Thurman, 1985). From these two operational definitions, it is difficult to determine a difference, if any, between the two humic matter fractions found in terrestrial and aquatic environments, apart from for the difference in isolation methods. Some maintain that the extraction procedures are in fact creating artifacts, and that the compounds isolated are not representative of humic matter under natural conditions (Gadmar *et al.*, 2005). However, one must first separate the humic matter from non-humic matter to further characterize the humic fraction, thereby potentially causing an alteration to the chemical structure due to harsh chemical conditions, (i.e., pH <2). The influence of potential artefacts resulting from chemical separation techniques makes it difficult to address whether there exists a distinct chemical structure for the humic molecule, or if

humic matter consists of a mixture of humified compounds that are separated temporally along the humification process.

The shortcomings in the present humic matter paradigm have been discussed by Wershaw (2000) who believes that the current approaches to understanding humic matter in soils are futile. Instead, Wershaw proposed a paradigm shift from trying to define and identify humic matter, to studying the process of humification, since Wershaw believes that humic matter is composed of a mixture of compounds and that a single characteristic humic molecule does not exist (Wershaw, 2000). Although there is still disagreement on what mechanisms govern the process of humification in soils, Wershaw believes that understanding the process of humification is achievable and humic matter research should focus on the process, instead of studying ill-defined intermediates (Wershaw, 2000).

The belief that humic substances were merely artificial compounds produced following an extraction procedure has, for the most part, been abandoned. Humic substances are now thought to be natural compounds and considered an integral part NOM in soil and water (Tan, 2011(a)). Unfortunately, the debate on humic matter and the humification process continues and it is beyond the scope of this manuscript to provide a definitive answer. The present author does tend to agree with the paradigm shift proposed by Wershaw and that greater focus be paid to understanding the process of humification, rather than trying to define this complex class of compounds. Therefore, this author will refrain from providing an in-depth review of the current definitions of humic matter, or humus, and will instead present the prevailing theories regarding the process of humification.

2.1.4.1 Formation of Humic Matter in Soils: Humification

There are two prevailing models to describe the humification process in soils. The first is known as the humic polymer model, where humic compounds are depicted as large covalently linked

polymeric molecules having unique chemical structures that no longer resemble the plant or microbial degradation products (Swift, 1999). The second model suggests that the humic molecule is not comprised of products held together by covalent bonds forming a large polymer; but is described as molecular aggregates of the plant and/or microbial degradation products held together by weak attractive forces (i.e., hydrogen bonds and hydrophobic interactions) (Wershaw, 2004; Piccolo, 2001). There is empirical evidence to support, as well as refute, both models and neither theory fully describes the mechanisms involved in the humification process. Disparity in the empirical evidence supporting each model largely centers around the model's explanation of the chemical composition, molecular size, and shape of humic molecules. The following sections provide brief descriptions of both the polymer and aggregation models, with attention given to the currently available empirical evidence that supports, or rejects, each model.

2.1.4.2 Polymer Theory

Polymer theory is the oldest concept for describing the synthesis of humic matter in soils (Tan, 2014). The basis of this theory describes the humic molecules as being synthesized from lignin, and its degradative products, as well as other biomolecules forming a large, covalently linked, polymeric compound.

The lignin theory, or ligno-protein theory, was postulated by Waksman and colleagues in the 1930s (Waksman & Iyer, 1936). This theory considers lignin and proteins, or amino acids, as initial reactants forming the core of the humic molecule to which other organic biomolecules can be attached. Waksman proposed that lignin was only partially degraded by soil microorganisms and the residuum undergoes further modifications within soil, such as demethylation forming *o*-hydroxyphenols and oxidation of aliphatic groups to form carboxylic acid moieties (Stevenson, 1994; Waksman & Iyer,

1936). The demethylation and oxidation of the lignin residuum first forms compounds described as humic acids, which undergo further unknown fragmentation reactions forming fulvic acids (Figure 12) (Waksman & Iyer, 1936). Waksman provided the following evidence to support his theory on the formation of humic acids from lignin (Waksman & Iyer, 1936):

1. Both lignin and humic acid are decomposed with great difficulty, or not at all, by the great majority of fungi and bacteria.
2. Both lignin and humic acids are partly soluble in alcohol and pyridine.
3. Both lignin and humic acids are soluble in alkali and precipitated by acids.
4. Both lignin and humic acids are oxidized by mild oxidizing agents, such as permanganate and peroxide.
5. Both lignin and humic acids are acidic in nature; both can combine with bases and are characterized for their capacity to undergo base exchange, although to a different quantitative extent.
6. When lignin is warmed with aqueous alkali solutions, it is transformed into methoxyl-containing humic acids. The humic acids have many of the same properties in common as oxidized lignin.

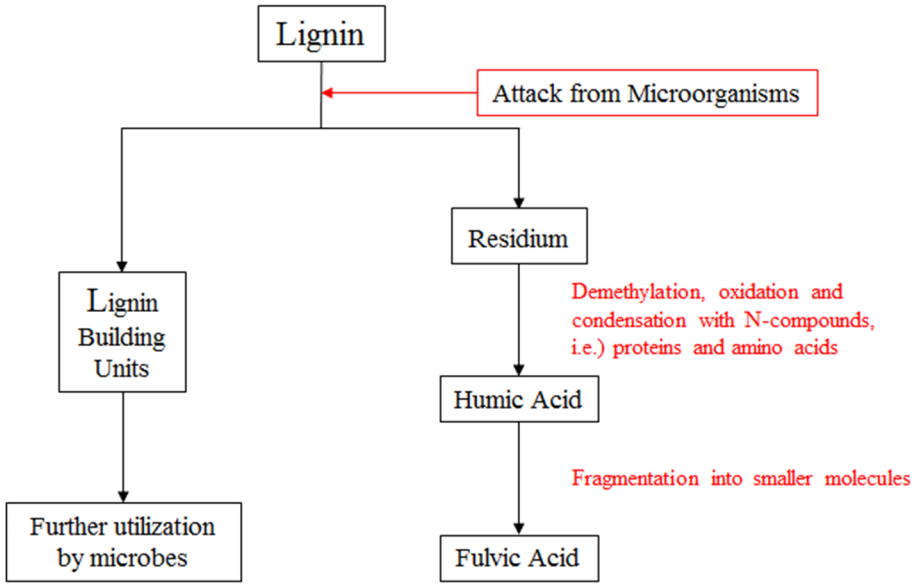


Figure 12: Schematic of the ligno-protein theory of humification according to Waksman. Figure recreated from (Stevenson, 1994). Permission granted by John Wiley and Sons through the Copyright Clearance Center Inc. See Appendix C for copyright permission.

Although there is some merit in the comparison between lignin and humic acids made by Waksman, Stevenson points out that although lignin is difficult to decompose compared to other plant biomolecules there are mechanisms in nature that can fully degrade lignin under aerobic conditions. Furthermore, if lignin remained only partially decomposed in soil it would accumulate over time until CO_2 was completely depleted from the atmosphere (Stevenson, 1994). Stevenson believed that partially degraded, or modified, lignin would only make a major contribution to humus under anaerobic conditions, such as poorly drained soils, peat, and lake sediments. In aerobic conditions, lignin can be completely degraded by soil microorganisms, forming low-molecular weight compounds which act as core building blocks for humus (Stevenson, 1994).

The ligno-protein theory evolved with notable works by Kononova (1966) and Flaig et al. (1975) who believed the humification process involved more complete degradation of lignin to primary structural units (i.e., monolignols) which would further undergo oxidation and demethylation reactions forming polyphenols (Figure 8) (Flaig, et al., 1975; Kononova, 1966). The polyphenols would be modified by extracellular phenol oxidase enzymes secreted by microorganisms forming quinones, or semiquinones, which can react with amino compounds to form humic substances (Flaig, et al., 1975) (Figures 13 and 14).

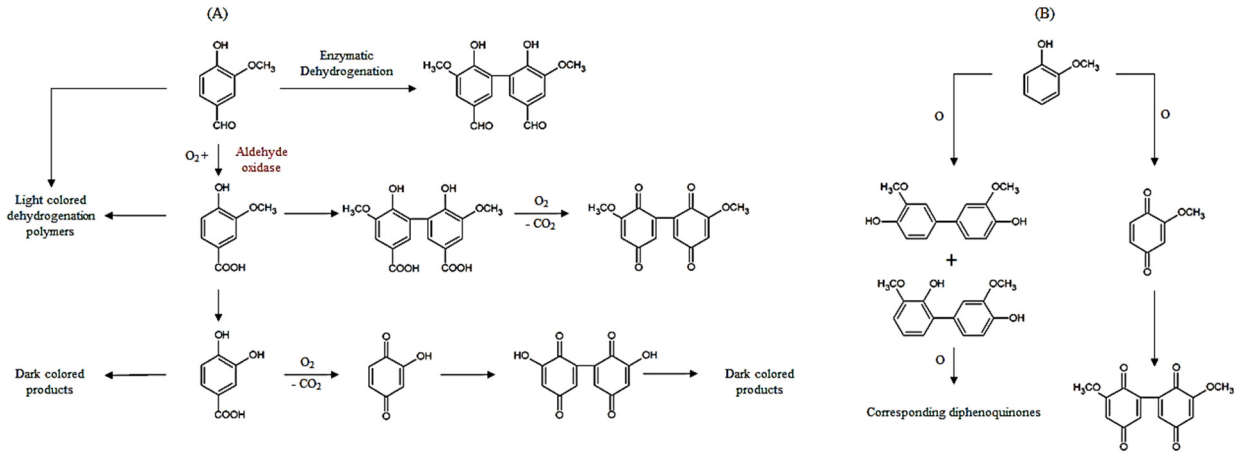


Figure 13: Formation of quinones from common lignin degradation products: (A) vanillin in the presence of microorganisms and (B) guaiacol in the presence of phenoloxidases. Figure adapted from Flaig et al. (1975) with permission from Springer-Verlag and the Copyright Clearance Center Inc. See Appendix C for copyright permission.

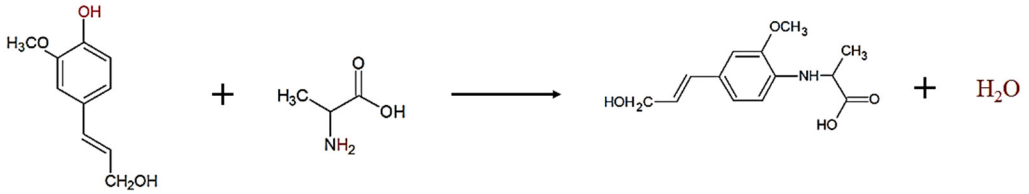


Figure 14: Reaction between a lignin degradation product and an amino compound (alanine) as described by the ligno-protein theory according to Flaig.

Stevenson (1994) introduced his version of the polyphenol theory for humification, noting the previous works by Kononova and Flaig, stating the formation of phenolic compounds from the degradation of lignin, cellulose, or other non-lignin sources such as those synthesised by microbes, was essential in the formation of humic substances. The polyphenol hypothesis does not differ greatly from the ligno-protein theory proposed by Flaig et al. (1975). The main difference in the polyphenol theory by Stevenson is that lignin is degraded to a variety of low-molecular weight aromatic acids and aldehydes formed by extracellular enzymes secreted by fungi. These compounds are converted to

quinones by enzymatic oxidation, which combine with other biomolecules, such as peptides and amino acids, to form humic polymers (Stevenson, 1994). Stevenson points out that the most likely mechanism involved in the formation of humic matter in soil occurs through the condensation of phenols and quinones; however, acknowledges there are undoubtedly several mechanisms occurring in soils which would produce humic matter (Stevenson, 1994). Figure 15 shows the major mechanisms for the formation of humic matter in soils according to Stevenson (1994). Pathways 2-4 represent the polyphenol theory, ligno-protein according to Flaig et al., and Waksman's lignin theory, respectively. Pathway 1 represents the sugar-amine condensation theory proposed by Maillard in the early 1900s. Maillard noted the formation of brown nitrogenous polymers following the condensation of reducing sugars and amines which occurred during the dehydration of food products. Maillard proposed the importance of this reaction, often referred to as the Maillard reaction or melanoidin pathway, in the formation of humic substances (Figure 16). The initial step in Figure 16 involves a reaction between an amine and the aldehyde group of a sugar forming a Schiff base and an *N*-substituted glucosamine. The glucosamine undergoes Amadori rearrangement to form an *N*-substituted-amino-1-deoxy-2-ketose which is further fragmented into smaller compounds. These highly reactive compounds can polymerize in the presence of amino acids to form brown-colored products which can be characterized as humic compounds (Stevenson, 1994).

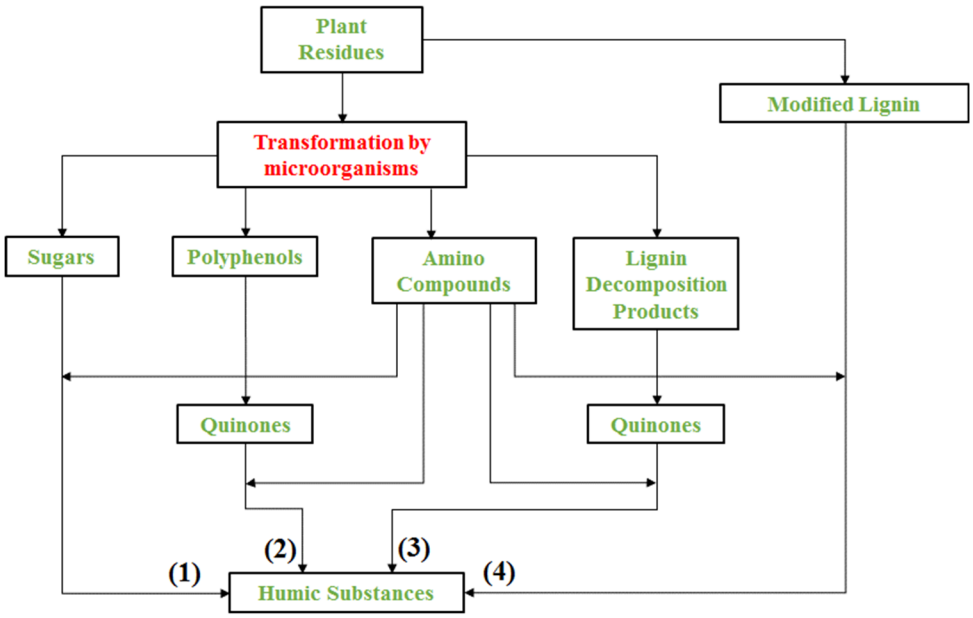


Figure 15: Major pathways for the formation of humic substances in soils according to Stevenson (1994). (1) sugar-amine theory according to Maillard (1916); (2) polyphenol theory according to Stevenson (1994); (3) ligno-protein theory according to Flaig et al. (1975); (4) ligno-protein theory according to Waksman (1936). Figure adapted from Stevenson (1994). Permission granted through the Copyright Clearance Center Inc. See Appendix C for permission.

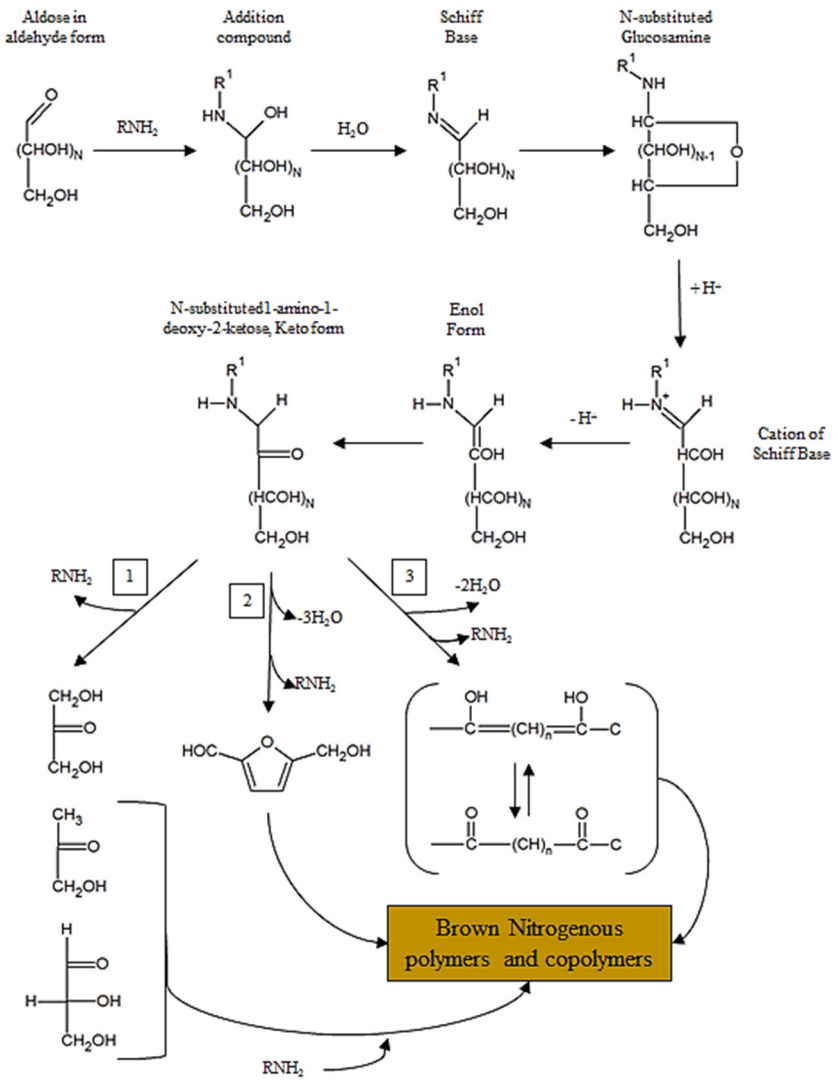


Figure 16: Pathways for the formation of brown nitrogenous substances via sugar-amine condensation. [1] Fragmentation followed by the formation of 3-carbon aldehyde and ketones; [2] the loss of three water molecules forming hydroxymethyl furfural; [3] the loss of two water molecules forming reductones. (Figure adapted from Stevenson, 1994). Permission granted through the Copyright Clearance Center Inc. See Appendix C for copyright permission.

Stevenson (1994) noted two attractive features of the sugar-amine condensation theory: (1) there are an abundance of these compounds found in soil and (2) it provides an explanation for the formation of humic substances in environments where there is limited or no source of lignin, such as aquatic or marine environments. However, Stevenson points out that this reaction occurs slowly under

natural soils conditions but suggests that drastic changes to temperature, along with the presence of catalytic minerals, may facilitate the condensation reaction (Stevenson, 1994).

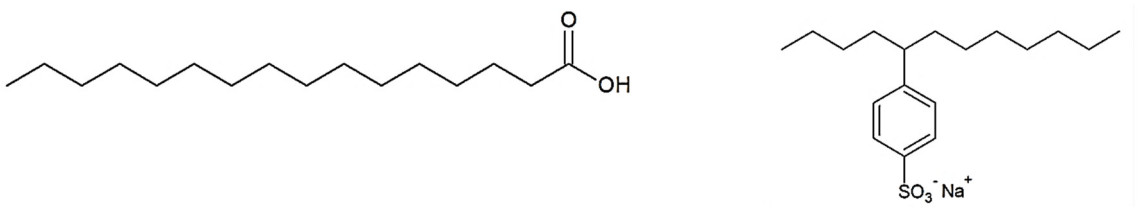
The polymer theories described above seem to provide suitable explanations for some of the physical and/or chemical characteristics of humic matter. For example, Tan (2014) points out that, with the polymer theory in mind, it would be expected that the humic polymer to assume an elemental composition similar to the monomeric units. This has been reflected in the consistencies in carbon and nitrogen content in organic matter. Soil organic matter is said to contain, on average, 58% organic carbon (C_{org}), which is used in the *van Bemmelen factor* (100/58 = 1.724) to convert SOM to soil organic carbon. Likewise, the nitrogen content in humic matter is thought to be derived from protein, or amino acid, precursors; therefore, one would expect similar N-content in each. This, in fact, appears to be the case where the N-content in proteins and humic matter is approximately 16% of the total elemental composition. The factor of 6.25 (=100/16) is commonly used in soil science to determine the protein content of SOM from the %N (Tan, 2014).

However, there are criticisms of the polymer theory's apparent inability to determine the molecular weight of a humic molecule, or to be able to provide a reasonable explanation for the large variability in the molecular weights and particle sizes of humic molecules reported in the literature (Tan, 2014). This apparent limitation has been the driving factor for some researchers to abandon the polymeric theory in search of one that would better explain the chemical and physical nature of humic matter. Several promising theories have been proposed in recent years including the micelle concept (Wershaw, 1999; von Wandruszka, 1998; Wershaw, et al., 1986), the supramolecular concept (Piccolo, 2001), and the nanotube membrane concept (Tan, 2011(b)), which view humic matter as an aggregation, or self-assemblage, of small biomolecules which are not covalently bonded, but are held

together by weak attractive forces (i.e., van der Waals forces, π-π interactions, hydrophobic bonding or electrostatic forces).

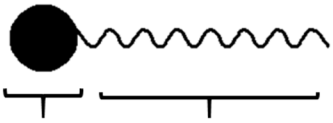
2.1.4.3 Aggregation Theories

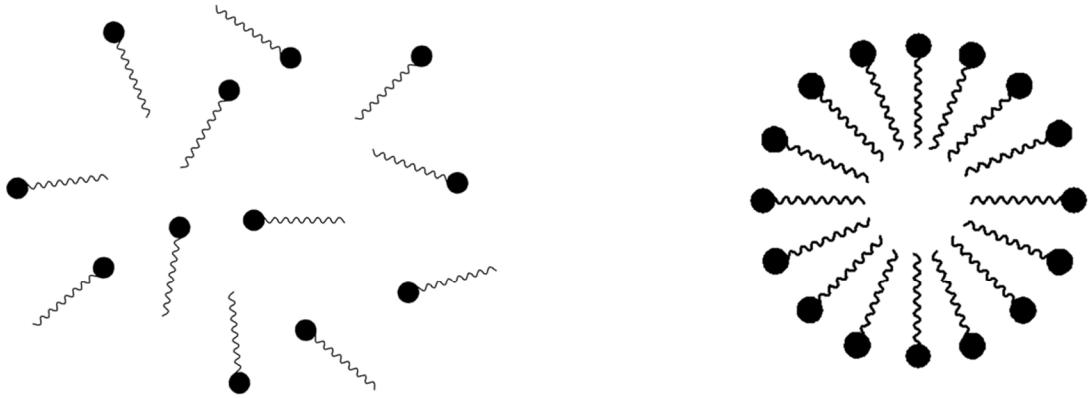
The concept of humic substances existing as large, covalently bonded, biopolymers was first challenged by Wershaw et al. (1986). He proposed that humic substances, including humic acids, fulvic acids, and humins exist as micellar aggregates of smaller, partially degraded, biomolecules, and not large molecular weight polymers as previously believed. The basic hypothesis for humic substances existing as micellar aggregates comes from the fact that the biopolymers assumed to be precursors to humic acids (i.e., lipids and amino acids) are amphiphilic in nature (Tan, 2014; Wershaw, 1999; von Wandruszka, 1998; Wershaw, et al., 1986). Furthermore, depolymerization and subsequent oxidation of biopolymers, such as lignin, produce smaller compounds which are also amphiphilic (Tan, 2014). Amphiphiles are compounds which have both hydrophilic (polar head) and hydrophobic (non-polar tail) moieties in their chemical structure, such as detergents or plant lipids (Figure 17). In aqueous solutions at low concentrations amphiphiles exist as monomers. However, if the concentration of amphiphiles increases above a certain concentration, known as the critical micelle concentration (CMC), the molecules will aggregate in a way where the hydrophilic head is in contact with the aqueous media while the hydrophobic tail would be orientated toward the center of the micelle (Figure 18) (Tan, 2014). Wershaw also believed that humic substances could form membrane-like bilayers around mineral grains, as the formation of micelles and bilayers represent the same aggregation phenomena (Wershaw, et al., 1986).



Palmitic Acid (Plant Fatty Acid) Sodium dodecylbenzene (Detergent)

Figure 17: Example of a natural and industrial amphiphile





Below Critical Micelle Concentration

Above Critical Micelle Concentration

Figure 18: Representation of the behaviour of amphiphiles in aqueous solutions below and above the critical micelle concentration.

Humic substances would differ from typical amphiphiles, such as the examples provided in Figure 17, in that the structure would not likely contain a specific hydrophilic head with a hydrophobic tail but would contain several polar and non-polar regions. Wershaw explained that the interior hydrophobic region of a membrane or micelle-like aggregate of humic matter would likely contain

polar functional groups; however, these groups would interact with each other through hydrogen bonding reducing the polarity and forming hydrophobic aggregates (Wershaw, et al., 1986). Metal ions (e.g., Ca^{2+}or Mg^{2+}) are also thought to enhance the hydrophobic region through charge neutralization of polar anionic functional groups (i.e., hydroxyl or carboxyl). The interaction between the metal cation and humic anion would cause the molecule to coil and form, what is described by von Wandruszka, a pseudomicellar structure (Figure 19) (von Wandruszka, 1998). However, it was noted by von Wandruszka that metal cations were not necessary for humic molecules to form micellar or membrane-like aggregates, their presence merely increased the hydrophobic interactions (von Wandruszka, 1998).

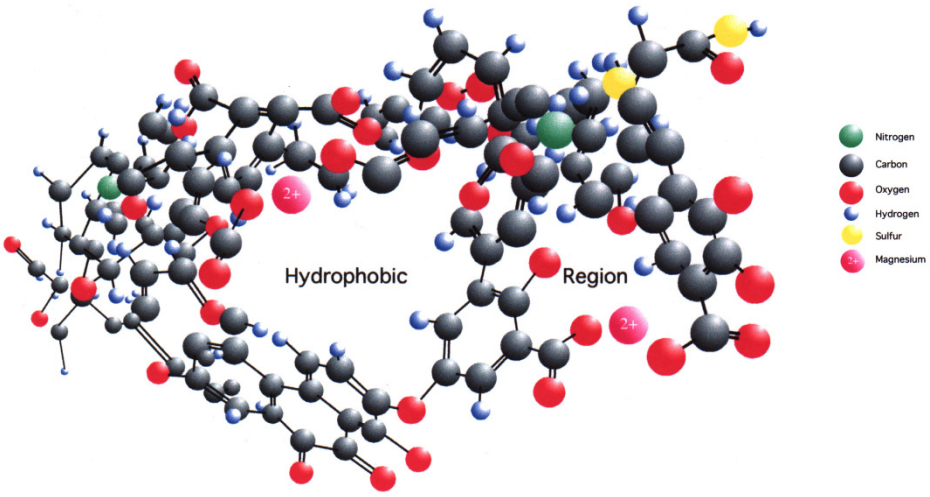


Figure 19: Pseudomicellar structure of humic molecule proposed by von Wandruszka (1998) showing the coiling of a humic molecule around a magnesium (Mg^{2+}) cation. Figure was provided by the original author. Permission to use the figure from (von Wandruszka, 1998) was provided by Wolters Kluwer Health Inc. and the Copyright Clearance Center Inc. See Appendix C for copyright permission.

Wershaw provided several explanations to support his theory of aggregation by interpreting results using an aggregation model for humic substances, opposed to the traditional polymer theory (Wershaw, et al., 1986). For example, soil humic acids fractionated using cross-linked dextran gels

(Sephadex) were separated into fractions with different chemical and physical properties (Wershaw & Pinckney, 1973(a); Wershaw & Pinckney, 1973(b)). The results from the two Wershaw and Pinckney studies found that the isolated humic fractions formed aggregates in solution and also that aggregation was pH and concentration dependant (Wershaw & Pinckney, 1973(a); Wershaw & Pinckney, 1973(b)). They reported that some of the fractions were found to increase in size with decreasing pH <3.5 (increased aggregation), with little change in size above a pH of 3.5. One the other hand, other fractions were found to continuously disaggregate up to pH of 7; however, aggregation occurred when pH was >7. Lastly, a third group of fractions were found to decrease in size with increasing pH. The Wershaw and Pinckney studies concluded that the observations were a result of the fractions being chemically different and that the differences resulted in aggregation behavior based on three types of bonding mechanisms: hydrogen bonding; π-bonding in the form of a stacked planar-π-donor-planar-π*-acceptor groups; and charge-transfer complexation (Wershaw, et al., 1986; Wershaw & Pinckney, 1973(a); Wershaw & Pinckney, 1973(b)). The weak interactions described above relate to isolated fractions of humic matter; however, in nature, humic matter consists of numerous fractions in a heterogeneous mixture. Wershaw postulated that humic substances exist as a hierarchy of structural elements, with the simplest being phenolic, carboxylic and quinoid groups, covalently linked in relatively small particles. Particles of similar chemical structure would be connected via weak interactions to form a somewhat homogeneous aggregate or particle. These relatively homogeneous particles would subsequently link, again, through weak bonds, forming a mixed aggregate or micelle (Wershaw, et al., 1977). Wershaw et al. (1977) compared X-ray scattering results of fractionated and unfractionated soil humic matter and found a reduction in particle size following fractionation. Wershaw et al. believed that because the fractionation procedure that was applied would not be expected to break covalently-linked bonds, the reduction in particle size observed must be due to

breaking weak bonds (i.e., hydrogen bonding or π-π interactions) disrupting the aggregate (Wershaw, et al., 1986; Wershaw, et al., 1977).

Schnitzer and Preston (1983) investigated humic acids by comparing ^{13}C-NMR before and after acid hydrolysis. They reported that the spectra for the acid hydrolyzed humic acids did not indicate the presence of protein or carbohydrate components. They concluded that acid hydrolysis could purify humic substances and remove protein and carbohydrate compounds chemically bound in the humic polymer, improving the ability to characterize humic substances (Schnitzer & Preston, 1983). Wershaw compared the Schnitzer and Preston results to ^{13}C-NMR results on fractionated and unfractionated humic samples and confirmed the fractions isolated using "soft" fractionation by means of Sephadex resins were chemically distinct, falling into three classes (Figure 20) (Wershaw, et al., 1988; Wershaw, et al., 1986). The ^{13}C-NMR spectra indicated the fractions contained carbohydrates (Group 1), melanin-like or lignin-like compounds (Group 2), and aromatic compounds (Group 3); components likely derived from degraded plant material (Wershaw, et al., 1988). Wershaw explained that soft fractionation was able to isolate the carbohydrate-rich fraction showing that chemical additions were not required to break covalently linked groups (i.e., carbohydrates) as humic matter existed as an aggregate, not a polymer (Wershaw, et al., 1986).

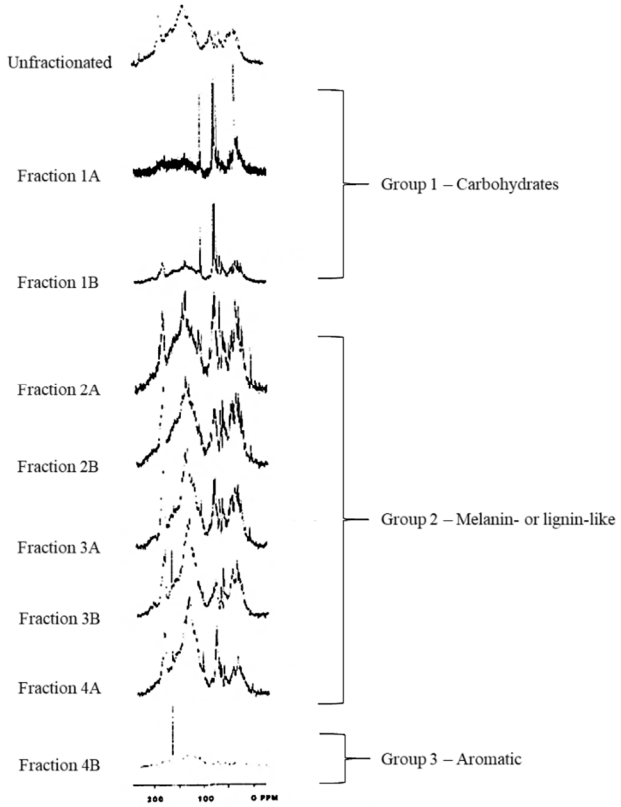


Figure 20: ^{13}C-NMR of Lakewood (North Carolina) soil humic acids fractions reported in (Wershaw, et al., 1986). Permission requested from PLS Clear. See Appendix C for permission request.

Wershaw published a series of articles that provided further empirical evidence to suggest that humic matter is comprised of amphiphilic compounds which form bi-layers or micelles by studying the formation of humus coatings on the mineral surfaces (Wershaw, et al., 1995; Wershaw, et al., 1996(a); Wershaw, et al., 1996(b)). The bilayer model proposes that the amphiphilic molecules would orient themselves in a way where a hydrophilic functional group would interact with the exterior surface of a mineral grain, while the hydrophobic tail extended away from the hydrophilic mineral surface creating the first layer. Hydrophobic structures in the first layer would then interact with hydrophobic structures of other nearby amphiphiles through weak attractive forces and would orientate in a way that shields the hydrophobic structures from the surrounding water, forming a

second layer (Figure 21). The outer layer of the bilayer would be comprised of charged polar functional groups what would interact with both water molecules and charged ions (e.g., Mg^{2+}, Ca^{2+}, and Na^{+}) (Wershaw, et al., 1995). To test this hypothesis, Wershaw measured the interaction of compost leachate with the surface of alumina. Adsorption measurements showed an increase in humic matter sorbed onto the surface of the alumina with increasing leachate concentration which indicated the possible formation of a multi-layer film. Furthermore, infrared linear dichroism of carboxylate groups indicated that the carboxyl groups were oriented towards the surface of the alumina and that they were not free to rotate. This implied the possibility of bidentate binding of organic molecules to the charged surface of the alumina (Wershaw, et al., 1995). After fractionating the compost leachate into hydrophobic and hydrophilic fractions, Wershaw confirmed that hydrophilic compounds were bound to the alumina though a bidentate mechanism, while the hydrophobic compounds were free to rotate about a single binding site (Wershaw, et al., 1996(a)). Using attenuated total reflectance (ATR) infrared spectroscopy to measure the isotherms of the leachate fractions, Wershaw found the hydrophobic fraction increased in surface excess with increasing concentration. Wershaw explained this as being evidence for the formation of aggregates on the surface of the alumina. The same phenomenon was not observed with the hydrophilic fraction (Wershaw, et al., 1996(a)).

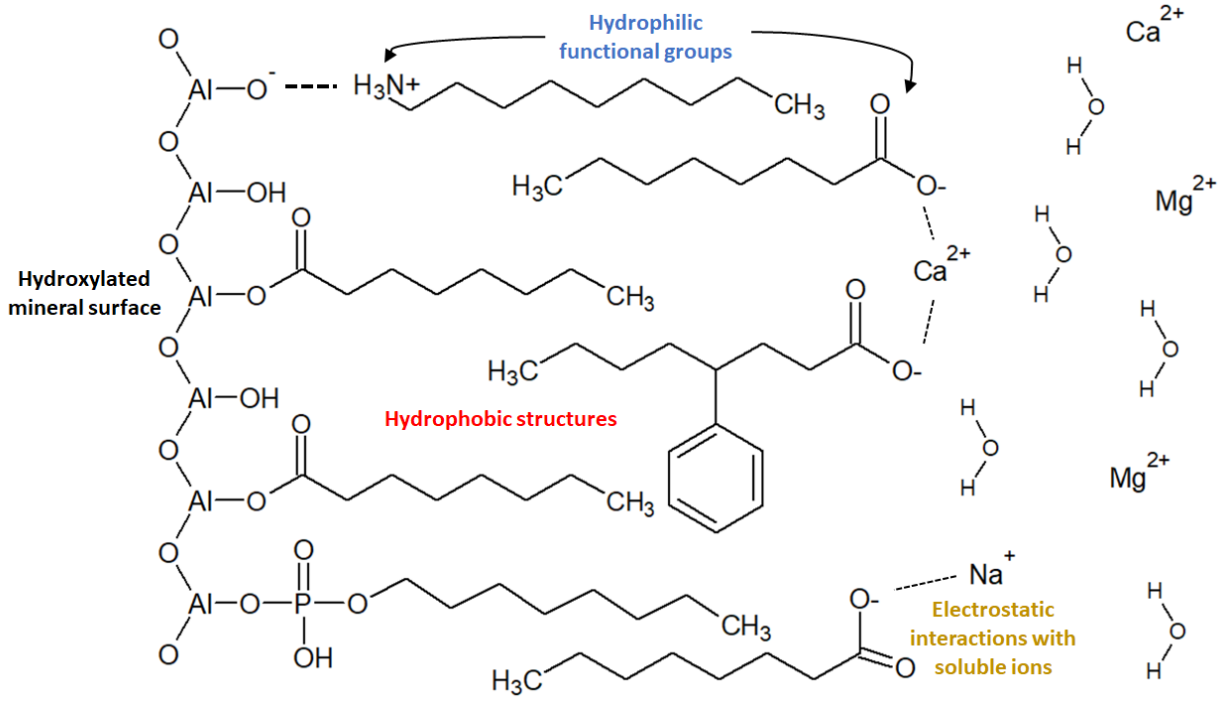


Figure 21: Bilayer model for the organo-mineral interactions as proposed by Wershaw et al. (1995, 1996(a) and 1996 (b)). Figure recreated from (Kleber, et al., 2007) with permission from Kluwer Academic Publishers (Dordrecht). See Appendix C for copyright permission.

The work of Wershaw did not initially receive great attention as the thought of humic matter existing as small aggregates required a large paradigm shift in thinking. Although there was hesitancy among most humic matter researchers, some were under what Tan (2014) described as *polymer fatigue* and were open to exploring this alternative hypothesis for humic matter.

Piccolo (2001) presented a theory to describe the process of humification and structure of humic molecules using a new field in chemistry, supramolecular chemistry. Unlike molecular chemistry, where the interaction between atoms or molecules is based on covalent bonding, supramolecular chemistry is used to describe intermolecular bonds and molecular assemblies through non-covalent interactions (Lehn, 1995). Supramolecular chemistry concepts have been largely applied to the biological or biochemical fields with regards to explaining the structures of proteins and DNA,

molecular recognition, and self-assemblage of the biological components that are believed to have created life as we know it (Tan, 2014; Lehn, 1995). In nature, the components of the system aggregate and self-organize spontaneously forming a multicomponent system, known as supramolecular assemblies. These assemblies would undergo a continuous assembly, disassembly, reassembly processes until the system found a thermodynamically stable conformation. This process is facilitated by weak interactions, such as van der Waal's forces, hydrogen bonding, π-π interactions, metal-ion bridging, and hydrophobic interactions (Tan, 2014). Piccolo believed that supramolecular interactions of humic molecules could better explain the downfalls of the humic polymer theory, such as molecular weight and size. In 1996, Piccolo *et al.* used low-pressure size exclusion chromatography (SEC) to study the changes in molecular size as a function of pH (Piccolo, et al., 1996). They reported that the molecular size of humic acids decreased at lower pH (pH = 2) with the use of organic acids which was explained by the formation of energy rich hydrogen bonds that altered the unstable conformation of humic molecules and prevented the reassembly into larger conformations (Piccolo, 2001; Piccolo, et al., 1996). In 2000, Piccolo et al. hypothesized that if humic matter were in fact an aggregation of small biomolecules held together by weak forces that could be disrupted by changing the pH or ionic strength; these molecules could also be stabilized by increasing the intermolecular connections via oxidative coupling (Piccolo, et al., 2000). To test this theory, Piccolo used a phenol oxidase enzyme to covalently link humic compounds collected from North Dakota lignite, forming a humic molecule that would be more appropriately described by the classic poly phenol theories (Piccolo, et al., 2000; Stevenson, 1994). These covalently linked humic polymers were subjected to SEC experiments using an analogous approach to their previous 1996 study. The results found that the control humic matter decreased in size with decreasing pH, which matched the previous results reported by Piccolo et al. (1996); however, the humic matter subjected to oxidative coupling before SEC separation did not

decrease in size when the pH was reduced (Piccolo, et al., 2000; Piccolo, et al., 1996). Piccolo believed that the results from the SEC experiments provided strong evidence that humic matter did not exist as a polymer that would coil and uncoil due to changes in pH or ionic strength, but that humic matter was an aggregation of small molecules that could disrupted which changing ionic conditions (Piccolo, et al., 2000). Although there is some pushback by those who still support the polymer theory (Swift, 1999), Piccolo maintained that the results from his work provide a better explanation to the wide range of molecular weights for humic matter reported in the literature (Tan, 2014; Piccolo, 2001).

The most recent theory for describing humic matter was introduced by Tan (2011) who felt that Piccolo was somewhat incorrect in implying that the supramolecular structure of humic matter produced random, poorly defined structures, that are in a state of constant disassembly and reassembly. Tan believed that if humic matter were truly a supramolecular structure as described by Piccolo than the theory of supramolecular assembly postulated by Lehn (1995) should also be maintained, in that the assembly process should form relatively ordered recognizable structures (Tan, 2011(a)). It was this limitation in the Piccolo theory that caused Tan to re-examine his own previous work in an attempt to "prove the impossible" and find an ordered arrangement for the molecular structure of humic matter (Tan, 2011(a); Tan, 2011(b)). According to supramolecular theory, molecules orient themselves into shapes and structures, forming supramolecular assemblies on the scale of nanometers to micrometers, which are known as nanoparticles (Tan, 2011(a)). Nanoparticles can exist in several shapes including spherical, such as the well-known buckminsterfullerene which is a molecule composed of sixty carbon atoms arranged in a soccer-ball shape, as well as nanotubes or nanomembranes (Tan, 2014). Tan noted works by Flaig and Beutelspacher (1951) and Visser (1964), who reported the size of humic acids to be on the order of 10 – 15 nm in diameter, which Tan believed

implied the possibility of humic matter existing as a nanotube (Tan, 2011(b)). Upon re-examination of his 1986 study of the structure of fulvic acids using a scanning electron microscope (SEM), Tan found the initial observation of the structure, being that of perforated sheets, could now be interpreted as a self-assemblage of humic matter into membrane nanotubes (Tan, 2014; Tan, 2011(a); Tan, 2011(b)). In 2011, Tan acquired scanning electron micrographs of humic acid isolated from lignite and found nanotube structures that resemble a characteristic honeycomb pattern (Figure 22 A-C) (Tan, 2014; Tan, 2011(a)). Tan also observed nanotube structures resembling spherical, or ball-like arrangements, which he described as nanobuds (Figure 22 D) (Tan, 2014; Tan, 2011(a)). SEM scans of aquatic humic matter showed the presence of long cylindrical fiber-like structures, or nanofibers, which form nano-bundles held together by van der Waals forces (Figure 23) (Tan, 2014).

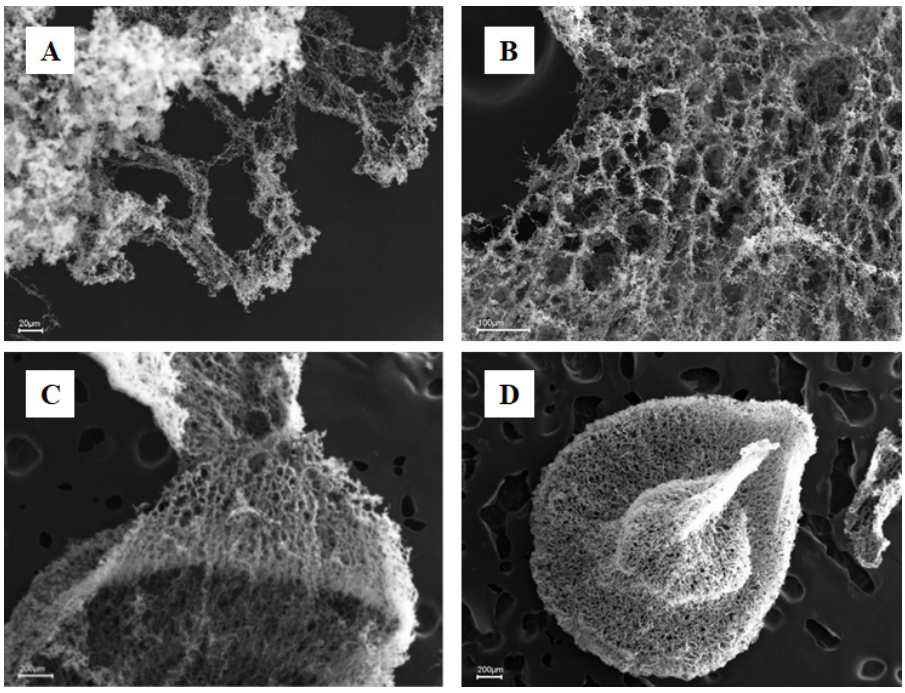


Figure 22: Scanning electron microscope images from of humic acid isolated from lignite showing honeycomb configuration (Images A-C). Humic acid in a cylindrical nanotube membrane configuration, or nanobud, showing a characteristic fishnet structure. Image created from (Tan, 2011(a); Tan, 2011(b)). Permission to use figures were provided on behalf of K.H. Tan. See Appendix C for permission details.

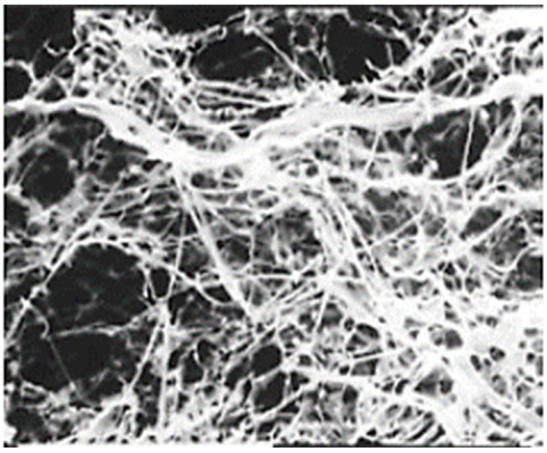


Figure 23: Scanning electron microscope image of a fulvic acid sample collected from Satilla River, Georgia, USA showing carbon nanotube bundles. (Tan, 2011(b)). Permission to use these figures was provided on behalf of K.H. Tan. See Appendix C for permission details.

Nanotubes and nanomembranes are found to occur in both animal and plant tissues, facilitating long-distance cell-to-cell communication and the transport of nucleic acids and cell components. The breakdown of these tissues would release the membrane nanotubes, forming smaller carbon nanotube fragments (Tan, 2014).

The concept of humic matter existing as nanoparticles does not discredit or contradict the previous theories on self-assemblage by Wershaw (1999) or Piccolo (2001), as the mechanisms involved are still non-covalent interactions (Tan, 2011(a)). What the nanotube theory does present that is different to the Wershaw or Piccolo theories is an orderly molecular assembly which Tan feels could allow for an explanation of the reproducible characteristics of humic matter, such as consistent carbon or nitrogen content, infrared spectroscopy, or nuclear magnetic resonance imaging (Tan, 2011(a)).

Although the debate on the exact definition of humic matter, and process of humification, still exists, it appears we are amid a paradigm shift from a polymeric concept to that of supramolecular self-assemblage. The recent studies by Tan suggest the possibility for an answer to what constitutes humic matter, what is the chemical structure, and how does it form in nature? Since humic matter has

a place in many fields of study, from soil science to environmental chemistry and engineering, and more recently noted implications in climate change, it is important to one day provide answers to those questions.

2.2 Aquatic Organic Matter

2.2.1 Composition of Aquatic Organic Matter

Aquatic organic matter (AOM) can be simply defined as the organic matter present in aquatic environments; however, as with SOM, there is much controversy in the actual meaning of term AOM as often terms such as DOM are used interchangeably (Tan, 2014). Of course, when evaluating the two, AOM and DOM are in fact describing different fractions of organic matter in aquatic systems. AOM, in this author's opinion, is accounting for the total organic matter within an aquatic system; which should include both dissolved organic matter as well as particulate organic matter (POM). DOM and POM are operational definitions, where DOM is defined as the fraction of AOM able pass though a 0.45 μm filter while POM is the fraction retained on the filter (Aiken, 1985). Although the concepts of AOM, POM, and DOM seem somewhat well defined, the terms total organic carbon, particulate organic carbon (POC), and dissolved organic carbon introduce a great deal of confusion as they are as well often used interchangeably with AOM, POM, and DOM, respectively (Tan, 2014). Although it seems reasonable to relate organic matter to organic carbon, Tan (2014) points out that the element, carbon, accounts for, on average, 57% of DOM. Therefore, DOM is approximately 1.7 times that of DOC, when describing dissolved AOM (Tan, 2014). Since the preceding sections of this manuscript have largely been dedicated to a review of the SOM, humic matter, and the controversy surrounding the definition and formation of humic matter, the following section will focus more on the composition and interactions of organic matter within aquatic environments as many of the

disagreements with regards to the meaning of AOM and humic matter in aquatic environments are similar to those found in SOM.

To avoid confusion, this manuscript will use AOM to describe the total organic matter present in an aquatic environment, including allochthonous and autochthonous organic matter. The terms DOM and POM will describe the operational definitions for dissolved and particulate organic matter based on their ability to pass through a 0.45 μm membrane filter (i.e., DOM) is the fraction able to pass through the filter, while POM is retained. The terms TOC, POC, and DOC will be avoided unless describing organic matter where the elemental carbon composition has been measured (i.e., using an organic carbon analyzer or other forms of elemental analysis). When referring to literature where the terms POC or DOC are used synonymously with POM and DOM, the later will be used to avoid confusion.

POM and DOM in the aquatic environment originates from both the terrestrial environment (i.e., allochthonous organic matter) and from within the waterbody (i.e., autochthonous organic matter) (Tulonen, 2004). The concentration of DOM exceeds that of POM in most natural water systems (Figure 24) and concentrations can vary based on the surrounding terrestrial environment and the magnitude of terrestrial runoff (Thurman, 1985; Wetzel, 1984). Large rivers can be an example of extreme situations where POM can be nearly equal to DOM resulting from increased organic loading following the erosion of the surrounding riverbank (Wetzel, 1984). The translocation of allochthonous organic matter from the terrestrial environment to a surrounding aquatic environment is slower for POM, compared to DOM, again accounting for a higher DOM concentration in aquatic environments. Since the aquatic environment is typically dominated by DOM, this review will focus more on the composition of DOM and its interactions with in the aquatic environment.

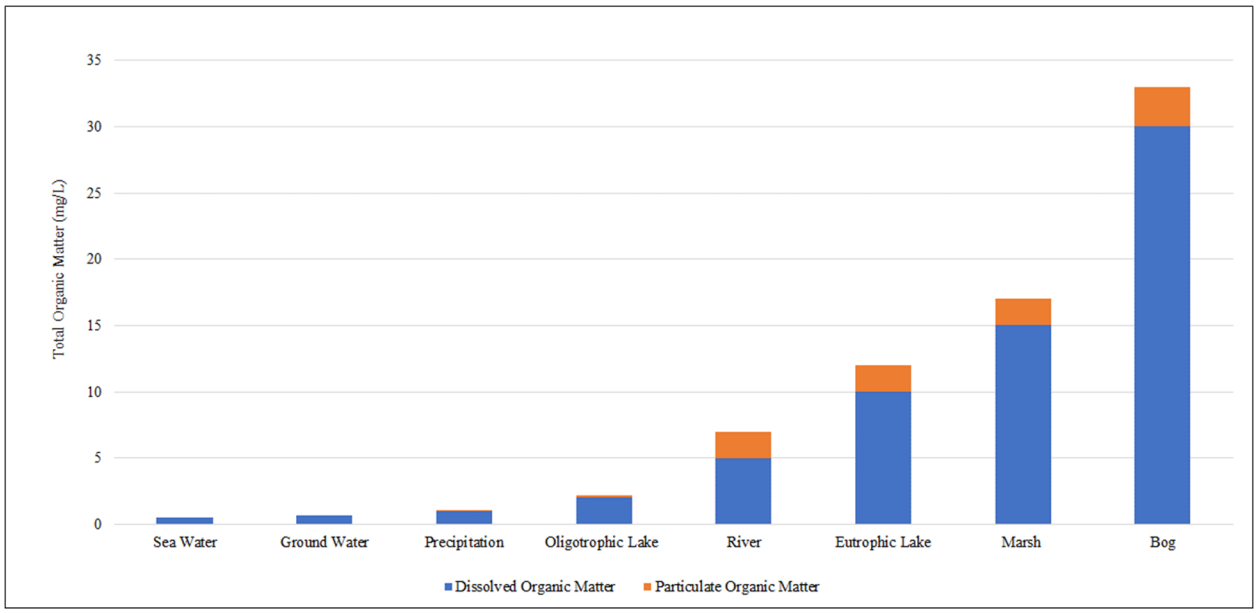


Figure 24: Approximate concentrations of particulate and dissolved organic matter in aquatic environments. Figure recreated from (Thurman, 1985). Permission to reproduce figure was provided by the Copyright Clearance Center Inc. See Appendix C for copyright permission.

As mentioned, the composition of DOM in aquatic systems is governed by both influx of allochthonous DOM from the surrounding terrestrial environments, as well as from autochthonous DOM synthesized within the waterbody by primary producers or heterotrophic organisms (Figure 25) (Tulonen, 2004; Cole, et al., 2002). The concentration and composition of DOM in a waterbody will therefore be subject to chemical interactions in both the surrounding terrestrial environment as well as within the waterbody itself. In general, the influx of allochthonous DOM into lakes exceeds the DOM contribution from autochthonous sources; a result of highly productive littoral zones or wetlands surrounding most lake ecosystems (Wetzel, 1984). A noted exception to this would be in that of a eutrophic lake, where high nutrient loading (i.e., phosphorous) typically from agricultural runoff or wastewater effluent, causes excessive growth of algae (Schindler, et al., 2016). However, the input of allochthonous DOM from rivers or littoral regions to the ocean, as DOM, is relatively small in comparison to the DOM contribution from primary producers in the pelagic zone (Wetzel, 1984).

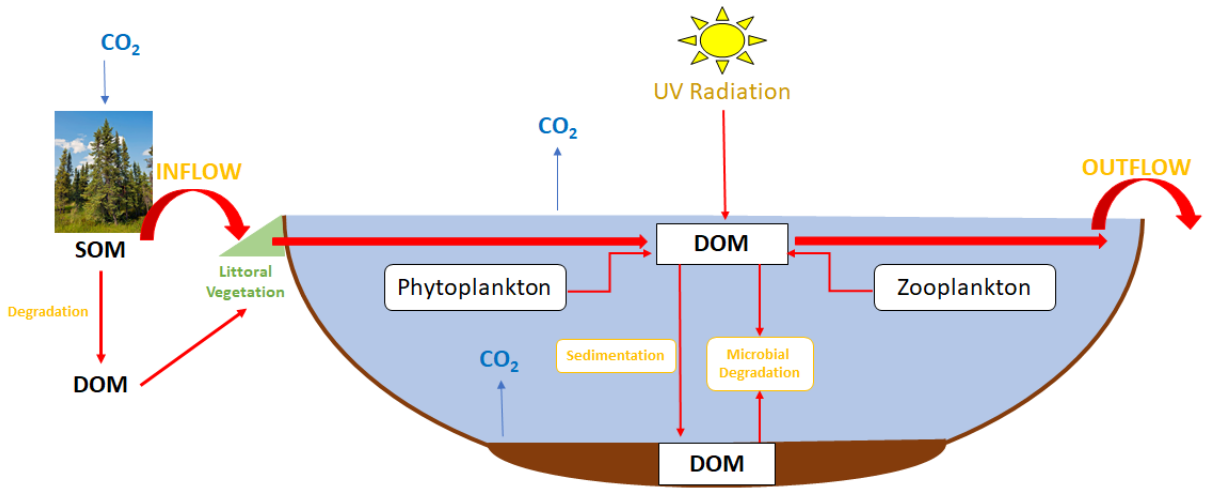


Figure 25: Flux of DOM in a fresh water lake. Diagram recreated from (Tulonen, 2004). Permission to reproduce this figure was provided by T. Tulonen. See Appendix C for copyright permission.

The composition of DOM in freshwaters is largely dominated by dissolved AHS which can be as much as 80% of the total DOM (or DOC) (Tan, 2014; Steinberg & Muenster, 1986). Since the concentration of DOM in lakes is largely allochthonous in nature, it is thought that the composition of aquatic humic substances is not drastically different from that soil humic matter, with low nitrogen content and high amounts of aromatic and phenolic moieties (Tan, 2014; Steinberg & Muenster, 1986). However, autochthonous humic matter would have higher nitrogen content and lower aromaticity and phenolic components as the synthesizing materials are low in lignin content (Tan, 2014). The formation of autochthonous humic matter in aquatic environments more likely follows the sugar-amine condensation theory outlined in Figure 16. It is probable that in nearshore regions allochthonous DOM would also affect the composition of autochthonous humic substances owing to why some feel humic substances in the aquatic environment are more complex than within SOM (Tan, 2014).

2.2.2 Isolation and Characterization of Aquatic DOM

Prior to the 1960s, much of the research into humic substances was centered around extracting and isolating these compounds from soil. However, in the last 50 or so years, greater attention has been given to the presence of these compounds in aquatic and marine environments due to growing public concerns for general water quality, largely those used as drinking water sources (Aiken, 1985; Thurman & Malcolm, 1981).

Techniques for isolating and fractionating humic substances from soils would, in many cases, be applicable for water analysis; however, with water, there is a need to concentrate the humic matter as it is generally in low concentration compared to humic matter is soils (Tan, 2014; Aiken, 1985).

There are several methods available to fractionate AOM based on either the molecular weight or size of the fraction, or on the chemical properties of a specific group, or fraction, of AOM. The following sections will describe some of the common techniques used to isolate fractions of DOM found in aquatic environments, along with their advantages and disadvantages.

2.2.2.1 Fractionation Based on Molecular Weight or Size

2.2.2.1.1 Membranes

AOM can be separated into different size fractions by filters, such as membrane or granular filters (Tan, 2014). This separation is achieved via a sieving action, where particles that are smaller than the pore size are able to pass through the membrane, while those larger than the pore size would be retained. A common example of this is the use of a 0.45 μm membrane filter to separate the dissolved from the particulate fraction of aquatic organic matter (Tan, 2014). Along with advective flow, the movement of molecules through a membrane is also based upon molecular diffusion, which is a function of both molecular size and shape. For example, large molecules will move across a

membrane slower compared to smaller molecules; likewise, spherical molecules will diffuse more readily than linear molecules of the same molecular weight. Another factor affecting the movement of molecules across a membrane would be the concentration gradient. The greater the difference in concentration between the feed side and the permeate side of the membrane, the more rapidly a given molecule will move through the membrane (Amy, et al., 1987).

A common problem with the use of membranes for isolating specific size fractions of DOM is pore clogging or fouling. As molecules begin to accumulate within the pores of the membrane, the pores become restricted and reduce the overall permeability of the membrane. Likewise, as molecules begin to buildup on the surface of the membrane a gel layer is formed. This phenomenon is known as concentration polarization, and as this layer increases it becomes the dominant resistance to flow. Another issue with measuring molecular weight using membranes comes from the fact that membrane pore size does not represent a distinct cut-off but is based on an average pore size (Amy, et al., 1987). A final limitation to using membranes to determine the size or molecular weight of a group of molecules is that molecular size is not necessarily equivalent to molecular weight. Molecular shape will affect the apparent size of a molecule, for example, a linear molecule will have a greater radius than a spherical molecule of the same molecular weight, affecting both the movement through, or the retention by, a membrane with a particular molecular weight cut-off (Tan, 2014; Amy, et al., 1987).

2.2.2.1.2 Size Exclusion Chromatography

Another method for fractionating organic molecules based on molecular weight is gel chromatography, or now commonly referred to as size exclusion chromatography. In SEC, the gel, or resin, is characterized by a range of molecular weight which can be fractioned by a specific resin. The most common resin used to fractionate humic matter is Sephadex, which is composed of cross-linked

polydextrane beads. As the degree of crosslinking increases, the pore size of the beads decreases, allowing for the isolation of smaller molecules (Tan, 2014; Amy, et al., 1987). In theory, the gel beads represent a stationary phase, while the solution containing the solutes represent the mobile phase. As the mobile phase moves through the column via gravity, solutes larger than the pore size of the stationary phase will be forced to move around the bead, equating to a shorter retention time. Smaller molecules will enter the pores of the beads equating to a longer retention time within the column (Figure 26) (Skoog, et al., 2007).

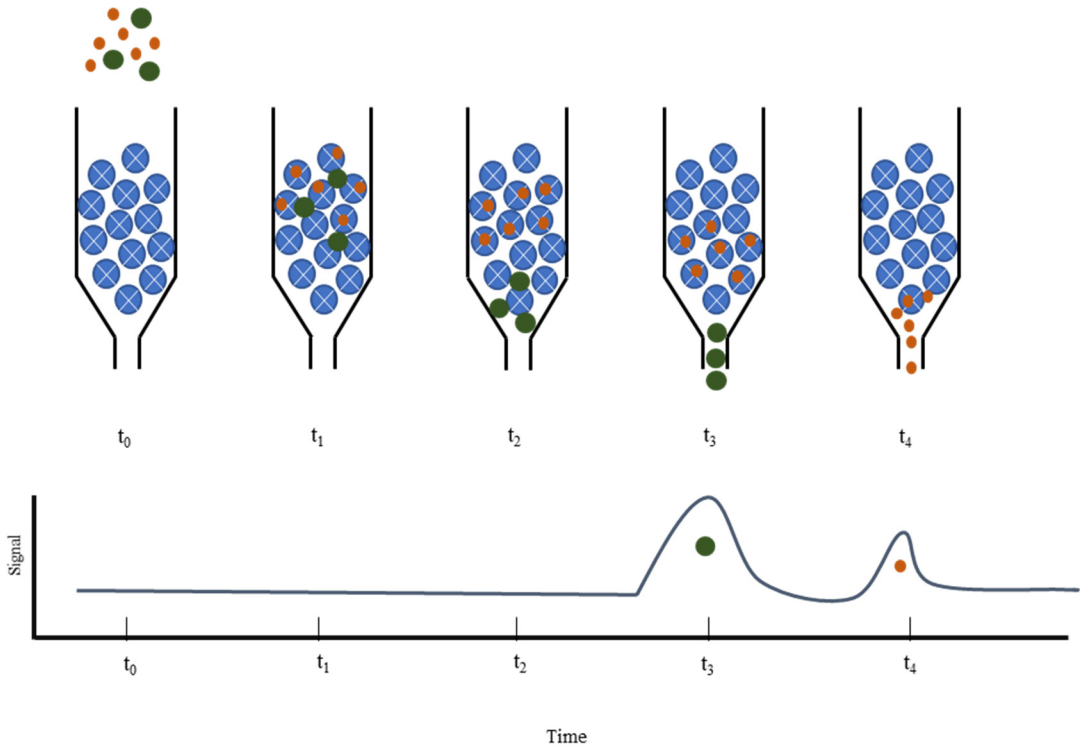


Figure 26: Graphical representation for the separation of high molecular weight (green) and low molecular weight (orange) molecules by size exclusion chromatography. Figure adapted from (Skoog, et al., 2007)

The SEC technique described above is known as low-pressure size exclusion chromatography (LPSEC), since the mobile phase flows through the column by gravity alone (Tan, 2014). High-pressure size exclusion chromatography (HPSEC) differs from LPSEC, as the mobile phase is forced

through the column at pressures exceeding that of gravity. Therefore, smaller gel beads, or pore sizes, can be used in HPSEC, significantly improving the separation of solutes within the mobile phase, increasing resolution (Tan, 2014).

The fractionation of humic matter by SEC is dependant on several factors: (1) the concentration of humic matter in the sample, (2) type of gel, (3) calibration of the column, and (4) the composition of the eluent (Amy, et al., 1987). Since the concentration of humic matter in natural waters is generally low, there is a need to preconcentrate the sample prior to SEC fractionation, in order to apply the sample to the column as a slug, or spike. The concern is that the concentration step could alter the properties of humic matter in the sample, causing an error in molecular weight determination. As discussed earlier, humic concentrations exceeding the CMC would cause an aggregation of smaller humic molecules, leading to an overestimate of higher molecular weight molecules (Tan, 2014; Amy, et al., 1987). Similar to membrane filtration, the shape and size of molecules can also cause an error in measuring the molecular weight using SEC. If large or linear molecules buildup in the resin pores, or on the surface of the beads, it would prevent small molecules from entering the pores, causing an underestimation of low molecular weight compounds (Amy, et al., 1987). Another issue with using SEC to determine molecular weight is that some resins contain carboxyl groups that could interact with humic molecules. This could result in retardation of negatively charged solutes by the resin, allowing them to move faster through the resin bed than uncharged solutes of the same size, resulting in an overestimation of molecular weight compounds (Amy, et al., 1987). The use of standard calibration chemicals for calibrating the retention times for various molecular weight humic compounds is another concern when using SEC. Since humic matter contains numerous unidentified compounds, there is no standard that can be used to calibrate the column. Often biochemicals or synthetic compounds, believed to be similar to the molecular weight of

humic fractions, are used to calibrate the column. However, these chemicals may have different physical and chemical properties than true humic matter, raising concern with the ability of SEC to accurately separate humic matter based on molecular weight (Amy, et al., 1987). The final limitation of SEC comes with the composition of the eluent used, as the eluent can affect both the humic material as well as with the humic-gel interaction. Humic-gel interactions are generally due to both electrostatic interactions and Van der Waal's forces, which are greatly affected by the ionic strength of the eluent. It has been reported that alkaline buffers are best suited to elute humic matter when using SEC (Amy, et al., 1987).

The use of SEC to determine the molecular weight of humic matter is a contentious topic among polymer theorists, and those who view humic matter as an aggregation of smaller molecules. Refining the current understanding of the composition of humic matter in aquatic environments, along with the development of adequate calibration standards and resins, the limitations of SEC could be overcome, allowing for accurate estimations of the molecular weights of humic material.

2.2.2.2 Fractionation Based on Chemical Sorption to a Solid Phase

The most common approach to isolate distinct fractions of DOM with specific chemical properties is through sorption onto a chromatographic media (Aiken & Leenheer, 1993; Aiken, 1985). Isolation of DOM occurs through preferential sorption onto the media based on specific chemical interactions between the stationary phase (sorbent) and the organic fraction of interest (sorbate). In the past 70 years, several types of sorbents, both ion-exchange and non-ionic macroporous resins, have been successfully used to isolate fractions of DOM, allowing for further investigation and testing of the reactivity and chemical properties of each fraction (Aiken & Leenheer, 1993). Adsorption chromatography methods have several advantages to other isolation or concentration methods. First,

large volumes of water can be easily handled allowing for high concentration factors for the isolated organic solutes. The fractionation method can be tailored to isolate a specific fraction of DOM based on sorption characteristics of the sorbent and chemical characteristics of the solute of interest. Finally, the sorbent can often be regenerated reducing laboratory costs (Aiken, 1985). Some disadvantages with sorption chromatography methods is that the sorbent may interact with the solute via multiple different sorption mechanisms, increasing the chance of irreversible sorption, or the occurrence of unwanted sorption/desorption processes (Aiken & Leenheer, 1993). Furthermore, changes in pH and the use of various solvents used during the fractionation process could alter the chemical structure of the isolated DOM fraction, raising concern to whether the isolated fraction is representative of the physiochemical properties of the fraction in natural conditions (Tan, 2014; Aiken, 1985). Finally, because DOM is composed of numerous organic compounds, each with varying degrees of molecular size, shape, polarity, and aqueous solubility there is no distinct cut-off between organic matter fractions, and likely some overlap between fractions occurs (Aiken & Leenheer, 1993).

2.2.2.2.1 Alumina, Polyamide and Carbon Sorbents

Sorbents, such as alumina, polyamide, or carbon, have be used to isolate humic matter from water with limited success. For example, alumina has acidic binding sites and is capable of charge-transfer interactions due to the presence of electron acceptor sites. This increases the irreversible binding of humic matter from alumina which equates to low percent recoveries (Aiken, 1985). Similar issues have been reported when using polyamide to isolate humic matter, where strong hydrogen bonding and hydrophobic interactions cause irreversible sorption and poor recovery (Aiken, 1985). The small pores of some sorbents are also problematic as they can exclude larger molecules, lowering capacity or clogging the pores of the sorbent impacting the elution step (Aiken, 1985). Another

sorbent that was used, with limited success, to isolate humic matter from waters is activated carbon. Although large volumes of water can be tested, activated carbon has several major drawbacks that made it an inadequate sorbent material for qualitatively or quantitatively isolating humic matter from water. Activated carbon is heterogeneous in nature and therefore can isolate organic compounds from water by several mechanisms which cause both irreversible sorption, slow elution rates, and poor recoveries of some groups of organic compounds (Liska, 2000; Aiken, 1985). To overcome some of the limitations with traditional sorbents, efforts to develop more homogeneous sorbents capable of targeting the removal of specific groups, or fractions, of DOM were pursued.

2.2.2.2.2 The XAD Resins

In the 1960s, a series of non-ionic synthetic polymeric macroporous resins developed by Rohm and Haas, the Amberlite XAD Series, gained interest as suitable replacements for previous SPE sorbents, such as carbon or polyamide (Liska, 2000; Thurman, 1985). The XAD resins are macroporous copolymers of either styrene divinylbenzene (ST-DVB) (XAD-1, XAD-2, and XAD-4) or acrylic esters (XAD-7 and XAD-8) with large surface areas, increasing the adsorptive capacity and recovery of humic substances (Aiken, 1985). For example, Mantoura and Riley (1975) tested the percent sorption and recovery of humic and fulvic acids from natural waters using either nylon (polyamide) or the XAD-2 resin. The results from the Mantoura and Riley study found that the polyamide column sorbed only 71% HA compared to 92% using XAD-2. Furthermore, the recovery of HA from XAD-2 was >95%, while only 54% of the HA was recovered from the polyamide resin (Mantoura & Riley, 1975). Similar results to the Mantoura and Riley study were reported by Stuermer and Harvey (1977) for the isolation of humic substances from seawater using the XAD-2 resin (Aiken, 1985).

The most noted advancements in the extraction and isolation of aquatic humic substances from natural water systems using the XAD resins was achieved by researchers at the USGS. The most recognized being the work by Thurman and Malcolm (1981), who used the XAD-8 resin to isolate AHS from surface and ground water. As mentioned, the XAD-8 resin is composed of a non-ionic polymethyl methacrylate resin, where the principle mechanism for isolating AHS are weak, hydrophobic interactions (Aiken, 1985; Thurman, 1985; Thurman & Malcolm, 1981). Since the carboxyl groups in humic material are in the ionic state at the pH of natural waters (~pH = 7), the sample must be acidified to increase the hydrophobicity of AHS (Thurman, 1985). Figure 27 presents the relationship between the pH of the sample and the distribution coefficient (K_D) for fulvic acid onto the XAD-8 resin (Aiken, 1985; Aiken, et al., 1979). The results show a significant reduction in K_D as the pH increases, due to the increase in polarity of the humic substances as carboxyl groups become deprotonated. Since the acid dissociation constant (pKa) is ~4 for carboxylic acid groups on humic matter, there is less change in the K_D at pH >4 (Aiken, et al., 1979). Although there is greater sorption of AHS to the XAD-8 resin at pH ~1.5, Aiken et al. (1979) suggest that pH< 2.0 be avoided as lower pH could cause denaturation of humic matter. The weak, non-ionic, hydrophobic interactions between the humic matter and the XAD-8 resin allows for easy desorption of the sorbed humic matter by increasing the pH, which causes deprotonation of the carboxyl groups of AHS, decreasing the favorable interactions between the sorbent and sorbed AHS (Aiken, 1985; Thurman & Malcolm, 1981). Figure 28 gives the generalized method for extracting AHS from natural waters using the XAD-8 resin, as developed by Thurman and Malcolm (1981).

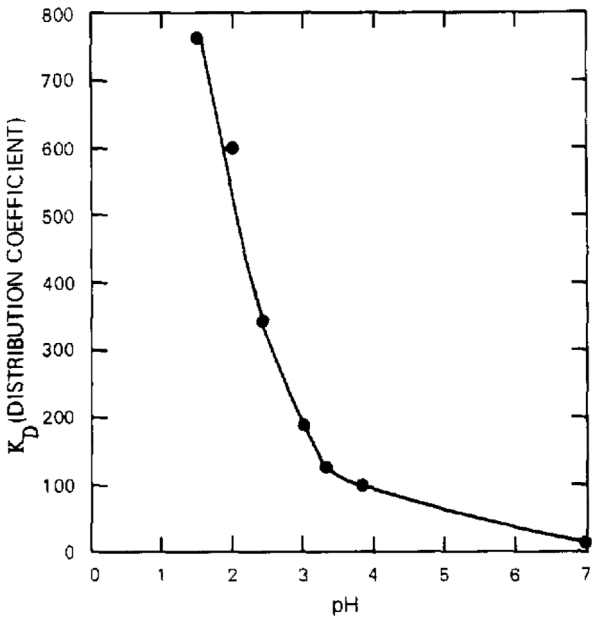


Figure 27: The relationship between pH and the distribution coefficient for fulvic acid on XAD-8 Resin. Figure reprinted from (Aiken, et al., 1979). Copyright (1979) American Chemical Society. See Appendix C for copyright permission.

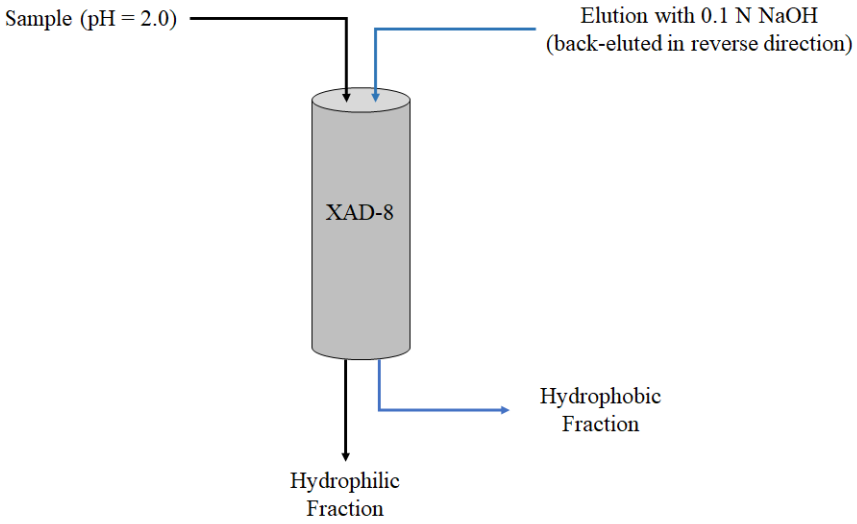


Figure 28: Fractionation of aquatic humic substances using the XAD-8 resin. Figure created from (Aiken and Leenheer, 1993).

Leenheer (1981) used the AHS isolation procedure of Thurman and Malcolm as part of a DOM fractionation method which used the XAD-8 resin, a strong cation exchanger (AG-MP-50), and a

weak anion exchanger (Duolite A-7) (Figure 29). This method isolates DOM into the following six fractions based on the hydrophobicity and acid/base functionality: hydrophobic acid (HPOA), hydrophobic base (HPOB), hydrophobic neutral (HPON), hydrophilic acid (HPIA), hydrophilic base (HPIB), and hydrophilic neutral (HPON) components. The HPO fractions are all retained on the XAD-8 resin while the HPI fractions are that which are not contained on the XAD-8 column. The HPI fractions are separated using the Bio-Rad AG-MP-50 column (strong cation exchange resin), which retains the HPIB, followed by the Duolite A-7 resin (weak anion exchange resin), retaining the HPIA fraction, while the HPIN fraction is that which does not adsorb to any of the three resins (Leenheer, 1981). The HPIN fraction is composed of short chain aliphatic amines, alcohols, ketones, aldehydes, and esters; <C5 aliphatic amides; carbohydrates and polysaccharides. Short chain (<C5) aliphatic carboxylic acids, polyfunctional carboxylic acids, and hydroxyl acids make up the HPIA fraction, while amphoteric proteinaceous materials such as aliphatic amino acids, amino sugars, proteins and peptides comprise the HPIB fraction. The HPOB fraction is made up of 1- and 2- ring aromatic amines, except pyridine which is a HPIB. HPON compounds are a mix of hydrocarbons such as >C5 aliphatic alcohols, amides, esters, ketones and aldehydes, as well as long chain >C9 aliphatic carboxylic acids and amines. Carboxylic acids and amines with >3 rings are also HPON in nature. The HPOA fraction contains humic substances, fulvic acids, C5-C9 aliphatic carboxylic acids, 1- and 2- ring aromatic carboxylic acids, and 1-and 2- ring phenols (Świetlik, 2004).

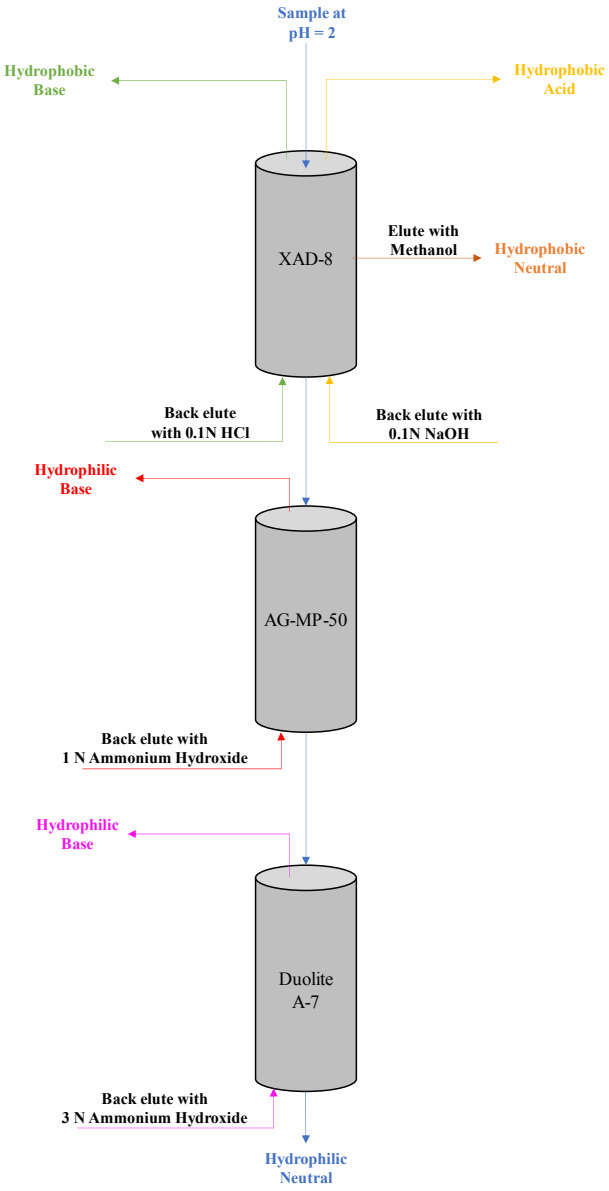


Figure 29: Leenheer method for isolating DOM into six fractions based on hydrophobicity and acid/base functionality. Figure adapted from (Leenheer, 1981).

These two methods are likely the most widely used DOM fractionation methods, with both works each being cited by over 1000 peer review journal articles. Furthermore, the Thurman and Malcolm method (1981) is the procedure recommended by the International Humic Substances

Society for the isolation of aquatic humic substances and the only one of two isolation methods found in *Standard Methods for the Examination of Water and Wastewater* (APHA, 2017).

Although the XAD-8 is widely used among soil and aquatic science researchers, the procedure itself is quite laborious and requires specialized equipment (e.g., Soxhlet reactor) which are typically only found in research laboratories. According to the procedure found in *Standard Methods 5510C*, the XAD resin requires a 0.1N NaOH rinse for 5-days, followed by successive daily washes in the Soxhlet reactor with methanol, hexane, acetonitrile, and finally methanol, totalling 9-days before the resin is ready for use (APHA, 2017). The method also indicates that the sample should be added to the XAD column at a flow rate of 1.0 mL/min equating to 16 hours to fractionate a 1L sample. These shortcomings impact the suitability of the XAD method for onsite monitoring of compositional changes in DOM character and limit the use of XAD fractionation to research settings.

2.2.2.2.3 Solid Phase Extraction

The XAD method is labor intensive, lengthy (9-12 days), and requires specialized equipment (Louchouarn *et al.*, 2000). Hence, the XAD method has been mainly limited to research studies to establish the DOM composition in natural environments and is not commonly used to monitor DOM composition on a continual basis in an operating water treatment plant. Recently, SPE has been shown to be an attractive alternative to the XAD method for isolating the HPO fraction (i.e., humic and fulvic acids) from fresh and marine waters (Green, et al., 2014; Tfaily, et al., 2012; Dittmar, et al., 2008). Silica-based sorbents, such as silica-C18, have advantages over the XAD method, including short resin preparation times and improved recovery and extraction efficiency (Kim, et al., 2003; Louchouarn, et al., 2000; Amador, et al., 1990). An *N*-vinylpyrrolidone (NVP) SPE was successfully used to isolate dissolved humic matter from nine eutrophic lakes in Poland (Glazewski & Wojcik, 2009). Polystyrene

divinylbenzene SPEs (Bond Elute ENV and Bond Elute PPL) were reported to improve extraction and recovery of DOM compared to silica-C18 SPEs (Dittmar, et al., 2008). The Dittmar *et al.* study concluded that the reduction in sample preparation and testing times for the ST-DVB SPE method made it more suitable than the XAD method for onsite monitoring of HPO DOM.

Simplification of the resin and sample preparation of commercially available pre-packaged SPE cartridges provide a robust method for isolating the HPO, or humic, fraction of organic matter from water. This has allowed researchers to apply SPE fractionation methods in a wider variety of situations (i.e., onsite separation of dissolved organic matter). One particular situation where it would be useful to have a simple technique for measuring the composition of DOM is in potable water treatment, where DOM, and its fractions, can negatively impact both water treatment and finished water quality.

2.3 Dissolved Organic Matter and Drinking Water Treatment

The presence of DOM in drinking water sources can negatively impact water treatment plant operations, by increasing the demand for chemical coagulants (Sadrnourmahamadi, et al., 2013) or fouling membranes and filters (Peiris, et al., 2010), the two most commonly employed methods for treating surface water. Elevated concentration of DOM in treated drinking water can cause aesthetic concerns, such as increased colour, taste and odour. Furthermore, DOM can bind with organic pollutants and inorganic contaminants decreasing the likelihood of removing these contaminants by conventional treatment methods (Leenheer & Croué, 2003). More importantly, if DOM is not adequately removed, there is greater potential to form halogenated DBPs following disinfection with chlorine. Many of these products are known, or suspected, carcinogens which are regulated reduce concentrations of these compounds in public drinking water supplies.

The following sections will largely focus on potable water disinfection, the role DOM plays in the formation of DBPs following disinfection with chlorine, the presence of DBPs in public water supplies and the potential health concerns and regulations regarding the most common group of DBPs found in public water supplies, THMs. The final section of this chapter will present methods for reducing organic matter in drinking water to prevent the formation of THMs and other DBPs.

2.3.1 A Brief History of Chlorination Disinfection of Drinking Water

Chlorine is a strong oxidizing agent that can destroy cell membranes and oxidize proteins and enzymes (White, 2010). Chlorine has been shown to destroy, or inactivate, many waterborne bacteria and viral pathogens such as *Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli, Campylobacter, Giardia lamblia, Cryptosporidium,* and hepatitis A (White, 2010). Disinfection of drinking water supplies began in the early 1900s following the discovery by Dr. John Snow that the cholera outbreak in London, England in 1855 was due to the presence of *Vibro cholerae* in a public well contaminated by sewage (White, 2010; United States Environmental Protection Agency, 2000). Chlorination disinfection of drinking water was first applied in a small town in Belgium in 1902 as a mixture of ferric chloride, used for coagulation, and calcium hypochlorite, forming ferric hydroxide floc and hypochlorous acid (White, 2010). This is believed to be the first documented use of continuous chlorine disinfection for drinking water.

Chlorine disinfection was first used in North America in 1908 at the Bubbly Creek Filter Plant in Chicago, USA (White, 2010). In 1908, Jersey City, New Jersey, also began to use chlorine as a primary disinfectant for public drinking water. In 1914, the US Public Health Service implemented federal regulations for the presence of bacteriological species in drinking water (United States Environmental Protection Agency, 2000). As a result of these standards, and more stringent

regulations set by the Public Health Service between the 1920s and early 1960s, more public water systems were disinfected using chlorine, or chlorination products, to reduce the presence of pathogens (United States Environmental Protection Agency, 2000).

Since then, nearly all public water supplies in developed countries are disinfected and nearly 90% of all public water supplies in Canada use chlorine as the primary disinfectant (Chowdhury, et al., 2011). Instances of disease outbreaks due to the presence of waterborne pathogens in drinking water supplies have largely been eliminated, except for isolated incidents (e.g., Walkerton, Ontario, Canada) in which the disinfection process or pathogen monitoring was compromised usually due to operator negligence (Hrudey, 2009).

2.3.2 The Discovery of Drinking Water Disinfection By-products

From the early 1900s it was largely believed by the general public that drinking water disinfected by chlorine was safe, and to a large extent it was. However, it wasn't until the 1960s and the publication of an influential work by Rachel Carson, *Silent Spring*, which evaluated the detrimental environmental effects of the use of dichlorodiphenyltrichloroethane (DDT) as a pesticide, that regulatory agencies and the public became concerned with the possible presence of man-made chemicals in public drinking water (Carson, 1962; Hrudey, 2009). Further public concerns came from a 1964 report by the World Health Organization (WHO) who suggested that three quarters of all cancers were caused by environmental factors, such as exposure to man-made chemicals (Hrudey & Krewski, 1995).

The increasing concern for public safety due to the possible presence of man-made carcinogenic compounds in drinking water prompted several ground-breaking studies to be conducted which evaluated, and identified, the presence of potential carcinogenic compounds in drinking waters.

Two independent publications in 1974 by Beller et al. and J.J. Rook found the presence of organo-halides in public water supplies that were disinfected using chlorine (Bellar, et al., 1974; Rook, 1974). Both studies identified the presence of chloroform (trichloromethane) and chlorinated-brominated THMs. These two studies provided strong evidence that these compounds were formed following a reaction between chlorine, used as a disinfectant, and DOM, more particularly the humic and fulvic fractions of DOM (Bellar, et al., 1974; Rook, 1974). In 1977, Rook added halogenated acetic acids (HAAs), chlorophenols, haloacetones, and chlorobenzenes to the growing list of compounds that are formed when chlorine disinfection was applied to surface waters (Rook, 1977). In 1976, the US Environmental Protection Agency (USEPA) conducted a national survey which found that chloroform and other THMs were present in nearly all drinking waters that disinfect surface waters with chlorine (Richardson, 2003).

Since the discovery of THMs, and other halogenated DBPs, by Beller et al. and Rook in the early 1970s, along with the advancement of analytical instrumentation and techniques, nearly 600 DBPs have been found identified in public water supplies, most a result of chlorine disinfection (Hrudey, 2009). Furthermore, it is suspected that many of these compounds have more toxic and carcinogenic potential than THMs and that anywhere from 50-80% of chlorinated DBPs are yet to be identified (Richardson, 2003).

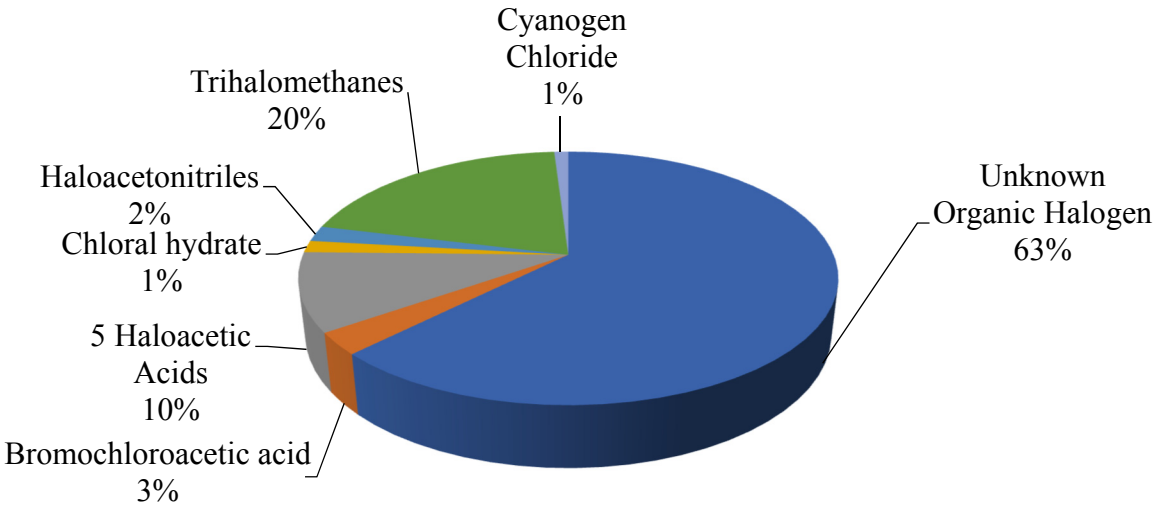


Figure 30: Relative percent of halogenated disinfection by-products as a proportion of total organic halogen (TOX) in chlorinated drinking waters. Figure recreated from (Richardson, 2003). Figure recreated with permission from Elsevier and the Copyright Clearance Center Inc. See Appendix C for copyright permission.

2.3.3 Dissolved Organic Matter and Trihalomethane Formation

In 1974, when Rook discovered the presence of THMs in chlorinated drinking waters, he proposed that these compounds were likely formed following a haloform reaction between chlorine and the natural polyhydroxybenzene compounds associated with colour in surface waters (i.e., humic matter) (Rook, 1974). In 1977, Rook provided further evidence that humic matter was the major precursor to halogenated DBP formation by investigating the formation of chloroform from model compounds of fulvic acid (Rook, 1977). Rook believed that hydroxylated aromatic rings with two free meta positioned OH-groups could be an available reaction site for haloform formation. Figure 31 is a proposed reaction mechanism by Rook (1977) for fulvic acids and resorcinol degradation to form chloroform.

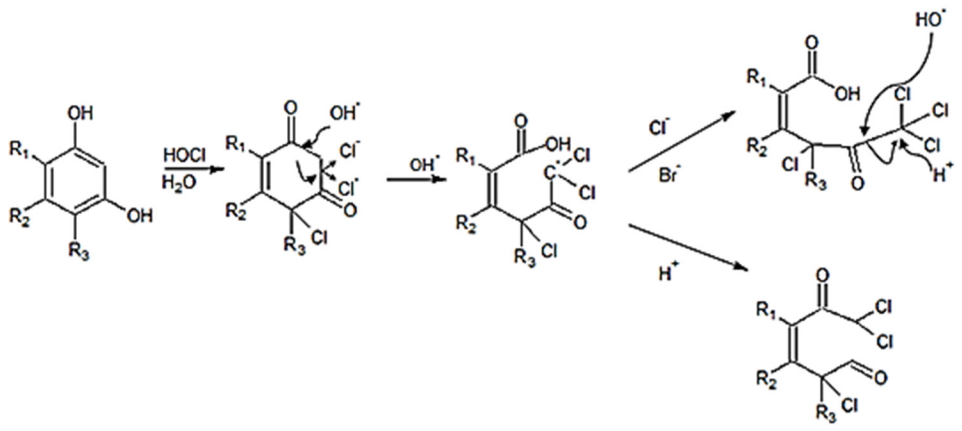


Figure 31: Degradative pathway of fulvic acids and resorcinol proposed by Rook (1977). Figure adapted from Rook, (1977). Copyright 1977 American Chemical Society. See Appendix C for copyright permission.

Given the vast complexity of DOM it is difficult to determine the exact reaction mechanism for the formation of these compounds, and likely several different mechanisms are occurring simultaneously. That being said, literature has largely implicated the humic fraction as being the most reactive to THM formation (Singer, 1999).

Reckhow et al. (1990) analyzed ten different humic and fulvic acid samples, from five water sources, for their halogenated by-product formation potential. This study wanted to establish a relationship between halogenated by-product formation potentials and the fundamental properties of different humic sources (Reckhow, et al., 1990). The ten samples of humic and fulvic acids ranged from 30-35% aromatic character for humic acids and from 14-17% for the fulvic acids. All samples were shown to have varying degrees of molecular size, acidic groups and specific UV absorbance at 254 nm and 400 nm. The results from the Reckhow et al. study found that each sample formed significantly different concentrations of halogenated by-products for the same concentration of total organic carbon (10 mg/L) of each humic and fulvic acid sample. Furthermore, the results found that in all five sources the humic acid fraction formed greater concentrations of all halogenated by-products

studied compared to the fulvic acid fraction from the same source. This group further reported that, in general, the activated or electron rich aromatics have a greater reactivity to react with chlorine and that humic matters rich in activated aromatic centers will react to a greater degree (Reckhow et al., 1990).

2.3.4 Trihalomethanes: Health Implications, Regulations, and Future Directions

Public health and regulatory agencies have set limits, or guidelines, for the presence of microorganisms and chemicals. In the USA, prior to the 1974, drinking water guidelines were largely based on aesthetics concerns (i.e., color, taste, and odour) the presence of some metals and elements, and disinfection to control microbial populations (United States Public Health Service, 1962). However, following the identification of THMs in public water supplies, the US Federal Government implemented the Safe Drinking Water Act (SDWA), which allowed the USEPA to set more stringent standards, limits, and recommendations to protect public drinking water and water supplies (Richardson, et al., 2002). In 1979, under the SDWA, a regulatory limit for the presence of four THMs (i.e., chloroform, bromoform, bromodichloromethane (BDCM), and dibromochloromethane (DBCM)) was set to 100 µg/L for a total of the four THMs based on an annual average with samples collected quarterly (Richardson, et al., 2002). In 1998, the USEPA instigated the Stage 1 Disinfectants/Disinfection By-product Rule (Stage 1-DBPR) which required that chlorine DBPs be reduced in potable water supplies. The focus of Stage 1-DBPR was to reduce the overall exposure of disinfection by-products, as well as the residuals for disinfectants, suspected of having carcinogenic properties (United States Environmental Protection Agency, 1998). The Stage 2-DBPR was implemented in 2006 which set the maximum contaminant level (MCL) to 80 µg/L for THMs based on a running quarterly annual average (United States Environmental Protection Agency, 2006).

In 1978, Canada implemented an MCL of 350 µg/L for total THMs. However, in 2006 Health Canada took a more assertive approach and reduced the limits to 100 µg/L to be more consistent with the USEPA limits (Hrudey, 2009; Health Canada, 2006).

The WHO's *International Standards for Drinking Water* prior to 1984 did not provide guidelines or limits for THMs (World Health Organization , 2008). In 1984, the WHO published the first edition of the *Guidelines for Drinking Water Quality*; however, there was only a guideline of 300 µg/L for chloroform as there was limited information regarding the other THMs (World Health Organization , 2008). The second edition, published in 1993, did not set limits for total THMs; however, did establish individual limits for the four THMs separately. The 1993 guideline limits for the four THMs were 100 µg/L for bromoform and DBCM, 60 µg/L for BDCM, and 200 µg/L for chloroform (World Health Organization , 2008). The WHO suggested to authorities wanting to establish a total THM regulation to use a fraction-based approach where the sum of each THM normalized to its guideline value be <1 (Equation 1). These guidelines were carried forward for the 3rd edition of the *Guidelines for Drinking Water Quality* in 2008.

$$\frac{Conc.chloroform}{Reg.Chloroform} + \frac{Conc.BDCM}{Reg.BDCM} + \frac{Conc.DBCM}{Reg.DBCM} + \frac{Conc.Bromoform}{Reg.Bromoform} \leq 1 \qquad \text{(Equation 1)}$$

In the 4th Edition of the *Guidelines for Drinking Water Quality*, the limit for individual THMs have remained the same, except for chloroform which was increased from 200 µg/L to 300 µg/L (WHO, 2011). A summary of the current regulations for THMs according to the WHO, USEPA, and Health Canada are presented in Table 1.

Table 1: Current THM regulations according to the WHO, USEPA and Health Canada.

Regulatory Agency	Chloroform	Bromoform	BDCM	DBCM
		(µg/L)		
World Health Organization	300	100	60	100
US Environmental Protection Agen		80		
Health Canada		100		

The regulations for THMs set by the USEPA, Health Canada, and the WHO are based on toxicological studies, both *in vitro* and *in vivo*, as well as epidemiological studies which looked at the potential impact of THMs in drinking water on human health. There is some who feel that THM regulations may be further reduced in the near future. It is suspected that the further reduction of THMs, and other regulated chlorination DBPs such as HAAs, is due to the belief that controlling THM formation may also prevent the formation of other halogenated by-products that are identified, but not yet regulated, or that remain unidentified. However, there is evidence suggesting that applying methods to reduce THMs (i.e., using alternative disinfectants or additional treatment processes) may in fact produce other DBPs which could be more toxic than THMs (Hrudey, 2009; Krasner, et al., 2006). Therefore, caution should be used when applying strategies to further reduce THMs in order to control other emerging DBPs.

The following sections will review some of the toxicological studies investigating the carcinogenicity of THMs, as well as, epidemiological studies looking at the impacts on increased cancer, along with reproduction and loss of pregnancy, conducted over the last few decades. Understanding the health implications of exposure to THMs, and other DBPs, is important for gauging the risks and associated regulatory limits set for these compounds in drinking water.

2.3.4.1 Health Risks Associated with Exposure to THMs

2.3.4.1.1 Carcinogenicity

Studies evaluating the carcinogenicity of chloroform have been conducted for over 65 years. Prior to the 1970s there were limited studies that looked at the carcinogenicity of chloroform. However, following the works by Beller et al. and Rook, greater attention was given to understanding the impacts chloroform, the main THM in drinking waters, had on causing cancer.

In 1945, the U.S. National Cancer Institute (NCI) conducted the first study to evaluate the carcinogenicity of chloroform (Eschenbrenner & Miller, 1945). This early study administered high doses of chloroform and carbon tetrachloride by stomach tube (gavage) to both male and female mice in order to assess the toxic effects. The chemicals were given to the mice every 4 days for a total of 30 doses, following which the animals were tested for the presence of liver and kidney necrosis. The Eschenbrenner and Miller study found that chloroform administered at the highest doses (400-1600 mg/kg) induced liver necrosis and hepatomas in both male and female mice. Furthermore, the study found that no dose administered was able to induce kidney necrosis in male mice but did cause kidney damage to female mice, again only at the highest doses (400 -1600 mg/kg). The male mice did not develop kidney necrosis likely due to the fact that they did not survive long enough to produce tumors. Although there were significant limitations of the Eschenbrenner and Miller study (i.e., number of animals tested was only 5 per sex and limited understanding of the mode of toxicity), this study suggested that cancer was caused following necrosis of liver and kidney cells and that doses low enough not to cause necrosis did not form cancer (Eschenbrenner & Miller, 1945).

In 1976, an NCI study reported that chloroform administered to B6C3F1 mice and Osborne-Mendel rats found positive results for hepatocellular carcinomas development in both species, but that

male rats also produced renal tumors (National Cancer Institute, 1976). It is important to point out a possible drawback to the NCI and the Eschenbrenner and Miller study it that the chloroform dose was administered by corn oil gavage (or with a toothpaste base). Jorgenson et al. (1985) pointed out this limitation in all previous toxicology studies of chloroform and re-evaluated the toxicological effects of chloroform on B6C3F1 mice and Osborne-Mendel by administering chloroform in the animal's drinking water (Jorgenson, et al., 1985). The results of the Jorgenson et al. study was significantly different than those reported in the previous NCI study (Table 2). Overall, Jorgenson et al. reported that the incidence of tumors in male rats did not change following chloroform treatment; however, did report a statistically significant increase in renal tumors in relation to chloroform dose, which was also observed in the NCI study (Jorgenson et al., 1985). The Jorgenson et al. study also reported no increase in the frequency of liver tumor formation in female B6C3F1 mice. This result does not agree with the previous studies that found chloroform increased liver tumor formation in female mice (Jorgenson, et al., 1985). The inability of Jorgenson et al. to reproduce the tumor formation reported in the NCI study suggests that there was a difference between the way the chloroform was administered (i.e., via corn oil gavage and drinking water). The differences in dosing could be related to chloroform being delivered in one high dose through gavage or more gradually through drinking water or that the vehicle itself may be contributing to the formation of tumors in the test animals (Jorgenson, et al., 1985; National Cancer Institute, 1976). The Jorgenson et al. study notes a study by Newberne (1979) that report increased liver tumors in rats when corn oil was introduced into the diet (Jorgenson, et al., 1985). A more recent study by Bruckner et al. (2010) investigated the influence of route of exposure of 1,1-dichloroethylene on target organ toxicity. The Bruckner et al. study suggests that the features and experimental design of toxicology studies can significantly impact the results by altering the toxicokinetics of the chemical being studied (Bruckner, et al., 2010).

Table 2: Incidences of liver and kidney tumor formation in female B6C3F1 mice from exposure to chloroform from corn oil gavage (National Cancer Institute, 1976) and drinking water (Jorgenson et al., 1985). Table reproduced from (Larson, et al., 1994) with permission of Oxford University Press and the Copyright Clearance Center Inc. See Appendix C for copyright permission.

	Chloroform in water (ppm)	Daily Dose (mg/kg)	Liver Tumor Incidence		Kidney Tumor Incidence	
			No.	%	No.	%
Oil Gavage (NCI, 1976)						
Female Mouse	-	0	0/20	0	0/20	0
Female Mouse	-	238	36/45	80	0/45	0
Female Mouse	-	477	39/41	95	0/40	0
Drinking Water (Jorgenson *et al.*, 1985)						
Female Mouse	0	0	0/20	0	NR	NR
Female Mouse	200	34	15/410	4	NR	NR
Female Mouse	400	65	9/142	6	NR	NR
Female Mouse	900	130	0/47	0	NR	NR
Female Mouse	1800	263	Jan-44	2	NR	NR

*NR = Not recorded

Butterworth et al. (1995) published a report aimed at establishing a strategy based on determining the mode(s) of action for assessing a chemical's carcinogenicity (Butterworth, et al., 1995). Butterworth et al. suggested that several of the default assumptions used in estimating a contaminants potential effects in people may not be applicable to all chemicals. Several assumptions that Butterworth et al. point out are: (1) that humans are as sensitive as the most sensitive rodent species to the carcinogenic effects of a chemical, (2) that all carcinogens act via a mutagenic mechanism and that they act in a manner analogous to radiation induced tumors where a linear, non-threshold, does-response relationship is assumed at low doses, and (3) a theoretical increase in risk can be calculated for exposure to one molecule of a chemical (Butterworth, et al., 1995). Butterworth et al. believed that it was more appropriate to classify carcinogens based on mode of action rather than the earlier approach which classified carcinogens as initiators, promoters, or complete carcinogens.

Butterworth et al. suggested a scheme which describes chemicals as genotoxicants or nongenotoxicants, which the authors point out is similar to the Cohen and Ellwein model (Butterworth, et al., 1995). Table 3 provides the carcinogen classification scheme proposed by Butterworth et al. based on mode of action.

Table 3: Butterworth et al. scheme for the classification of carcinogens based on mode of action. Figure recreated from (Butterworth et al., 1995) with permission from Elsevier and the Copyright Clearance Center Inc. See Appendix C for copyright permission.

Classification	Mode of Action
Genotoxic carcinogen	• Deoxyribonucleic acid (DNA) reactive or DNA reactive metaboli • Direct interaction to alter chromosome structure or number • May also be cytotoxic • Regenerative cell proliferation will enhance mutagenic/carcinoge activity
Cytotoxicant (Nongenotoxic)	• Not DNA reactive • Cytolethal at carcinogenic doses • Induce regenerative growth • Mutations may occur secondary to regenerative proliferation • Accompanying critical effects may occur such as inflammation • Circulating growth factors may cause preferential growth of preneoplastic cells
Mitogens (Nongenotoxic)	• Not DNA reactive • Not cytolethal at carcinogenic doses • Mitogenic stimulation of growth • May act through a specific receptor • May stimulate preferential growth of preneoplastic cells

At the time of the Butterworth et al. report the carcinogen risk assessment used the linearized multistage (LMS) model, which was based on the premise that the mode of action for carcinogenesis was through DNA reactivity, and that disruption in DNA reactivity would lead to the formation of cancer. LMS has been used to assess the risk between radiation exposure and the formation of cancer. The LMS model was also accepted for use with nongenotoxic chemicals, such as THMs, because limited information was available at the time with regards to the carcinogenic process. Furthermore, it was felt that the LMS model would provide a conservative estimate of the risk for developing cancer.

However, Butterworth et al. believed that the advances in understanding the mode of action, and to some extent the mechanisms, in the process of carcinogenesis warranted a more appropriate model of risk assessment. Butterworth et al. felt that applying a threshold risk approach, which assumes there is a minimal chemical dose to elicit a toxic response and below that would produce no effects, would provide a better assessment of risk. A risk assessment based on establishing a no-observable-adverse-effect-level (NOAEL), in some cases, may be more appropriate for setting regulations for chemical exposure. Butterworth et al. suggest that if a NOAEL could be established process causing cancer, there should be no increased risk of tumors above this exposure limit (Butterworth, et al., 1995). The Butterworth et al. study went on to present examples of chemicals that would deviate from the LMS model, and were more appropriately evaluated under a threshold model, such as chloroform.

Butterworth et al. (1995) also reviewed the results found from the NCI (1976) study, the Jorgenson et al. (1985) study, and the Eschenbrenner and Miller (1945) study which reported significantly different results for cancer formation in the livers and/or kidneys of mice and rats from exposure to chloroform. Butterworth et al. felt that the previous studies support the belief that chloroform carcinogenicity was secondary to chemically induced cytotoxicity followed by cell proliferation in target tissues (Butterworth, et al., 1995). Further evidence to support the theory that cancer was secondary to cytotoxicity was provided by Larson et al. (1994) who evaluated chloroform toxicity in female B6C3F1 mice by both bolus gavage in corn oil and drinking water at doses equivalent to those used in the NCI (1976) study. Larson et al. measured the increase in liver enzymes, alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH), as well as the histological testing, to determine the toxicological effects chloroform had on mice kidney and liver. The results from the Larson et al. study found no increase in ALT or SDH occurred after 3 weeks of exposure via corn oil gavage at concentrations less than 34 mg/kg; however, there were some hepatic changes seen in the

histology testing suggesting a no-observable-effect-limit (NOEL) of 10 mg/kg/day (Larson, et al., 1994). Furthermore, Larson et al. reported no statistically significant changes in ALT or SDH in mice exposed to chloroform through drinking water and histological changes in the liver of treated mice were mild (Larson et al., 1994). These results provide evidence suggesting higher concentrations, where there is cell death followed by subsequent cell proliferation, caused an increase in cancer formation (gavage results); however, with more gradual exposure (drinking water results) the liver is able to detoxify and repair damage (Larson, et al., 1994). Therefore, if exposed to concentrations below levels which induce cell proliferation there should be no risk to cancer formation. Larson et al. concluded that risk assessment for chloroform from corn oil gavage data would give a 1/100,000 increase in cancer at 4.3 μg/L in drinking water using the LMS model. However, if one were to assess the risk using a more conservative approach, where no risk of cancer was associated with doses that did not induce cell proliferation, than a significantly higher concentration in drinking water can be viewed not to increase the risk of cancer (Larson, et al., 1994). The Butterworth et al. report concluded that if one were to use the NOAEL, (or NOEL), of 1800 mg/kg/day found in the Larson et al. or Jorgenson et al. studies, a drinking water concentration of 1.8 mg/L should not pose a risk to cancer formation (Butterworth et al., 1995). It should be noted that this value is taken from the 1800 mg/L dose in drinking water where NOAEL was seen divided by an uncertainty factor of 1000 to account for individual to individual variation (factor of 10), mouse to human extrapolation (factor of 10), and subchronic to long term exposure (factor of 10). Figure 32 gives the does-response curve for liver tumor formation from chloroform exposure to B6C3F1 mice from corn oil gavage and drinking water routes found from the NCI (1976), Jorgenson et al. (1985), and Larson et al. (1994) studies, along with the predicted tumor incidences according to the LMS model and the threshold model (Butterworth, et al., 1995). The results in Figure 32 clearly show that the prediction for cancer risk assessment in

drinking water is extremely over estimated using the LMS model, while the threshold (cell proliferation) model strongly agrees with the results for liver tumors in mice following chloroform exposure in drinking water.

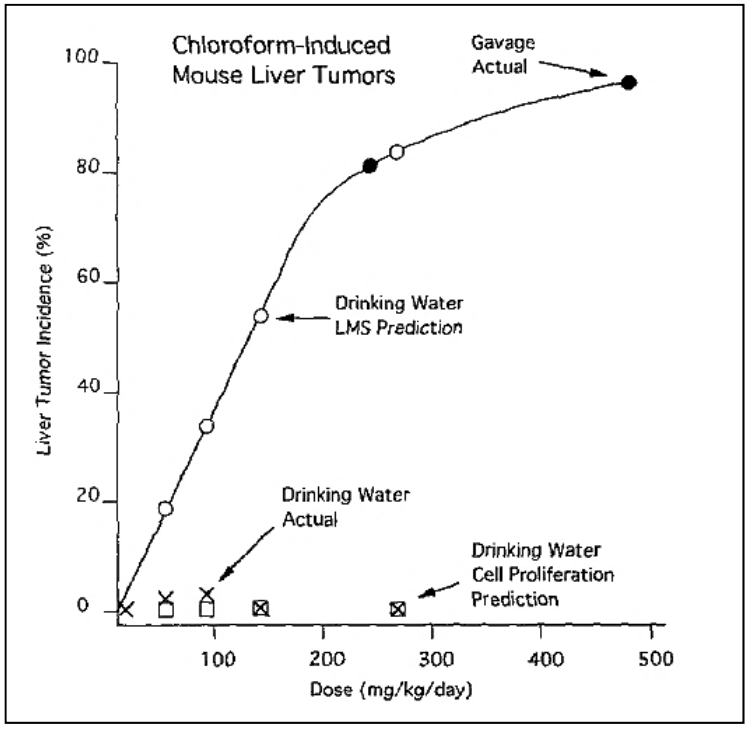


Figure 32: Dose-response curve for liver tumor formation from chloroform exposure to B6C3F1 mice from corn oil gavage and drinking water routes. Results presented are from the NCI (1976) study (●), Jorgenson et al. (1985) study (○) showing the predicted rate of tumor incidences from drinking water exposure, and the Larson *et al.* (1994) study (□) prediction using cell proliferation. The (x) are the actual incidences of liver tumor formation in mice (Butterworth *et al.*, 1995). Figure reprinted with permission from Elsevier and the Copyright Clearance Center Inc. See Appendix C for copyright permission.

From the Jorgenson et al. (1985), Larson et al. (1994) and the Butterworth et al. (1995) studies it is clear that the use of the no-threshold LMS model for predicting tumor formation from chloroform exposure in drinking water is inappropriate and may have caused unnecessary regulations to be set for the THMs. Unrealistic regulations can have a negative impact on drinking water treatment by either significantly increasing expenses to comply with low regulatory limits or increase the use alternative

disinfectants known to decrease the formation of THMs. The drawback to the use of alternative disinfectants that form lower concentrations of THMs is that the alternative disinfectant may form other groups of by-products that may be unregulated, or unidentified, and which may be more toxic than chloroform, or THMs.

2.3.4.1.2 Epidemiology

Epidemiological studies have been conducted looking at the relation of THM concentrations in drinking water to a wide range of adverse human health effects such as liver, kidney pancreas, prostate, stomach, bladder, as well as, adverse reproductive outcomes (International Programme on Chemical Safety, 2000). A complete review of this epidemiological evidence would be extensive and somewhat out of the scope of this particular work. Nevertheless, epidemiological evidence is significant in the assessment of human risks; therefore, this work will review epidemiological studies but limited to adverse reproductive outcomes. A review of adverse reproductive outcomes seems appropriate as previous sections investigated the carcinogenicity and toxicity of THMs, mainly chloroform, with little attention given to potential negative reproductive outcomes. Reproductive outcomes also provide evidence for a sensitive demographic that is not necessarily evaluated in cancer studies. Furthermore, this review will largely limit itself to studies conducted in Canada and the United States of America (USA) due to the stringent THM limits imposed by the Health Canada and the USEPA. Extensive reviews of the epidemiological evidence for a number of negative health impacts from THM and other DBP exposure are available (Nieuwenhuijsen, et al., 2011; Hrudey, 2009; Tardiff, et al., 2006; International Programme on Chemical Safety, 2000).

Canadian epidemiological studies have been conducted mainly in Nova Scotia and Ontario by Dodds and King (Dodds, et al., 2004; King, et al., 2000; Dodds, et al., 1999). The first study by Dodds

et al. (1999) was a retrospective cohort study from 1988-1995 using Nova Scotia health records which evaluated the relationship between birth weight, preterm births, neural tube defects, cleft defects, cardiac defects, stillbirth, and chromosomal abnormalities. They related these defects and conditions to total THM concentrations taken from utility records (Dodds, et al., 1999). Confounding factors such as age and weight of mother, smoking habits, prenatal class attendance, and family income were identified and eliminated based on their effect on the coefficient pertaining to THM levels. The results from the Dodds et al. study found no significant risk to any of the conditions evaluated for all concentrations of THMs used in the study which ranged from 0 - >100 µg/L. The study also found no relation to neural tube or cardiac defects from THMs (Risk ratio (RR) = 1.18 and 1.0 at THM >100 µg/L) which was previously reported to be the most significant risk from a New Jersey study (Odds Ratio (OR) = 3.0 and 1.8) (Dodds, et al., 1999; Bove, et al., 1992). Dodds et al. indicated THM exposure estimations as a potential misclassification in their study due to the fact that only quarterly THM concentrations were available. King et al. (2000) used the same Nova Scotia health and utility records to evaluate the risk of total THMs, as well as chloroform and bromodichloromethane to asphyxia and unexplained stillbirths during the same time period as the Dodds et al. (1999) study (King, et al., 2000). The other two regulated THMs (i.e., bromoform and chlorodibromomethane) were not found in significant concentrations in the area studied therefore the authors did not account for these compounds individually. The results found a slight risk for stillbirths for total THMs (RR = 1.69) and chloroform (RR = 1.59) at concentrations exceeding 100 µg/L and for bromodichloromethane (RR = 1.99) at concentrations greater than 20 µg/L (King, et al., 2000). As the King et al., study used the same source of information as the Dodds et al. study, similar confounders and limitations were assumed. None the less, the results from the King et al. study suggest that bromodichloromethane may increase the risk for stillbirth (King, et al., 2000). Dodds et al. (2004) conducted a population-based

case control study in Nova Scotia and Eastern Ontario from the beginning of 1999 to the end of 2001. This study improved the estimation of exposure to total THMs, chloroform, and BDCM by including the amount of bottled drinking water the mother drank or if a home charcoal filter was used. Furthermore, they estimated dermal and inhalation exposure to THMs from bathing or showering habits (Dodds, et al., 2004). Dodds et al. also determined THM concentrations by collecting tap samples from each subject's house one year later to determine seasonal changes to the THM concentrations. Although sampling one year later may produce an error in THM concentration estimations, the authors collected samples two years after the study and compared them to the previous year finding a correlation of 0.87 suggesting the error would be minimal (Dodds, et al., 2004). Slightly higher risks were seen in the 2004 study compared to the previous Dodds et al. study which used Nova Scotia health and utility records. The 2004 study reported that women who consumed >5 glasses of cold tap water per day with > 50 μg/L THMs were four times likely to experience a stillbirth. Furthermore, when accounting for dermal and/or inhalation exposure women in the upper most quintile had increased risks for stillborn birth for total THMs (OR = 2.4), chloroform (OR = 2.0), and BDCM (OR = 2.5). Dodds et al. concluded that there was a slight risk for stillbirth when accounting for THM exposure though drinking water, as well as, dermal and inhalation routes (Dodds, et al., 2004). The Dodds et al. study shows that accounting for multiple exposure routes is important in reproductive risk assessments.

The epidemiological studies reviewed here do not provide conclusive evidence with regards to the risk associated with THM exposure and adverse reproductive outcomes; however, an extensive epidemiological review by Tardiff et al. (2006) suggested only minimal risk is associated with THM exposure and reproductive outcomes from epidemiological evidence (Tardiff, et al., 2006). Regardless of the conflicting epidemiological evidence regarding negative health affects associated with chronic

exposure to THMs, epidemiological and toxicological studies, such as those presented here, are used to guide and set regulatory limits for THMs in public water supplies.

2.3.4.2 Future Direction Regarding THM Regulations in North America

The USEPA has significantly more stringent guidelines than the WHO. Furthermore, there has been suggestion that Health Canada was considering changing THM regulations to meet the limits set by the USEPA; however, there is no indication a change in regulations in Canada is forthcoming. A review of the different approaches used by the USEPA to set current THM regulations, as well as the estimation of future goals, will provide some insight into the appropriate direction for future regulations in Canada.

The USEPA National Primary Drinking Water Regulations: Stage 1 and 2 Disinfectants and Disinfection By-products Rules provided MCLs and maximum contaminant level goals (MCLGs) for chloroform (United States Environmental Protection Agency, 2006). MCLs are an enforceable regulation while MCLGs are non-enforceable health goals. The MCLGs are estimated based on health risk and exposures, while applying a margin of safety. The MCLs are set as close to the MCLGs while considering the costs and benefits, as well as, the ability to detect and remove contaminants. These guidelines and goals were established by evaluating the current toxicological data available and applying an appropriate risk assessment based on the results of certain studies deemed to have the strongest evidence for toxicity (United States Environmental Protection Agency, 2006; United States Environmental Protection Agency, 2001). In the Stage 1-DBPR MCLGs were set at 60 µg/L for DBCM and 0 µg/L for both bromoform and BDCM; however, did not provide a MCLG for chloroform (United States Environmental Protection Agency, 2001). The MCL for total THMs (sum of all four) under the Stage 1-DBPR was 80 µg/L. The MCLs for total THMs did not change for Stage

2; however, the Stage 2-DBPR provided a MCLG of 70 µg/L for chloroform (Equation 2) (United States Environmental Protection Agency, 2006). The reference dose (RfD) used was found using the lowest-observable-adverse-effect-level (LOAEL) of 15 mg/kg/day reported by Heywood et al. (1979) who found the presence of fatty cysts in the livers of beagles following long term exposure to chloroform (Heywood, et al., 1979). The RfD also applies a safety or uncertainty factor (UF) of 1000 to account for species-species differences, sensitive populations, and using a LOAEL instead of a NOAEL (Equation 3) (United States Environmental Protection Agency, 2006). Although other studies were considered, the USEPA felt that the use of beagles had an advantage over small rodents such as mice and rats when evaluating risk to humans. The relative source contribution (RSC) of chloroform exposure in drinking water was 0.2 or suggesting 20% of the daily exposure to chloroform is from drinking water consumption (United States Environmental Protection Agency, 2006). One of the questions noted by reviewers of the Stage 2-DBPR was the application of an RSC of 20%. In the Stage1-DBPR MCLG for DBCM applied an RSC of 80%; however, the USEPA chose 20%, not 80%, for chloroform due to the possible exposure from other sources, which is assumed to be dermal and inhalation exposure during showering. Weisel and Jo (1996) evaluated the concentration of chloroform in the body from dermal and oral exposure during showering or bathing finding that a 10-minute shower or a 30-minute bath was approximately the same exposure as ingestion of 2 L of water (Weisel & Jo, 1996). If one were to assume an adult drinks 2 L of water a day and takes a 10-minute shower, there would be a 50% contribution to chloroform exposure from each source. If you were to apply this to the MCLG for chloroform it would increase from 70 µg/L to 175 µg/L. Under the current Stage 2-DBPR chloroform and DBCM have MCLGs of 70 µg/L and 60 µg/L, respectively. However, the MCL for total THMs is set to 80 µg/L which is oddly lower than the goals the USEPA would like to meet (i.e., sum of MCLGs of chloroform and DBCM = 130 µg/L). Furthermore, if one were to

consider the equal source contribution from oral, dermal, and inhalation exposures (MCLG = 175 µg/L) the MCLGs for total THMs would be 235 µg/L, nearly three times the current USEPA guideline of 80 µg/L.

$$MCLG\ for\ chloroform = \frac{RfD\ x\ weight\ of\ person\ x\ RSC}{Consumption\ of\ water} \qquad \text{(Equation 2)}$$

$$RfD = LOAEL\ /\ UF \qquad \text{(Equation 3)}$$

In a review of health risks associated with DBPs, Hrudey (2009) stated that the USEPA proposed to raise the MCLG for chloroform to 300 µg/L due to chloroform exhibiting a threshold limit for toxicity. However, this proposed change was met with protest from the Chlorine Chemical Council who convinced the US District Court that the USEPA was in violation of the Safe Drinking Water Act by failing to use the best available science (Hrudey, 2009). Following this decision, the USEPA imposed the 80 µg/L MCLG, likely to prevent further protest.

It is unclear whether the MCLGs proposed for chloroform and DBCM will be set as MCLs in the future. One possible reason that a reduction may be imposed would be due to a belief that THMs, and HAAs, are indicators for the presence of other chlorinated DBPs and that if you control THM and/or HAA formation there would be an overall reduction in DBPs (Hrudey, 2009). Unfortunately, this is not the case. Several researchers have found that the use of alternative disinfection, such as chloramination which is known not to form THMs to the same degree as hypochlorite disinfection, has the potential to form other DBPs (Krasner, et al., 2006; Richardson, 2003). Some of these by-products, such as nitrosamines which predominantly form during chloramination disinfection, have been found to be more toxic and carcinogenic than THMs or HAAs (Hrudey, 2009). Disinfection with chlorine dioxide has been shown to increase the formation of MX (3-chloro-4-(dichloromethyl)-5-hydroxy-

2(5H)-furanone) which has been reported to be the most mutagenic DBP identified in drinking water (Hrudey, 2009; Richardson, 2003). If one were to forecast a risk assessment under the current regulatory guidelines it would be expected that the overall THM and HAA concentrations in public water supplies will decrease with the use of alternative disinfectants to control THMs and HAAs; however, the formation of other unregulated and potentially more harmful DBPs would increase thereby rising the health risk found from exposure to public drinking waters. Although many emerging DBPs are under consideration by the USEPA and Health Canada, there are limited analytical techniques available to accurately measure the presence of these compounds in drinking water. Furthermore, there is limited toxicological and epidemiological evidence for emerging DBPs which would be required for regulatory agencies to determine appropriate MCLs. Until improvements are made for the analytical detection of emerging DBPs, along with relevant toxicological and epidemiological research studies, it is unlikely to see significant additions to the short list of regulated DBPs. In saying that, it would be unlikely to see changes to the current Stage 2-DBPRs by the USEPA until a time comes where the exposure to DBPs, other than THMs, outweigh the risks associated with the presence of THMs in public water supplies.

Given there are negative health implications with the presence of DBPs in public water supplies, there is a need for greater understanding the formation mechanisms of these toxic by-products and the associated organic precursors that form them. This will allow for more strategic removal of the organic precursors during potable water treatment.

2.4 Removal of THM Precursors from Drinking Water

There are a number of water treatment methods that are used to reduce raw water DOM, such as chemical coagulation or filtration using media or membranes. Although these treatment methods

reduce raw water organic matter, in situations where the source water is high in DOM, or there are other treatment limitations such as low alkalinity or high hardness, DOM removal may be difficult leading to a greater chance for DBP formation following the disinfection stage.

The following section will describe the two most common potable water treatments used for DOM removal, chemical coagulation and filtration, with focus given to the removal of specific fractions of DOM by these technologies.

2.4.1 Chemical Coagulation

Conventional chemical coagulation is a treatment technique which commonly uses aluminum or iron-based salts to remove impurities such as clays or other inorganic materials, DOM and pathogenic microbes from raw waters (Pernitsky, 2011; Duan & Gregory, 2003). When coagulants are added to water, they dissociate forming trivalent ions, Al^{3+} and Fe^{3+}, respectively. These ions further hydrolyse to form soluble complexes that have an increase positive charge (e.g., $Al_{13}(OH)_{34}^{5+}$). The removal of DOM using aluminum or iron salts is achieved through charge neutralization of the largely negatively charged surface of DOM, due to hydroxyl and carboxylic acid functional groups, by the positively charged metal complexes (Matilainen et al., 2010). Charge neutralization destabilizes the surface of DOM allowing smaller particles to coagulate and undergo flocculation.

There are other mechanisms involved in the removal of DOM by chemical coagulation such as entrapment in the metal complexes, adsorption onto the surface of metals, or complexation to form insoluble aggregates (Matilainen et al., 2010). The ability of coagulants to remove DOM depends on several factors such as coagulant type, dose, pH and alkalinity of the raw water, temperature, and the chemical composition of NOM found in the water (Matilainen et al., 2010). Therefore, it is important

to understand the characteristics of the source water, as well as the coagulant, in order to establish the optimal conditions for DOM removal.

There are limited studies which study the removal of DOM fractions by chemical coagulation (Sharp, et al., 2006). One such study was conducted by Sharp et al. (2006) who investigated DOM-coagulant interactions at various pH by evaluating zeta potentials of the various DOM fractions and floc characteristics, such as size, strength and settling. Sharp et al. fractionated natural water samples into hydrophobic and hydrophilic fractions. The hydrophobic fraction was further separated into a humic acid fraction and a fulvic acid fraction, while the hydrophilic fraction was separated into a hydrophilic acid and non-acid fraction, respectively. Sharp et al. indicated that the zeta potential of the raw water source was largely dominated by the hydrophobic fraction, which is negatively charged at neutral pH. Furthermore, there was a larger change in zeta potential in the hydrophobic fraction, compared to the hydrophilic fraction, as pH was varied from 2 – 9 (Sharp, et al., 2006). Sharp et al. attributed these observations to the pKa of the carboxyl functional groups on humic and fulvic acids (pKa = 2 to 4), compared to hydrophilic acids. Charge density is an important factor to consider when coagulating with metal salts as the introduction of ionized metal coagulants (i.e., Fe^{3+} or Al^{3+}) neutralizes the negative surface charge of DOM and reduces the zeta potential. Sharp et al. observed that optimal removal of DOM occurred when the zeta potential of the bulk solution was between -10 and +3 mV concluding that the hydrophobic fractions with the greatest charge density was more effectively removed with the addition of coagulants compared to the hydrophilic fractions (Sharp et al., 2006).

2.4.2 Magnetic Ion Exchange Resin

In the 1990s, a technology was developed by Orica Watercare which used an ion exchange resin with magnetic properties, under the tradename MIEX, to promote the removal of DOM and control the formation of DBPs (Boyer, 2015; Karpinska, Boaventura, Vilar, & Bilyk, 2013; Brezinski & Gorczyca, 2018). MIEX is comprised of a hydrophilic polyacrylic macroporous structure with strong quaternary ammonia functional (Karpinska, et al., 2013; Singer & Bilyk, 2002). The MIEX resin has magnetic properties through the inclusion of iron oxide particles into the bead and is much smaller than conventional ion exchange resins (180 – 200 µm) (Boyer, 2015; Karpinska, et al., 2013). The small bead size and magnetic properties of MIEX allow it to be partially regenerated after its use by adding a regeneration solution, typically sodium chloride to release bound DOM, and a magnetic field to recapture the resin (Figure 33) (Singer & Bilyk, 2002).

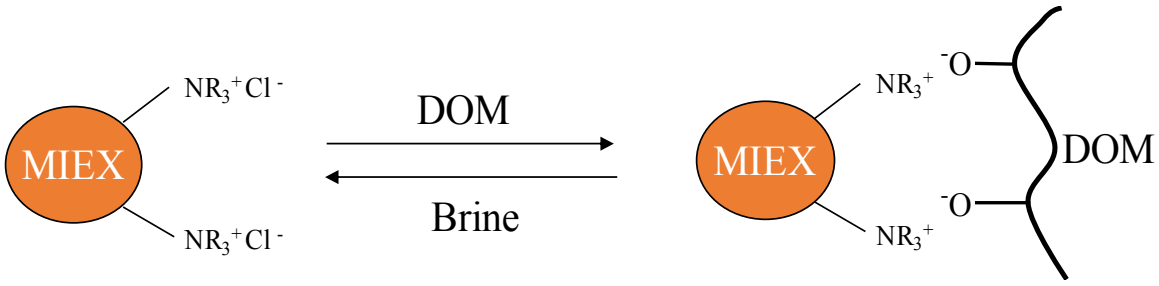


Figure 33: MIEX treatment process showing interactions between the MIEX resin and a negatively charged DOM molecule. Figure adapted from (Lee et al. 2002). Permission requested from the American Water Works Association. See Appendix C for permission request details.

MIEX resin has been shown to remove a wider range of DOM fractions compared to traditional coagulants (Boyer, 2015; Boyer & Singer, 2005). Boyer and Singer (2005) and Lee et al. (2002) tested the removal of DOC fractions by MIEX using jar tests. Both studies fractionated MIEX treated water into polarity fractions (hydrophobic, hydrophilic and transphilic) and by molecular weight (<1 kDa and 1-10 kDa) (Boyer & Singer, 2005; Lee et al., 2002). The conclusions from the Boyer and Singer

study found that MIEX significantly reduced all polarity fractions to a relatively equal extent. Chemical coagulation, as previously discussed, is generally better at removing hydrophobic DOM. Furthermore, MIEX reduced the high molecular weight fraction (1-10 kDa) by 80%, as well as the low molecular weight fraction (<1 kDa) by 60% (Boyer & Singer, 2005). Boyer and Singer noted that traditional coagulants were not generally effective at reducing compounds <1 kDa, showing the advantage of MIEX over traditions coagulants. The Lee et al. study also reported that MIEX reduced all fractions of DOM, but large molecular weight fractions, containing proteins and polysaccharides, were not effectively reduced by MIEX (Lee, et al., 2002). Nonetheless, Lee et al., like the Boyer and Singer study, suggested MIEX was more suited to reduce total DOM concentrations and thus reduce the potential for DBP formation.

2.4.3 Activated Carbon

A common media used for removal of DOM is activated carbon. Granular Activated Carbon (GAC) filtration has been shown to improve the removal of DOM over traditional filtration media, such as sand or anthracite (Collins, et al., 1996). There are several factors that can govern the removal of DOM by GAC including, pore size, type of GAC media, pH, temperature, ionic strength and hardness (Dastgheib, et al., 2004; Michaud, 1988). DOM carries a negative charge at pH values found in typical natural waters yet GAC media can have a positive, negative, or neutral surface charge which will affect the electrostatic interactions between NOM and GAC (Velten, et al., 2011; Weber, 2004). Pore size of the GAC media is believed to have the greatest effect on the removal of NOM from water. The pore size of GAC that has been reported to have the greatest capacity for NOM adsorption is the mesopores (2-50 nm) and is negligible on activated carbon with a pore size less than 1 nm (Velten, et al., 2011). Large molecular weight organic compounds (>10,000 Da) adsorb less to GAC filters due to

size exclusion effects, whereas medium molecular weight (MW) compounds (500-4000 Da) would adsorb more readily. Large molecular weight compounds are more easily removed by other treatment processes such as coagulation and therefore GAC is tailored to remove medium and lower weight compounds. On the basis of pore size, low molecular weight compounds (<500 Da) should be readily removed during GAC filtration however low molecular weight compounds tend to be more hydrophilic which will influence the adsorption rate (Velten, et al., 2011; Dastgheib, et al., 2004).

2.4.4 Membrane Filtration

The use of membranes in the treatment of potable water has been applied since the 1980s and have become increasingly popular in the last decade due to improvements to performance and reduction in costs (Ates et al., 2009; Guo et al., 2009). Since membranes are generally classified by their molecular weight cut-offs, the major rejection mechanism for organic matter is sieving; however, polarity, molecular conformation, and the dielectric constant impact the removal of DOM and DOM fractions. Some trends have been noted for the removal of organic compounds by membranes, such as compounds with ionizable groups are removed to a greater extent when the compounds are ionized. Also, phenolic and low molecular weight chlorinated hydrocarbons are poorly removed while organic acids and amines are better removed when they are present as a salt. Lastly, low molecular weight polar organics are more readily removed by nonpolar membranes (Wiesner & Aptel, 1996).

However, with membrane treatment the impact of irreversible fouling by DOM is a concern when applying treatment to surface waters. Fouling is largely due to the deposition of DOM to the surface or pores of the membrane. Surface waters, high in humic acids, can have a greater impact on membrane performance than clays or inorganic matter (Wiesner & Aptel, 1996). Several properties of DOM increase the tendency to foul membranes including the affinity for the membrane material,

molecular weight of the DOM, and the presence of specific functional groups (Wiesner & Aptel, 1996). The composition of the membrane can impact the removal of certain DOM fractions, for example, polysulfone, cellulose acetate, and thin film composite membranes are, to so degree, negatively charged. The negatively charged surface of the membrane would repel negatively charged functional groups (e.g., COO^-) of humic acids. Zularisam et al. demonstrated the fouling effects of hydrophobic and hydrophilic NOM on a hydrophobic polysulfone membrane. The study found that the hydrophilic NOM exhibited greater flux decline but less NOM removal while the hydrophobic NOM was better removed by the negatively charged polysulfone membrane surface (Zularisam et al., 2007). The Zularisam study suggests that charge-charge interactions, as well as size exclusion, can play an important role in removing NOM. Not only have studies found direct evidence of NOM fouling, indirect fouling with the presence of high organic matter has also been shown to occur. Polyphenolic compounds, proteins, and polysaccharides can bind colloids that deposit on the membrane effectively cementing the concentration polarization layer to the membranes surface. The stabilized cake layer cannot be easily removed through hydraulic cleaning and requires chemical methods to remove or dissolve the layer (Wiesner & Aptel, 1996).

Although there is the risk of irreversible organic fouling when treating surface waters with high DOM concentrations, membrane treatment, particularly with nano-membranes and reverse osmosis membranes, can reduce DOM to very low concentrations (<0.5 mg/L), largely reducing the risk for DBP formation. More insight into fouling mechanisms by DOM, and DOM fractions, on membranes of various compositions (e.g., polysulphone or cellulose acetate) may allow for better decisions of the most appropriate membrane to use for a particular water source. However, this requires organic matter compositional testing to be conducted on the surface water source prior to deciding on a particular

membrane type. Given the difficulties in rapidly measuring DOM composition using fractionation methods, it is unlikely these predesign studies will be adopted by engineers or water treatment utilities.

2.5 Monitoring DOM in Water Treatment using Specific UV Absorbance

To ensure water treatment process are removing DOM, monitoring methods, such as Specific UV Absorbance (SUVA) or UV_{254}, are used to gauge the removal of DOM following treatment. Although SUVA is widely applied to drinking water treatment systems, there are some limitations to this method which suggest caution should be used when determining treatment objectives based on UV absorbance. Furthermore, these limitations indicate a need for more reliable and accurate DOM measurement method in order to better gauge the propensity for treated water to form THMs, or other DBPs, after the disinfection stage.

UV radiation at 254nm (UV_{254} or $SUVA_{254}$) is strongly absorbed by aromatic and other conjugated molecules. Because DOM in surface water is a mixture of organic compounds all having different structure, bond arrangement and functional groups, the resulting UV absorbance is mainly an average of those molecules that preferentially absorb UV radiation (Weishaar et al., 2003). Although there have been studies showing a positive correlation between $SUVA_{254}$ and THMFP (Reckhow et al., 1990), other studies show the limitations and errors assessing THMFP using $SUVA_{254}$ absorbance. (Weishaar et al., 2003; Fram et al., 1999). This limitation was identified by Weishaar et al. (2003), who reported that four waters with similar SUVA values were found to have significantly different THMFP ($r^2 = 0.54$ for organic fractions and $r^2 = 0.4153$ for whole water). The authors attribute these results to varying degrees of lignin content in each water body. The differences in DOM molecular structure in a natural water sample are not accurately reflected in SUVA analysis nor can it cannot provide a DOM concentration (Weishaar et al., 2003; Her et al., 2002). This implies that the

usefulness of SUVA to predict THMFP is limited and that the relations are site specific and can not be used to gauge the formation of THMs in other waterbodies.

Chapter 3: Experimental Methods

3.1 Experimental Approach

The following general approach was used to determine, and develop, an alternative to the XAD method (Figure 34).

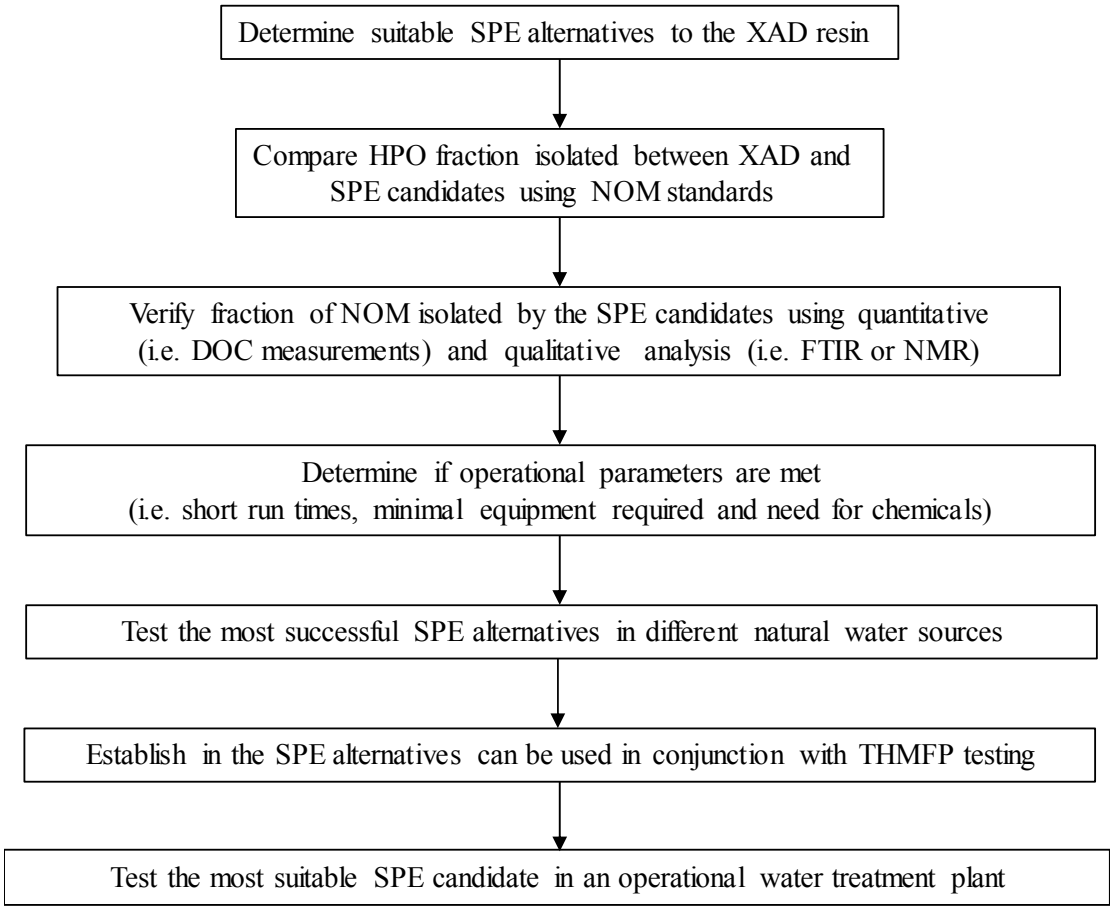


Figure 34: General methodology used to determine an alternative HPO isolation method to the XAD method using SPE.

3.2 Materials and Methods

3.2.1 Solid Phase Extraction

3.2.1.1 XAD Method

Supelite™ DAX-8 resin was purchased from Supelco (Bellefonte, PA, USA) and was used to isolate the humic fraction of DOM following the procedure found in Method 5510C in *Standard Methods,* and is outlined as follows (APHA, 2012). It should be noted that the DAX-8 resin was used alternatively to the XAD-8 resin, as the XAD resin is not commercially available. Comparative studies between XAD-8 and DAX-8 have indicated these two resins isolate very similar fractions of DOM and are considered equivalent in most cases (Chow, 2006; Peuravuori, et al., 2001). In order to maintain consistency in this thesis reference to the XAD-8 method, or XAD-8 resin, will be synonymous with the DAX-8 resin.

First the XAD resin was rinsed in 0.1N NaOH for 5-days. Following the NaOH rinse, the resin was sequentially rinsed in a Soxhlet reactor using hexane, methanol, acetonitrile, and methanol, for 24-hours each. The resin remained in methanol following the final rinse in the Soxhlet reactor. The methanol-resin slurry was packed into a glass column and rinsed with deionized (DI) water until the effluent DOC concentration decreased to <0.5 mg/L. In most cases, the DOC concentration of the DI rinse was non-detectable prior to initiating the fractionation procedure. After the resin was rinsed with DI water, the resin was precleaned with three cycles of 0.1N NaOH and 0.1N hydrochloric acid (HCl). Following the base/acid cleaning cycles, the column remained saturated with HCl until fractionation. Prior to fractionation, the 1L sample was acidified with 0.1N HCl to a final pH of 2, then pumped into the top of the column at approximately 1 mL/min. The column effluent was collected and used to determine the HPO/HPI fraction concentrations by measuring the DOC concentration and comparing it to the DOC concentration of the unfractionated sample (Equation 4 and 5). The fraction of DOM

that interacts with the XAD resin (i.e., sorbed) is the HPO fraction while the fraction of DOM that does not interact with the resin in the HPI fraction.

$$HPO_{Fraction} = DOC_{Unfractionated\ Sample} - DOC_{Column\ Effluent} \quad \text{(Equation 4)}$$

$$HPI_{Fraction} = DOC_{Column\ Effluent} \quad \text{(Equation 5)}$$

The HPO fraction was recovered from the resin by back eluting the column with either 0.1N NaOH (DOC measurement and THMFP testing), or methanol for Fourier Transform Infrared Spectroscopy (FTIR) Spectrochemical Imaging.

3.2.1.2 Solid Phase Extraction Candidates

Seven SPE cartridges were selected to test for their ability to isolate the HPO fraction from water containing Suwannee River standards purchased from the International Humic Substance Society (IHSS) (Table 4). The SPE candidates were all prepackaged from the manufacturers, containing 1 gram of sorbent, except Oasis HLB which had 150 mg of sorbent and Bond Elute Plexa which had 500 mg of sorbent, and were selected based on their capability to isolate hydrophobic compounds. Suwannee River standards were used because they are one of the most well characterized NOM samples available, as the composition of the Suwannee River has been studied for several decades (Perdue, 2002). The isolation of the HPO fraction by the seven SPE candidates were measured against the standard XAD method for comparison.

Table 4: SPE candidates used for the isolation of HPO-DOM

SPE	Supplier	Sorbent	Pore Size (Å)	Bed Mass (mg)	Volume (mL)
Strata C18-U	Phenomenex	Un-capped C18	70	1000	6
Strata C18-E	Phenomenex	End-capped C18	70	1000	6
Strata X	Phenomenex	N-Vinylpyrrolidone	85	1000	12
Bond Elute ENV	Agilent	Polystyrene divinyl benzene	450	1000	6
Bond Elute PPL	Agilent	Surface Modified polystyrene divinylbenzene	150	1000	6
Bond Elute Plexa	Agilent	Modified polystyrene divinylbenzene	160	500	12
Oasis HLB	Waters	Divinylbenzene-N-vinylpyrrolidone copolymer	80	150	6

All SPE cartridges were conditioned with 10 mL of methanol and allowed to dry under 10 mmHg vacuum pressure. The SPEs were then rinsed with deionized water (>1 L) until effluent DOC concentration from the SPE cartridge was <0.5 mg/L. A 1L was reduced to pH 2 using 0.1N HCl and pumped through an SPE candidate under 10 mmHg vacuum pressure. The concentration of the DOM sample was taken before and after SPE fractionation (measured as DOC) to determine the concentration of DOM isolated by each sorbent (see Equation 4). Following the extraction, sorbed DOM was eluted from each SPE using either 10 mL acetonitrile or methanol (FTIR imaging) or 20 mL 0.1N NaOH (THMFP). NaOH was used for THMFP testing to avoid organic contamination from acetonitrile or methanol. However, NaOH could not be used for eluting DOM for FTIR imaging as it promoted the formation of interfering salts. Methanol was used as the elution solvent for recovering the fraction of DOM sorbed to Strata X in preparation for FTIR imaging, as it was found that elution with acetonitrile resulted in poor recovery (visual observation). Methanol was found to remove the organic matter from the Strata X effectively. For isolation of the HPO fraction isolated from natural

water, methanol was used as the extraction solvent for elution of the HPO fraction from the SPEs to allow for more accurate comparisons between SPE extracts.

3.2.2 Suwannee River NOM Standards

Suwannee River (Georgia, USA) NOM standards were purchased from IHSS (St. Paul, Minnesota, USA) (Reference material 1R101N). Standard solutions were prepared by dissolving the IHSS NOM standard in DI water and diluting to approximately 10 mg/L. The NOM standard solutions were filtered through 0.45 μm nitro-cellulose filter paper to achieve the DOM fraction. SR HA and FA standards were also purchased from the IHSS (Reference materials: FA-1S101F and HA-2S101H) and used to establish reference infrared spectra for SR, HA and FA standards.

3.2.3 Capacity Testing for SPE Candidates

Capacity testing were conducted on the SPE candidates that were found suitable for field testing to confirm that the capacity to retain the HPO fraction would not be exceeded under typical DOM concentrations in natural waters, which was assumed to be <40 mg/L (measured as DOC). Suwannee River DOM samples were prepared at concentrations ranging from approximately 10-40 mg/L which were fractionated using each SPE candidate selected for field testing. The HPO fraction isolated by a candidate SPE was compared at increasing concentrations of DOM with the premise that a similar %HPO fraction should be isolated as the concentration of DOM increased.

3.2.4 SPE Field Testing on Natural Water Sources

Field testing of natural water bodies located in Manitoba, Canada were tested periodically over an approximate 13-month period (May 2014 - July 2015). The surface waters were monitored using the SPE candidates that were determined to be the most suitable for field trials, Strata X, Bond Elute ENV and Bond Elute PPL. It is important to note that the PPL was not included in the initial field

testing. It was recommended by an anonymous reviewer of this work that PPL may also be valuable to this investigation. As a result, PPL underwent preliminary testing using IHSS NOM standards and was determined to be suitable for field work. PPL was only tested on natural waters in late-2015. Seasonal DOM fractionation testing also included the XAD-8 resin to use as a comparison with the SPE fractionation results.

Water samples were collected from three surface waters in Manitoba, Canada; Lake Winnipegosis, the Waterhen River, and the La Salle River. La Salle River water was collected on from the raw water intake of the Macdonald Water Treatment Plant (Sanford, Manitoba). Lake Winnipegosis samples were collected from raw water intakes at the Duck Bay Water Treatment Plant, Camperville Water Treatment Plant, and the Pine Creek First Nation Water Treatment Plant. Waterhen River samples were collected from the Waterhen Water Treatment Plant. The general raw water quality for the three surface waters is summarized in Table 5.

Table 5: Typical raw water quality parameters. Values were taken from 2011- 2015 historical plant raw and treated water quality data provided by the Duck Bay Water Treatment Plant (Lake Winnipegosis), the Waterhen Water Treatment Plant (Waterhen River), and the Sanford Water Treatment Plant (La Salle River). Figure taken from (Goss et al, 2017).

Water Quality Parameter	La Salle River	Lake Winnipegosis	Waterhen River
Color, True (TCU)	> 20	< 5.0	< 5.0
Turbidity (NTU)	3 – 50	<1.0	<1.0
pH	7.9 – 8.3	8.0 – 8.5	8.0 – 8.7
Alkalinity (mg/L $CaCO_3$)	100 – 500	80 –125	70 – 125
Dissolved Organic Carbon (DOC mg/L)	12 – 25	10 – 18	8 – 15
*Total THMs (µg/L)	100 – 160	150 – 250	150 – 220

* Treated water

Lake Winnipegosis and the Waterhen River are located in the Manitoba Interlake and are characteristic of surface waters found on the Canadian Shield. The La Salle River is located in southwest Manitoba and is typical of rivers found on the Canadian Prairies (Figure 35). All three water bodies have different water qualities in terms of colour, turbidity, alkalinity and pH; however, all have elevated concentrations of DOM (measured as DOC) which leads to THM concentrations exceeding provincial guidelines.

Natural water samples were collected in 20 L plastic containers which were thoroughly cleaned and rinsed with deionized water prior to collection. All samples were filtered through 0.45µm nitrocellulose filter paper to obtain the DOM fraction of NOM. Samples were stored at 4°C until use.

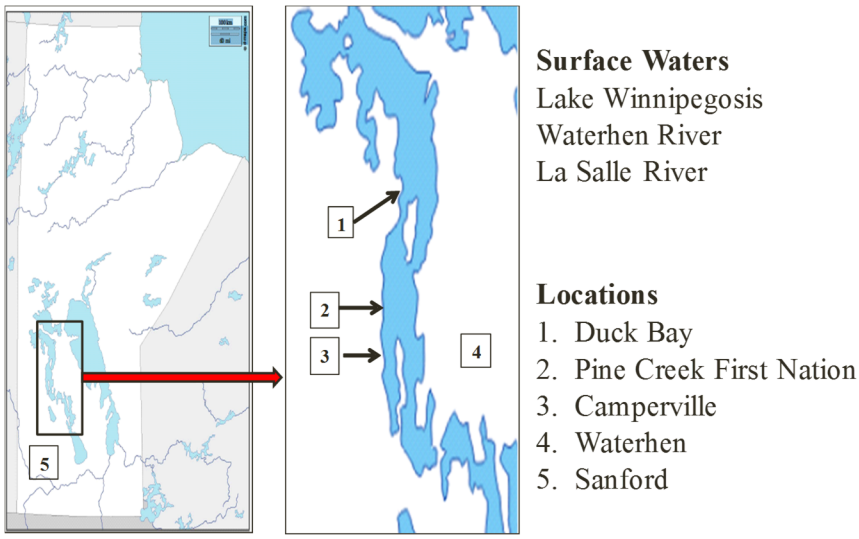


Figure 35: Manitoba surface waters studied during field testing of solid phase extraction candidates.

3.2.4.1 Seasonal Monitoring of Waterhen River DOM

The Waterhen River is in the Manitoba Interlake Region and is the primary outfall for Lake Winnipegosis which flows south approximately 22 km into the northern part of Lake Manitoba. This river is typical of surface waters located on the Canadian Shield and is characterized as having pH ranging from 8.0-8.7, low alkalinity (80-125 mg/L $CaCO_3$), colour, low turbidity, and DOM concentrations ranging from approximately 8-15 mg/L (measured as DOC). The Waterhen River is the potable water source for the community of Waterhen (Waterhen, Manitoba). The Waterhen Water Treatment Plant (WWTP) has frequently reported THM concentrations which exceed the provincial regulatory limit of <100 µg/L. Previous investigations into the WWTP have identified poor organic removal by this system, making it a prime candidate to conduct SPE field testing (Goss, 2012).

Raw water samples were collected from the Waterhen River on June 17, 2014, Aug 12, 2014, September 29, 2014, January 22, 2015 and August 19, 2015, via the raw water intake located in the WWTP.

3.2.4.1.1 Sampling at the Waterhen Water Treatment Plant

The WWTP is a small package treatment plant (BCA Model ST&GAC-50 system) with a flow of 3.2 L/s. The treatment process at Waterhen can be found in Figure 36 and is as follows: raw water enters the packaged water treatment plant to a small coagulation tank where polyaluminum chloride (PACl) and polymer aid (LT22S) are added. The chemically treated water then undergoes flash mixing followed by flocculation and clarification. The water then in pass through a dual media filter (sand and anthracite), followed by GAC (FILTRASORB 400) filtration. Water is disinfected with sodium hypochlorite, which is maintained above the provincial guideline of 0.5 mg/L free chlorine. It should be noted that the GAC filter was not in use during the time of this study due to poor performance of the GAC filter rapidly reaching capacity and requiring constant backwashing impacting production volumes.

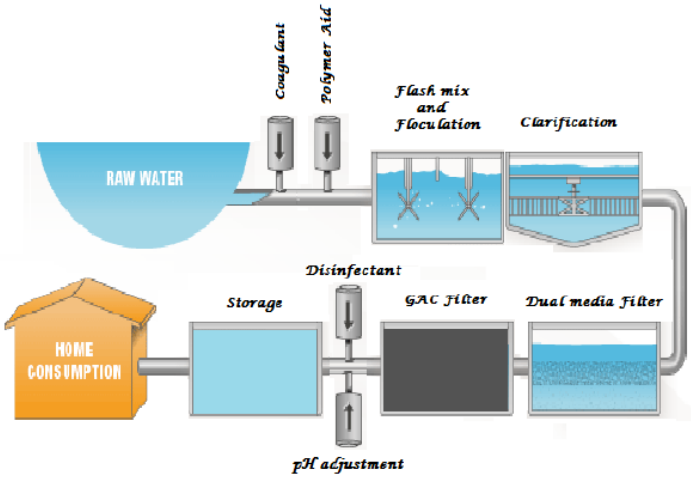


Figure 36: Treatment train for the Waterhen Water Treatment Plant.

The WWTP has frequently experience elevated levels of THMs and overall poor removal of DOM by the treatment system (Goss, 2012). As a result, the WWTP provided an opportune scenario

to both monitor the seasonal changes in DOM, as well as the reduction in DOM, and DOM fractions, by the WWTP.

Sample were collected on August 12, 2014, September 29, 2014 and January 22, 2015 from the following locations:

1) Raw water (prior to chemical addition)
2) Top of the coagulation tank
3) Top of the flocculation tank
4) Tap

The samples collected were filtered with 0.45 μm filter paper to isolate the dissolved fraction of NOM and stored at 4°C until use. Samples collected following each treatment process were fractionated using Strata X and Bond Elute ENV.

3.2.4.2 Seasonal Monitoring of Lake Winnipegosis

Lake Winnipegosis has an area of approximately 5,400 km^2 making it the 2nd largest lake in Manitoba and the 29th largest lake in the world (Lake Manitoba Regulation Review Advising Committee, 2003). The main influx of water to Lake Winnipegosis is through rivers and streams that flow down from the Manitoba Escarpment which first empties into Dauphin Lake, which then flows through the Mossey River, emptying into Lake Winnipegosis. The only outfall from Lake Winnipegosis is the Waterhen River which flows south and empties into the north basin of Lake Manitoba (Lake Manitoba Regulation Review Advising Committee, 2003). The raw water quality of Lake Winnipegosis is similar to that of the Waterhen River, having low colour and turbidity, pH ranging from approximately 8.0 to 8.5, low alkalinity (80-125 mg/L $CaCO_3$) and DOM ranging from 10-18 mg/L.

Lake Winnipegosis samples were collected from the following three different locations throughout this study: Duck Bay, Camperville and Pine Creek First Nation. The dates samples were collected from each location is presented in Table 6. Raw water samples were collected from raw water lines feeding each communities water treatment plant.

Table 6: Lake Winnipegosis sampling dates and locations

Date	Duck Bay	Camperville	Pine Creek First Nation
June 17, 2014	√		
August 11, 2014	√	√	
September 29, 2014	√	√	√
January 22, 2015	√	√	√
August 19, 2015	√		

Of the three sample locations along Lake Winnipegosis, Duck Bay was sampled most often providing better insight into seasonal changes in the lake during the sampling period

3.2.4.2.1 Sampling at the Duck Bay and Camperville Water Treatment Plants

The Duck Bay and Camperville WTPs are both equipped with the same prepackaged water treatment system as the WWTP, a BCA Model ST&GAC-50 system. Samples were collected from both water treatment plants at the following locations:

1) Raw water (prior to chemical addition)
2) Top of the coagulation tank
3) Top of the flocculation tank
4) Tap

3.2.4.2.2 Sampling at the Pine Creek First Nation Water Treatment Plant

The Pine Creek First Nation WTP is of similar design as the Camperville, Duck Bay and Waterhen WTPs, with the addition of dissolved air flotation (DAF) and reverse osmosis (RO) membrane filtration. Samples were collected from the Pine Creek First Nation WTP at the following locations (Figure 37):

1) Raw water (prior to chemical addition)
2) Top of the coagulation/flocculation tank
3) After Dissolved Air Floatation
4) After RO membrane
5) Tap

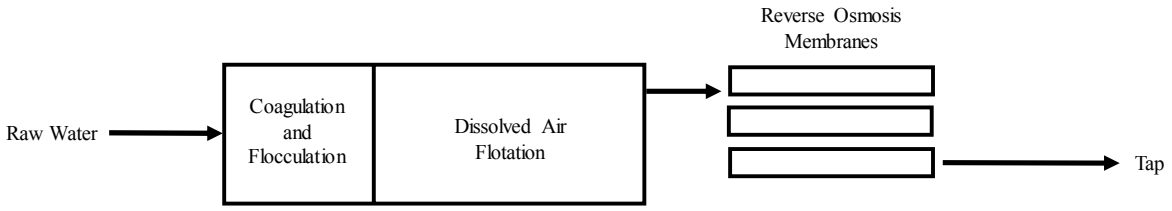


Figure 37: Treatment Train at the Pine Creek First Nation Water Treatment Plant.

It should be noted that samples were not collected from the DAF on September 29, 2014 as the DAF was not operational at the time. Furthermore, it should be noted that the historical records for raw and treated water quality for the Pine Creek First Nation was unavailable as the operators were not required to keep records for the system. It is also important to note that there were no operators present at the WTP on either of the two sampling days. For both the September 29, 2014 and January 22, 2015 sampling days, access to the WTP was given by council members who were unfamiliar with the operation of the WTP. As a result, intimate details on the operation of the WTP (i.e., coagulant type, coagulant dose, operational pH, DAF operation, and RO membrane operation) were unavailable. Raw water quality for the plant was never provided by the plant operators; therefore, it was assumed that

raw water quality was similar to Duck Bay and Camperville given the close proximity of the Pine Creek First Nation to both Camperville and Duck Bay.

3.2.4.3 Seasonal Monitoring of the La Salle River

The La Salle River is in the located southwest portion of Manitoba and is characteristic of many rivers located in the Prairie Region of Manitoba. The La Salle River is characterized as having high colour which can range from 50 TCU to greater than 100 TCU, high hardness (200-600 mg/L $CaCO_3$), pH ranging from 7.0-8.5, and high DOM which can often exceed 20 mg/L in the summer. This water source has different water quality compared to Lake Winnipegosis and the Waterhen river which offered an alternative natural surface water to those found on the Canadian Shield.

The DOM composition of the La Salle River was measured using the SPE candidates, as well as using the XAD resin, for comparison purposes. Of the three surface waters tested in this study, the La Salle River was most frequently tested given its proximity to the University of Manitoba (~20 km from Winnipeg, Manitoba). The La Salle River DOM composition was tested seven times from May 2014 to July 2015. Raw river water samples were collected from the raw water line located in the Macdonald Water Treatment Plant, which uses the La Salle River as a potable water source.

3.2.4.3.1 Sampling at the Macdonald Water Treatment Plant

In 2010, the Macdonald Water Treatment Plant (MWTP) underwent an upgrade from a lime soda softening plant to a dual ultrafiltration/reverse osmosis (UF/RO) facility to meet growing demands and more stringent water quality regulations. The UF/RO membranes used by the MWTP in 2010 were the Z-PAK Ultrafiltration System and the MUNI Reverse Osmosis System, both General Electric products. The raw water source is the La Salle River; however, the MWTP has a large raw

water storage pond to provide adequate water in time of low water levels in the La Salle River. Raw water is drawn from the pond and enters the WTP where a small amount of coagulant (alum) is added (2-6 mg/L) to increase removal of DOM by the UF system. UF permeate water is then treated by the RO membrane system to remove DOM for THM control. The RO system also removals minerals and alkalinity, therefore, approximately 25% of the UF permeate is blended with RO permeate to increase the alkalinity in the treated water to prevent corrosive water from entering the distribution system. The blended water is then treated with UV and chlorine to achieve the required disinfection (Figure 38).

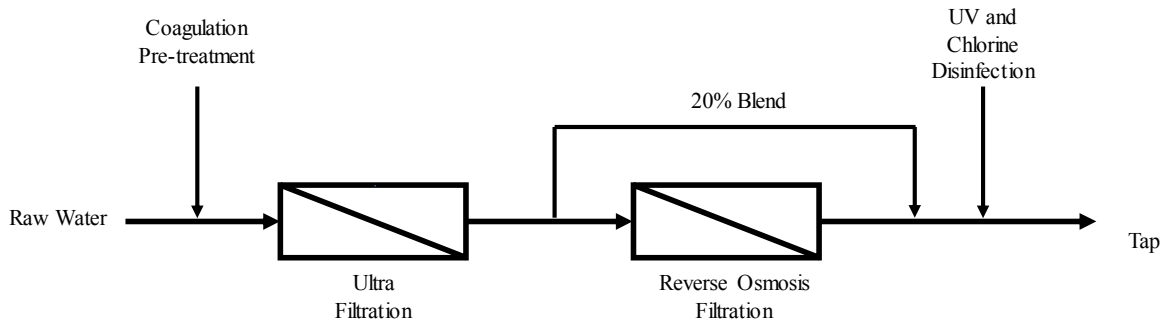


Figure 38: Flow diagram of the Macdonald Water Treatment Plant.

Samples were collected on July 10, July 24, and September 24, 2014 and on March 4 and June 30, 2015 from the following locations in the MWTP:

1) Raw water (prior to chemical addition)
2) Ultrafiltration Permeate (Post-UF)
3) Reverse Osmosis Permeate (Post-NF)
4) Tap (UF/RO Blend)

The samples collected were filtered using 0.45 μm filter paper to obtain the dissolved fraction. Samples were then fractionated using the Bond Elute ENV, Strata X, and Bond Elute PPL (June 30 sample set only) to determine the removal of DOM fractions by the MWTP. It should be noted that the DOM concentration in the RO permeate was low, ranging from non-detectable to approximately 1.0

mg/L. As a result, the RO permeate could not be fractionated due to the low overall DOM concentration.

3.2.5 THMFP and Specific THMFP of DOM Fractions Isolated Using SPE

The THMFP was determined for each of the DOM fractions (HPO and HPI) isolated from each surface. A 7-day THMFP was conducted according to *Standard Methods 5710B* (APHA, 2012), with one alteration, the chlorine demand was not determined prior to the THMFP test due to small sample volume (1L). Instead, all samples were chlorinated with 20 mg/L sodium hypochlorite to ensure there was sufficient free chlorine available to react with organics. The chlorine residual was >1.0 mg/L following the 7-day reaction time in all samples. THMs were measured using gas chromatography equipped with an electron capture detector according to *Standard Methods 6232B* (APHA, 2012). The results are reported as total THMs which is the sum of the four regulated THMs: chloroform, bromoform, bromodichloromethane, and dibromochloromethane.

The specific trihalomethane formation potential (STHMFP) was determined by normalizing the concentration of THMs (µg/L) measured to the concentration of organic matter (mg/L as DOC) in the sample (i.e., total DOM, HPO, or HPI).

3.2.5.1 Waterhen River THMFP and STHMFP Testing

DOM fractions collected from the Waterhen River on January 22, 2015 and August 19, 2015 were tested for the THMFP and STHMFP of the HPO and HPI fractions isolated with either the XAD resin, Bond Elute ENV, Strata X and Bond Elute PPL (August 19 sample set only). Unfractionated raw water samples were also tested for the THMFP and STHMFP for each sample period. The sample periods would represent winter (January) and summer (August) water conditions.

3.2.5.2 Lake Winnipegosis THMFP and STHMFP Testing

Raw water was collected from Lake Winnipegosis, via the raw water intake from the Duck Bay, Camperville and Pine Creek First Nation WTPs, on January 22, 2015 and from the Duck Bay WTP on August 19, 2015. The HPO and HPI fractions were isolated with XAD resin, Bond Elute ENV, Strata X and Bond Elute PPL (August 19 sample set only), respectively. The DOM fractions were chlorinated and the THMFP and STHMFP was measured for the DOM fractions isolated with each sorbent. Unfractionated raw water samples were also tested for the THMFP and STHMFP for each sample period. Each sample period would represent winter (January) and summer (August) water conditions.

3.2.5.3 La Salle River THMFP and STHMFP Testing

DOM fractions collected from the La Salle River on March 4, 2015 and July 23, 2015 were tested for the THMFP of the HPO and HPI fraction isolated with either the XAD resin, Bond Elute ENV, Strata X and Bond Elute PPL (July 23 sample set only). Unfractionated raw water samples were also tested for the THMFP and STHMFP for each sample period. Each sample period would represent spring (March) and summer (July) water conditions.

3.2.6 Fourier Transform Infrared Spectroscopy: Spectrochemical Imaging of SPE Fractions

The HPO fraction isolated from each SPE was prepared for FTIR analysis by first removing the methanol using roto-evaporation at 40°C. Elution with methanol for FTIR analysis was preferred over 0.1N NaOH as methanol extracts the HPO fraction in its acidic form, as well as simplifies FTIR sample preparation by eliminating the need for a desalting step with a cation-exchanger (Zherebker et al., 2016). The dried organic fraction was re-dissolved in deionized water (~1 mL); the resultant solution was transferred to a microscope slide dropwise using a Pasteur pipette and dried in a vacuum

desiccator. The samples collected from the Waterhen River had low DOM; therefore, 3 L of raw water sample were fractionated by each SPE in order to have enough HPO material for FTIR imaging. Images were collected using an Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array (FPA) mercury cadmium telluride (MCT) detector which was cooled with liquid nitrogen. The spectra were obtained with a slide-on Attenuated Total Reflectance unit, with a germanium crystal, that yields a 70 x 70 μm^2 area image with a 64 x 64 array and a depth penetration of ~2 µm. Co-addition of 64 scans yielded good quality spectra at 4 cm^{-1} resolution in the mid-IR range, 900-4000 cm^{-1}. Spectral images were analyzed using Agilent ResolutionsPro software.

3.2.6.1 Spectrochemical Imaging of HPO Fractions Isolated from Natural Waters

Water samples were collected from Lake Winnipegosis, the Waterhen River, and the La Salle River for FTIR spectrochemical imaging of DOM fractions isolated with the three SPE candidates; Strata X, Bond Elute ENV and Bond Elute PPL. La Salle River water was collected on July 23, 2015 from the raw water intake of the MWTP. Lake Winnipegosis and Waterhen River samples were collected on August 19, 2015 from raw water intakes at the Duck Bay Water Treatment Plant and WWTP, respectively.

Water samples were collected in 20 L plastic containers which were thoroughly cleaned and rinsed with deionized water prior to collection. All samples were filtered through 0.45µm nitrocellulose filter paper to obtain the dissolved fraction of natural organic matter. Samples were stored at 4°C until use.

3.2.7 Recovery Efficiency

The recovery efficiency (reported as % recovered) was determined as the amount of sorbed DOM recovered following elution with 20 mL of 0.1N NaOH:

$$\text{Recovery Efficiency} = \left(\frac{\text{DOM}_{\text{Eluted}}}{\text{DOM}_{\text{Sorbed}}}\right) \times 100\% \qquad \text{(Equation 6)}$$

The recovery efficiency was not determined for elution of sorbed DOM with methanol as all of the entire eluted sample was required for FTIR-FPA imaging.

3.2.8 Onsite Monitoring Using SPE by WTP Operators

From July 23, 2015 to January 21, 2016 operators at the MWTP tested Bond Elute ENV onsite. The SPE equipment and methodology required to conduct the DOM fractionation was provided to the operators (See Appendix B for procedure). The operators were asked to incorporate the SPE method into their general water quality testing, without interrupting their normal routine. During the 6-month period the MWTP operators fractionated the La Salle River treated by the UF filter a total of nine times, approximately once or twice per month. UF permeate was fractionated as this was the source of organic matter in the treated water following the blending of UF/RO permeate prior to chlorination.

3.2.9 Statistical Analysis

Statistical analysis was conducted on sample sets with multiple trials (n>1). The results for testing when n>1 are presented as the average of all trials with the error reported as the standard deviation from the mean. The average and standard deviation were calculated using the appropriate functions in Microsoft Excel.

The significant difference between datasets was evaluated using the t-Test function in Microsoft Excel, with the assumption the two datasets were of equal variance using a 95% confidence interval.

ANOVA analysis was used to measure the one-way variance between the candidate SPEs and the XAD resin for the results collected during the field testing of natural waters, including fractionation, THMFP and STHMFP. ANOVA results were calculated at a 95% confidence interval using online software (VassarStats, 2019). Along with ANOVA, Tukey Honestly Significant Difference (HSD) test results were calculated to provide insight into the significant difference between individual datasets. The Tukey HSD test was also calculated using available online software (VassarStats, 2019). The Tukey HSD test measures the significance between all datasets within an ANVOA analysis at 95% and 99% confidence intervals.

Chapter 4: Results & Discussion

4.1 Quantitative Isolation of HPO-DOM by Solid Phase Extraction

The HPO composition of the IHSS Suwannee River NOM standard was first measured using the XAD method (n=3). The SR NOM standard was found to have 76.2±2.3% HPO when isolated using the XAD method, which is comparable to results reported in literature (Table 7) (Averett, Leenheer, & McKight, 1994). Replicate trials (n=6) were conducted on each SPE sorbent and quantitatively compared to the results found using the XAD method. All SPEs were found to isolate a portion of DOM. Bond Elute ENV (ENV) was found to be equivalent ($p = 0.48$) to the XAD resin; isolating 75.5±0.4% and 76.2±2.3% total DOM, respectively. ENV was first used by Ratpukdi et al. (2009) as part of a five SPE cartridge method to isolate DOM into six common fractions based on hydrophobicity and acid/base functionality. Three ENV cartridges were used in the Ratpukdi method to isolate the acid, base, and neutral hydrophobic compounds under sample conditions of pH=2, 7, and 10 (Ratpukdi et al., 2009). ENV showed good precision with a standard deviation of 0.4% ($n = 6$). Bond Elute PPL (PPL) also isolated a percent DOM that was comparable to the XAD resin ($p = 0.06$), indicating PPL was suitable to undergo field testing on natural waters. Strata X was not found to isolate a concentration of DOM that was statistically similar to the XAD resin ($p = 0.0009$); however, Strata X was found to isolate the highest percent HPO DOM (82.2±1.1%) suggesting there was warrant in testing this sorbent in natural water conditions. The remaining four SPE candidates isolated HPO DOM in the following order Bond Elute Plexa> Oasis HLB> C18-U > C18-E and were not found to isolated percent DOM that was statistically similar to the XAD method ($p < 0.05$).

Table 7 also presents the results for the percent of the DOM fraction recovered from each SPE candidate following elution with 0.1N NaOH. The two C18 sorbents and the Oasis HLB had recovery percentages that exceeded 100% indicating there was contamination from the sorbent itself. Given this

result, it is determined these SPE candidates would not be suitable for further testing in field trials as contamination from the sorbent could impact THMFP and FTIR results. Bond Elute Plexa had greatest recovery (96.2%) of all SPEs tested; however, did not isolate a comparable HPO concentration to the XAD method. PPL, ENV and Strata X showed similar HPO recoveries as the XAD-8 resin, again showing their potential for field trials.

Table 7: Fractionation of Suwanee River DOM standard using the seven SPE candidates. Error is presented as standard deviation from the mean (n=6).

Sorbent	Raw DOC (mg/L)	Isolated DOC (mg/L)	Percent DOC Isolated	Percent HPO Recovered
XAD-8 [1]	9.97 ± 1.13	7.61 ± 1.15	76.2 ± 2.3	88.3 ± 4.7
Bond Elute ENV	10.51 ± 0.38	7.94 ± 0.28	75.5 ± 0.4	85.6 ± 5.8
Bond Elute Plexa	8.40 ± 2.29	5.30 ± 1.57	62.7 ± 2.2	96.2 ± 7.9
Strata - X	9.69 ± 1.56	7.95 ± 1.18	82.2 ± 1.1	89.1 ± 5.3
C18-U [2]	9.73 ± 1.29	4.03 ± 1.77	41.3 ± 3.8	135.8 ± 16.7
C18-E	9.39 ± 0.20	1.45 ± 0.89	15.3 ± 1.4	192.4 ± 20.0
Oasis HLB	8.41 ± 1.41	4.32 ± 0.38	52.1 ± 6.4	138.1 ± 10.0
Bond Elute PPL	10.69 ± 0.48	7.82 ± 0.36	73.1 ± 1.8	91.6 ± 2.6

[1] n = 3
[2] n = 5 – one trial was eliminated due to instrumental error during analysis.

Considering one objective of this study was to have the successful SPE method used by water treatment plant operators as a method for gauging the removal of THM precursors; the time required to complete a sample run by each candidate was investigated. This was determined by comparing the flow rates of each candidate at 10 mmHg pressure, which would affect both the time for DI rinse and overall sample run-time. The flow rates for the SPE candidates were significantly more rapid than the XAD resin fractionation which supports literature in that SPE has an advantage of shorter run-times over column fractionation (Table 8). The SPE methods would thus be more applicable to WTP

scenarios than that of the XAD method, as the total test time would be reduced from 9-12 days (XAD method) to between 2 to 4 hours (SPE methods), depending on the SPE.

The Oasis HLB had the shortest run-time between 50-60 minutes to complete the DI rinse and sample fractionation. This was due to the high flow rate of 40 mL/min and limited DI required to remove residual DOC for the SPE, likely due to less sorbent (150 mg). However, this SPE had low recovery of the HPO fraction and was found to contaminate the sample as the sorbent appeared unstable at high pH (Table 7). Bond Elute Plexa and the two C18 SPEs had the longest run-times which ranged between 120 to 160 minutes. This was due to the low flow rates and larger volumes of DI required to rinse residual DOC from the SPE prior to sample fractionation. Strata X had the lowest flowrate of approximately 18 mL/min under 10mmHg vacuum pressure. This implies there may be some limitation of this SPE as a method for WTP operators; however, given this SPE isolated the most DOM of all SPEs tested, and was found to be stable following elution at high pH, field testing with Strata X was determined to be valid.

Table 8: Flow rates and approximate SPE run-times for the seven SPE candidates. Run-time includes the time required for DI rinse and to fractionate the sample and does not include sample or resin preparation times.

Sorbent	DI Water Rinse (L)	Flow Rate (mL/min)	Approximate Run-time (minutes)
XAD-8	1-2	1	1500
Bond Elute ENV	1	30	60-80
Bond Elute Plexa	1	28	60-80
Bond Elute PPL	1-2	23	80-110
Strata - X	1-2	18	100-140
C18-U	2+	20	120-160
C18-E	2+	20	120-160
Oasis HLB	<1	40	50-60

4.2 Qualitative Analysis Using FTIR-FPA Spectrochemical Imaging

4.2.1 NOM Standards

FTIR has been used to identify characteristic functional groups associated with bulk NOM as well as with the humic fraction (Hay & Myneni, 2007; Cabiniss, 1991). HA and FA are large hydrophobic compounds mainly comprised of aromatic hydrocarbons with carboxyl and phenolic functional groups. FTIR is useful in identifying the presence of these functional groups due to the vibrational modes associated with C-O, C=O, and C-OH bonds (Hay & Myneni, 2007; Croué et al., 2000).

Both HA and FA show a pronounced band around 1710 cm^{-1} caused by a C=O stretch from carboxylic acids, aldehydes, esters, and/or ketones. The band between 1620-1640 cm^{-1} is assigned to C=C stretch in aromatic rings or C=O stretch of cyclic and acyclic ketones and quinones. This peak is typically very weak in spectra of FA, due to the lower content of these compounds (Rodriquez et al., 2016). Both HA and FA spectra exhibit a broad, poorly defined absorption from around 3500 cm^{-1} to 2800 cm^{-1}. The higher energy region (~3400 cm^{-1}) is associated with OH stretching vibrations of

alcohols, phenols, and carboxylic acids, NH stretching around 3200 cm^{-1} from amides, along with a weak multicomponent band around 2950 cm^{-1}, attributed to CH stretching modes (Rodriquez et al., 2016; Musikavong & Wattanachira, 2013; Giovanela, et al., 2010; Simjouw et al., 2005). The band around 1400 cm^{-1} is due to CH deformation modes of CH_2 and CH_3 groups (Giovanela et al., 2010). The band at ~1200 cm^{-1} is due to C-O stretching or OH deformation in carboxylic acid groups (Rodriquez et al., 2016; Musikavong & Wattanachira, 2013; Chen et al., 2002). Finally, the band between 1030 cm^{-1} and 1050 cm^{-1} is caused by stretching of C-O groups in alcohols, ethers, and polysaccharide or polysaccharide-like compounds (Rodriquez et al., 2016; Musikavong and Wattanachira, 2013; Giovanela et al., 2010). Figure 39 shows the single element IR spectrum for the Suwannee River NOM standard with peaks at 1716 cm^{-1} and 1622 cm^{-1} representing carboxyl groups and aromatic carbonyl groups, respectively. The broad band located between 3300-3400 cm^{-1} is attributed to the presence of OH bonds, while the weak, broad peak at 2935 cm^{-1} is due to the presence of CH_n groups. Suwannee River NOM is comprised largely of humic and fulvic acids which account for approximately 77% of the total NOM (Croué et al., 2000). The single element IR spectra for the Suwannee River NOM standard (Figure 39) represents the average signal for the entire area scanned.

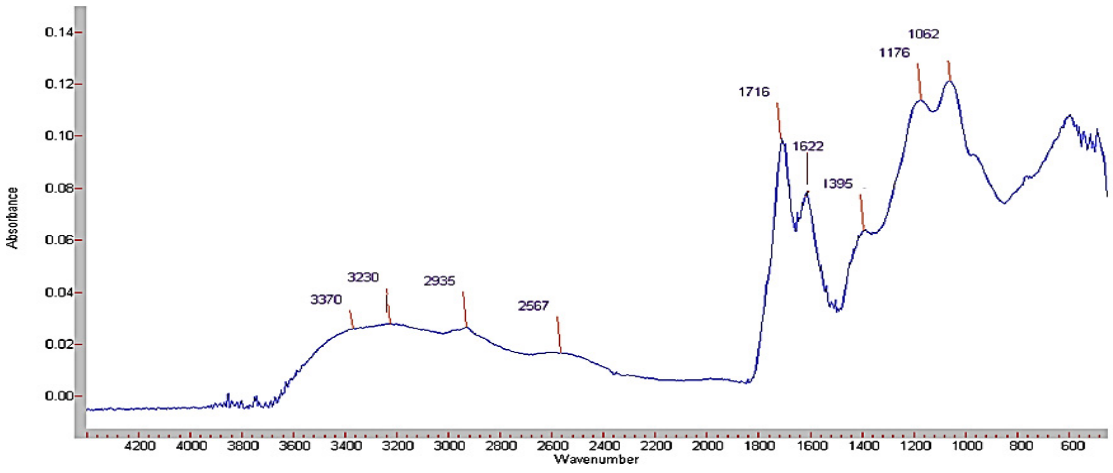


Figure 39: Single element FTIR spectra for IHSS Suwannee River NOM standard

An FTIR-FPA ATR spectrochemical image yields 4096 individual spectra which can be used to investigate chemical differences within the sample. One advantage of FTIR-FPA is the ability to determine areas of the scan where there was poor ATR contact on the sample.

The samples did not dry uniformly to the surface of the slide. After ATR touchdown there were areas of the FTIR-FPA scan which indicate irregular contact with the sample (Figure 40). Such areas are distinguished by poorer signal to noise levels but are still suitable for full analysis. One minor disadvantage to FTIR-FPA imaging is the reduced signal to noise ratio compared to single element IR spectra, since the contact area per pixel for the FTIR-FPA is 2.5×10^{-4} times smaller than the total area imaged with the single element. However, the signal is still very good and reduced signal to noise does not affect the applicability for ascertaining heterogeneity at this spatial resolution.

Typical spectra from the HA and FA standards (Figure 41 A and B) exhibit a significant difference in the relative height of peaks at ~1707 cm^{-1} and ~1615 cm^{-1}, indicating that these peaks can be used as markers to differentiate between HA and FA within the NOM sample. Images were processed by calculating the integrated bands areas as defined in Table 9. In the HA standard (Figure 41A), the two marker peaks have relatively the same intensity, while the peak at 1707 cm^{-1} is stronger than the peak at 1615 cm^{-1} in the FA standard. This is ascribed to a lower aromatic carbonyl character in FA (Reckhow et al., 1990). The false color images of the FA and HA standards (Figure 41 A & B, inserts) illustrate the intensity of each of the marker bands across the contact area. The similar colour patterns in each pair indicate that the standards are relatively homogenous. The variation in color is a result of differential contact between the germanium crystal and the rough surface of the dried standards, resulting in lower total signal. However, the spectral profiles are the same. The NOM standard was found to have both HA character (Figure 41 C, red spectrum) and FA character (Figure

41 C, blue spectrum). The FTIR-FPA false color image for SR NOM was created from the ratio of the integrated peak areas at 1707 cm^{-1} and 1615 cm^{-1}, to highlight regions of NOM with more HA or FA character a distinction that could not have been achieved with the single element IR scan.

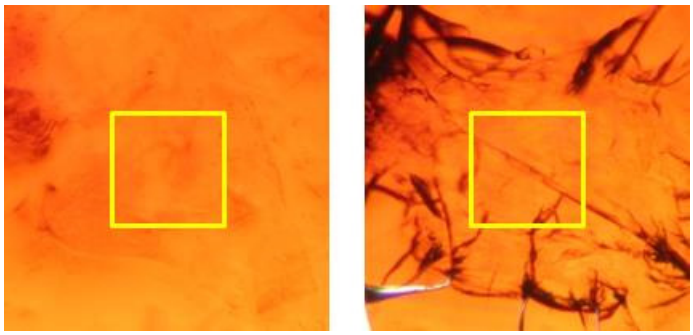


Figure 40: Before and after ATR touchdown on IHSS NOM standard dried on a microscope slide. Image at 10x magnification. Yellow box indicates approximate touchdown area. Further images showing before and after ATR touchdown are presented in Appendix A.

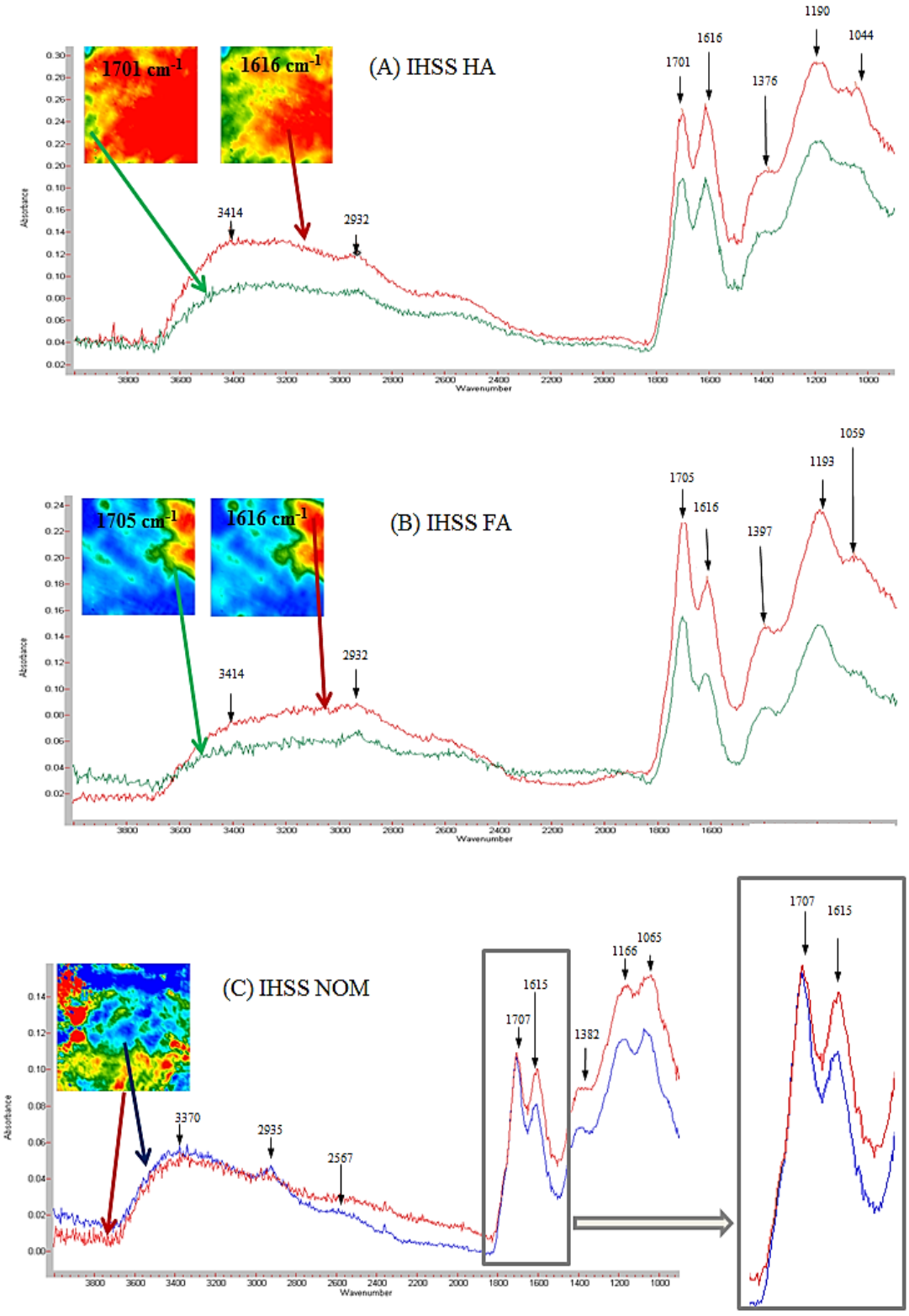


Figure 41: FTIR-FPA spectrochemical imaging of Suwannee River (A) Humic acid standard and (B) Fulvic acid standard and (C) NOM standard. Inserts are the false color images.

Table 9: FTIR-FPA processing parameters used to compare marker bands at 1707 cm^{-1} and 1608 cm^{-1}.

Peak (cm^{-1})	Left Edge (cm^{-1})	Center (cm^{-1})	Right Edge (cm^{-1})	Left Baseline (cm^{-1})	Right Baseline (cm^{-1})	Upper Threshold	Lower Threshold
1707	1727	1707	1687	1860	1500	6	0
1615	1628	1615	1588	1860	1500	4	0
Reference 1707	1727	1708	1687	1860	1500		
1608 ratio to 1707						0.8	0.6

4.2.2 Solid Phase Extraction Isolates

FTIR-FPA spectrochemical imaging was used to establish whether the SPE candidate was isolating the HPO fraction, mainly comprised of HA and FA. Spectrochemical imaging was also used to determine the presence of HA or FA in the isolate using the markers at 1615 cm^{-1} and 1707 cm^{-1}. Initially, the organic fraction sorbed to each SPE candidate was eluted with 0.1N NaOH and dried for spectral analysis. However, the spectra showed deprotonated carbonyl groups when eluted with NaOH. Elution with methanol was chosen to extract the sorbed HPO fraction from the SPEs in their protonated forms for comparison with the protonated IHSS standards.

All seven SPE candidates analyzed using FTIR-FPA imaging (Figure 42 A-G) were found to isolate organic matter from SR DOM samples with similar IR profiles as those found in the SR standards (Figure 40 A-C). C18E, C18U, Oasis HLB extracts, Starta X and Bond Elute PPL (Figure 42 A-C, F & G) show strong FA character based on the marker peaks at 1707 cm^{-1} and 1615 cm^{-1}. The marker peaks for the FPA images of the Bond Elute Plexa and Bond Elute ENV extracts show more HA character compared to the other SPEs.

The HPO fraction has the greatest potential to form chlorinated disinfection by-products (Reckhow & Singer, 1990). The overall objective of this research was to investigate a simple SPE method for monitoring the concentration and seasonal variation of the HPO fraction in surface waters, in the field. Of the seven SPE candidates tested in this study, only the Bond Elute ENV and PPL isolated the HPO fraction both quantitatively and qualitatively. The Bond Elute ENV was also shown to be very reproducible with a standard deviation of 0.4% (n=6), showing that the Bond Elute ENV method is more reliable than the XAD method for isolating HPO organic matter. The ENV method was simple to conduct using minimal equipment and only required methanol and DI for SPE conditioning. It is believed that a WTP operator would be able to conduct this test in a reasonable amount of time (~ 2 hours) and with minimal equipment (flask, vacuum pump, and tubing) and training.

The Strata X SPE also isolated the HPO fraction qualitatively; however, had a slightly higher quantitative result compared to the fraction isolated with the XAD method, with marginally higher error (Table 7). However, Strata X SPE exhibited better reproducibility than the XAD method. Furthermore, this SPE isolated the most DOM of all SPEs tested; therefore, field testing of the Strata X was deemed warranted.

All other SPE candidates tested were unable to both quantitatively and qualitatively isolate the HPO fraction of DOM. It was determined these candidates are not applicable for Phase II, field testing on natural waters.

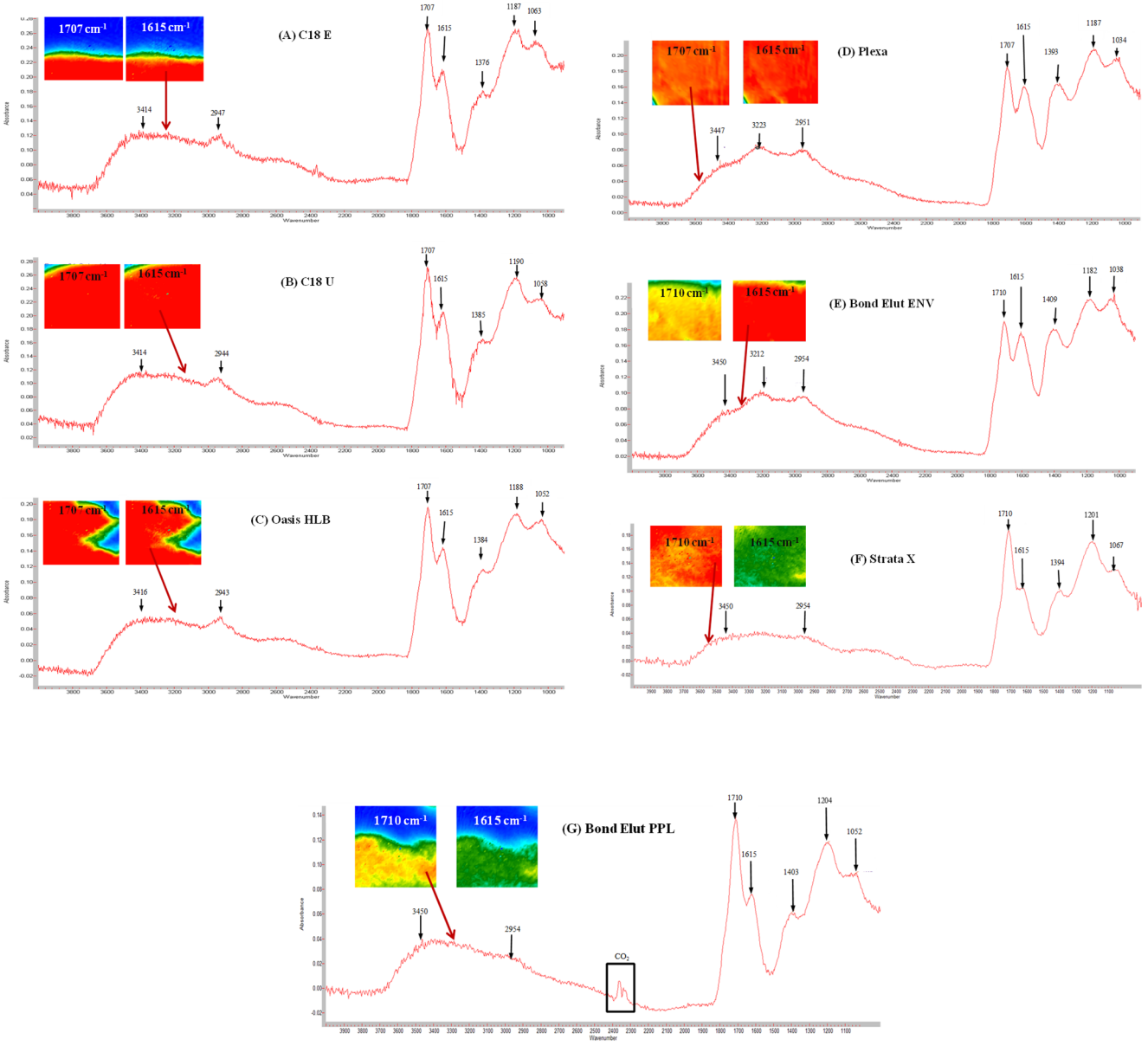


Figure 42: FTIR-FPA images and spectra for DOM eluted from (A) C18-E, (B) C18-U, (C) Oasis HLB, (D) Bond Elute Plexa, (E) Bond Elute ENV, and (F) Strata X and (G) Bond Elute PPL. Image inserted into spectra (A-E) are the FTIR-FPA image of area for peak at 1707 cm^{-1} and 1615 cm^{-1}. Individual spectra for the DOM fraction isolated with each SPE are presented in Appendix A.

4.3 Capacity Testing

The Bond Elute ENV showed the greatest variability of 2.1%; however, there was an increase in the %HPO fraction isolated as the concentration increased suggesting this sorbent has not reached capacity (Table 10). The result does imply that there may be some increase retention of HPO compounds as the Bond Elute ENV sorbs more HPO compounds, possibly from some co-adsorption phenomena. However, the increase is only minor and not believe to impact natural water testing. Strata X also showed some variability; however, as with the Bond Elute ENV there was no significant loss of capacity at increasing concentrations of DOM. The Bond Elute PPL showed the lowest variability in HPO capacity of all three SPEs having a standard deviation of 0.6%. Capacity testing results confirm that the candidate SPEs should not exceed adsorptive capacity under typical DOM concentrations in natural waters.

Table 10: Capacity testing for SPE candidates selected for testing of natural waters. Average %HPO is presented as the average HPO fraction isolated at increasing DOM concentrations. Error is presented as standard deviation from the mean.

Sample	Concentration Raw DOM (mg/L)	Concentration HPI (mg/L)	Concentration HPO (mg/L)	Percent HPO	Average % HPO
Strata	10.5	2.1	8.4	80.0	78.6±1.5
	18.8	4.4	14.4	76.7	
	27.7	5.7	22.0	79.5	
	38.9	8.5	30.4	78.1	
ENV	11.0	3.7	7.3	66.4	68.9±2.1
	20.7	6.6	14.1	68.1	
	30.6	8.8	21.8	71.3	
	39.7	12.1	27.7	69.6	
PPL	11.8	2.6	9.2	77.9	77.1±0.6
	21.6	5.1	16.5	76.5	
	29.8	6.9	22.9	76.9	
	36.5	8.5	28.1	76.8	

4.4 Field Testing of Natural Waters Using SPE

4.4.1 Seasonal Field Testing of the Waterhen River Using SPE

The raw water DOM concentration in the Waterhen River ranged from approximately 8 mg/L to 14 mg/L throughout the study period (Table 11 and Figure 43). The highest DOM concentration was measured winter 2015 (January) and the lowest DOM concentration occurred in summer 2014 (June). The seasonal minimum and maximum raw water DOM concentrations recorded indicate that the DOM concentration increased from spring to winter (June 2014 to January 2015) which is consistent with results reported by Karapinar et al. (2014). The increased DOM concentration in winter may be a result of high river flow under ice cover which could cause sediment on the bottom to become suspended. Historically, higher TOC concentrations have been observed in the Waterhen River during winter compared to summer.

When comparing seasonal changes in DOM composition in the Waterhen River, the results show the HPO fraction was changing throughout the study period for each of the SPEs used to isolate the HPO fraction. Comparing each HPO isolation method, there was no statistical difference between XAD, ENV and Strata X for the June 17, 2014 and August 12, 2014 sample sets ($p>0.05$).

However, there was a statistical difference measured for the samples collected on September 29, 2014 ($p = 0.0016$). The Tukey HSD results show Strata X isolated a higher concentration of DOM compared to both ENV ($P <0.01$) and XAD ($P <0.05$). Strata was also shown to isolate more DOM compared to ENV and XAD during Phase I of this study (Table 7).

For the January 22, 2015 samples, there was also a significant difference between XAD, ENV and Strata ($p = 0.0029$). The Tukey HSD test results show that ENV and Strata X statistically different ($P = <0.01$) isolating 37.9±1.9% and 42.7±3.9%, respectively. However, for the August 19, 2015

samples, although variance test results showed a significant difference between the data sets, the Tukey HSD test results show ENV and Strata were not significantly different and that both appeared to be isolating less HPO DOM compared to the XAD resin. PPL was found to be similar to Strata and XAD; however, was found to be isolate a significantly higher HPO concentration compared to ENV ($p < 0.05$). Considering that PPL was only tested on the raw water collected on August 19, 2015, it is unclear if this trend would have remained consistent throughout the sampling period.

Table 11: Seasonal DOM fractionation results showing changes in the HPO fraction in the Waterhen River from 2014-2015. Error reported as standard deviation from the mean (n=3).

Date	DOM (mg/L)	Fractionation Methods			
		XAD-8 (% HPO)	Bond Elute ENV (% HPO)	Strata X (% HPO)	Bond Elute PPL (% HPO)
June 17, 2014	8.2	39.2±4.0	39.9±1.3	43.4±2.4	Not Tested
August 12, 2014	11.3	30.6±10	35.2±1.7	34.5±1.0	Not Tested
September 29, 2014	10.1	38.4±1.1	40.8±0.3	48.0±1.4	Not Tested
January 22, 2015	13.6	30.3±0.9	37.9±1.9	42.7±3.9	Not Tested
August 19, 2015	11.4	40.1±2.8	28.6±2.2	31.9±3.9	37.3±2.9

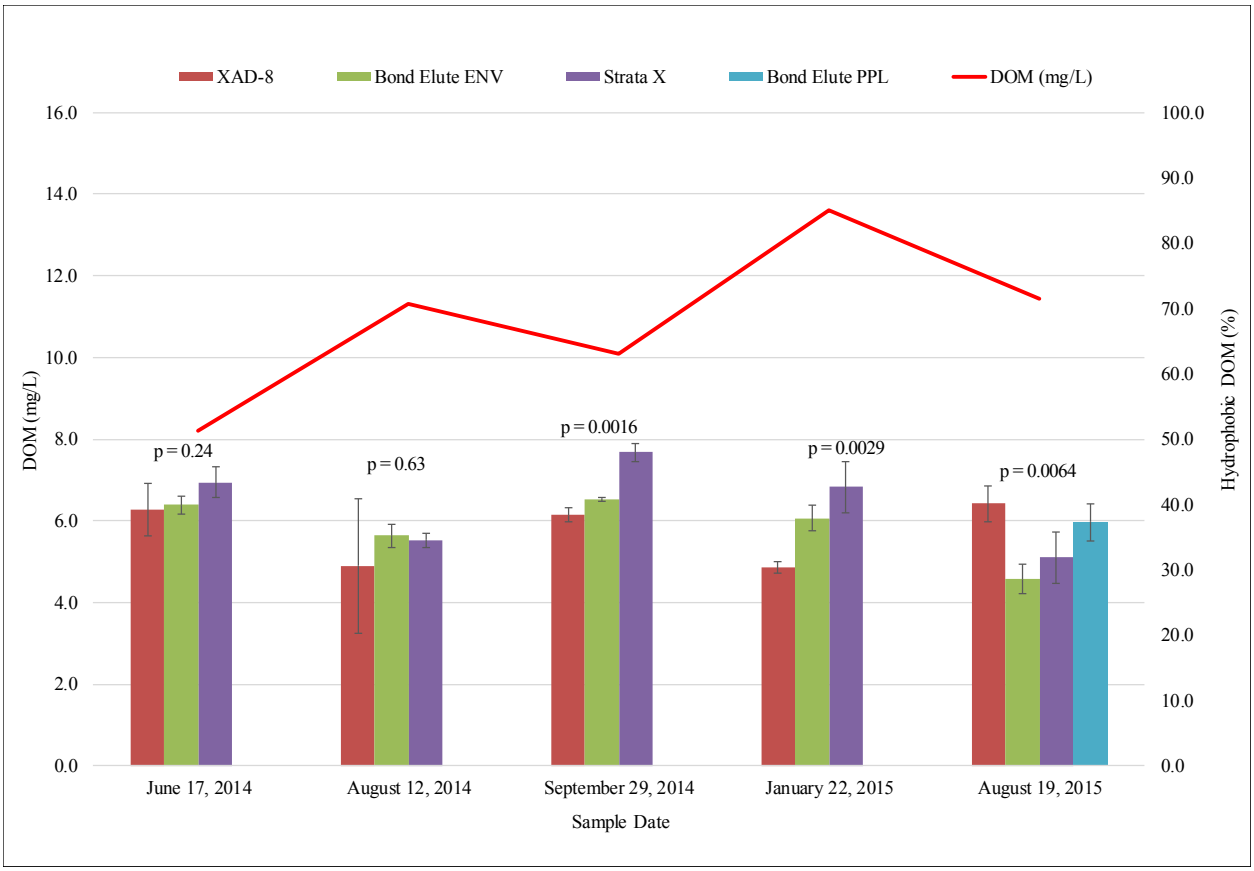


Figure 43: Seasonal DOM fractionation results showing changes in the HPO fraction in the Waterhen River from 2014-2015. Error bars are presented as standard deviation from the mean for the % hydrophobic DOM. The p = value reported represents the significant variance measured using one way ANOVA.

4.4.1.2 Removal of DOM Fractions by the Waterhen WTP

Seasonal removal of DOM and DOM fractions by the WWTP are presented in Figures 42-44. For the August 12, 2014 sample set (Figure 44) the raw water DOM concentration was 11.2 mg/L. When comparing the fractions of raw DOM isolated using the Strata X and ENV, there were similar concentrations of the HPO fraction isolated by each sorbent of 34.5±1.0 and 35.2±1.7, respectively. Likewise, similar DOM concentrations were isolated by Strata X and ENV for samples collected from the top of the coagulation tank. For samples collected from the top of the filter (prior to filtration), it

was observed ENV isolated a higher concentration of HPO DOM compared to the Strata X ($p = 0.01$). This result implies that different components of the HPO fraction are removed by coagulation and that the ENV and Strata X are isolating different components of HPO DOM. Following sand filtration there is an increase in the total DOM, which appears to be largely HPO in character. The WWTP has reported that the GAC media frequently reaches sorption capacity and requires frequent cleaning or replacement of the media. The increase in DOM following filtration (measured in the tap) is likely an indication that the media has exceeded capacity and is leaching DOM, mainly HPO. The increase in DOM, and in particular the HPO fraction, is likely the cause for elevated THMs reported by the WWTP.

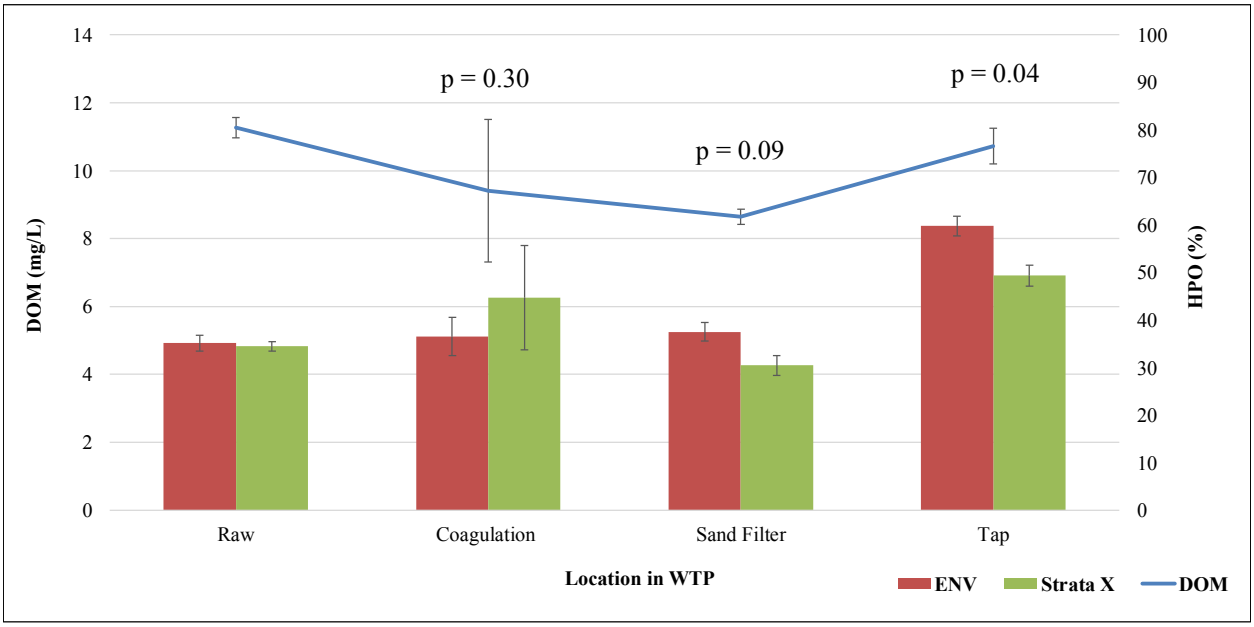


Figure 44: Changes in DOM and HPO fraction by treatment processes at the Waterhen WTP. Samples were collected on August 12, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

The fall results (September 29, 2014; Figure 45) found that the Strata X was isolating a higher concentration of DOM from the raw water compared to ENV. The IHSS fractionation results indicated that the Strata X was isolating some HPI DOM (i.e., fractionation results found Strata X isolated more

DOM compared to XAD). The difference in raw water HPO concentration found between the Strata X and ENV in Figure 45 may be a result of the Strata X isolating some HPI DOM, which may not interact with the ENV sorbent. This can further be exemplified by the samples collected from the top of the coagulation tank. The results show a large reduction in the HPO fraction isolated by Bond Elute ENV; however, there is more DOM isolated by the Strata X for the same sample, which is likely a result of the sorbent isolating some HPI. Coagulation is known to remove the HPO fraction preferentially over the HPI fraction, therefore, it is expected that there would be a greater reduction in the DOM fraction isolated by Bond Elute ENV compared to Strata X. The sample collected from the top of the sand filter measured an increase in the HPO fraction, as well as an increase in overall DOM. The increase in DOM and the HPO fraction could be due to buildup of organic matter on the filter which has exceeded capacity and is releasing DOM back into the treated water. The concentration of HPO measured in the tap by Strata X and Bond Elute ENV show a similar trend as the August 12, 2104 results where there was an increase in DOM following filtration, and that the remaining DOM had approximately 40-45% HPO character, which is likely contributing to increased THM concentrations in the finished water.

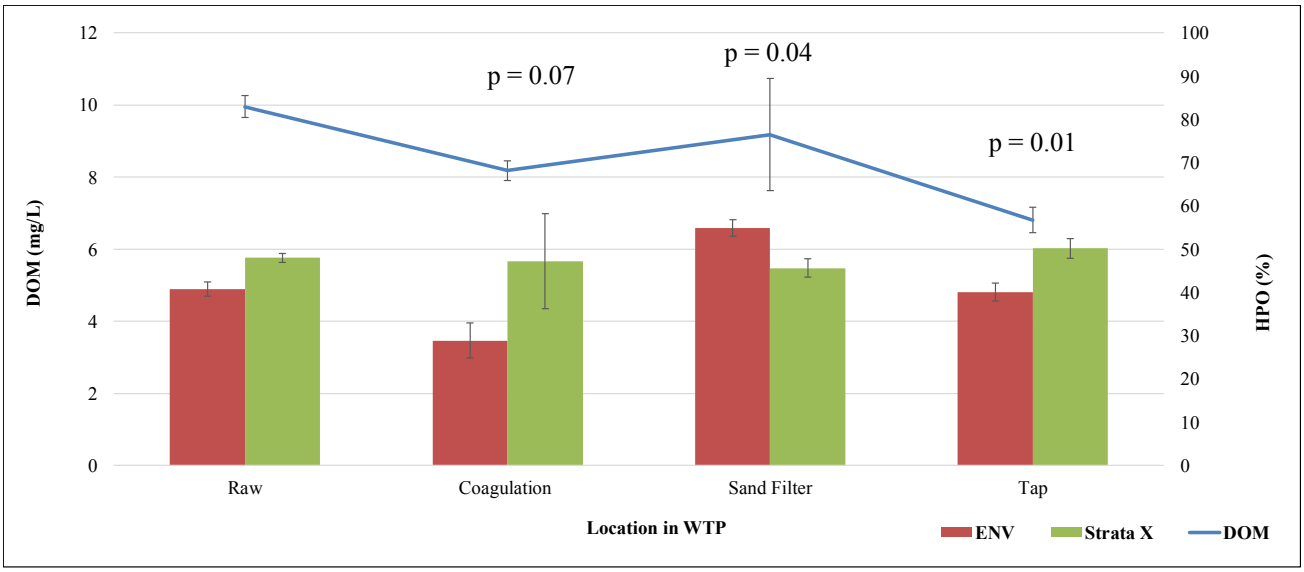


Figure 45: Changes in DOM and HPO fraction by treatment processes at the Waterhen WTP. Samples were collected on September 29, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

The results for samples collected on January 22, 2015 found that coagulation and filtration were not reducing DOM (Figure 46). This is potentially due to the reduction in coagulant efficiency typically observed in cold water conditions with aluminum-based coagulants (Jasim et al., 2008; Morris & Knocke, 1984). Poor removal of DOM by the filters indicated, again, that the GAC filter has likely reached sorption capacity and is unable to reduce DOM. The concentration of DOM in the treated water is approximately 10.8 mg/L, with around 40% of the DOM being HPO in character. These results provide evidence for the cause of elevated THMs consistently reported by the WWTP.

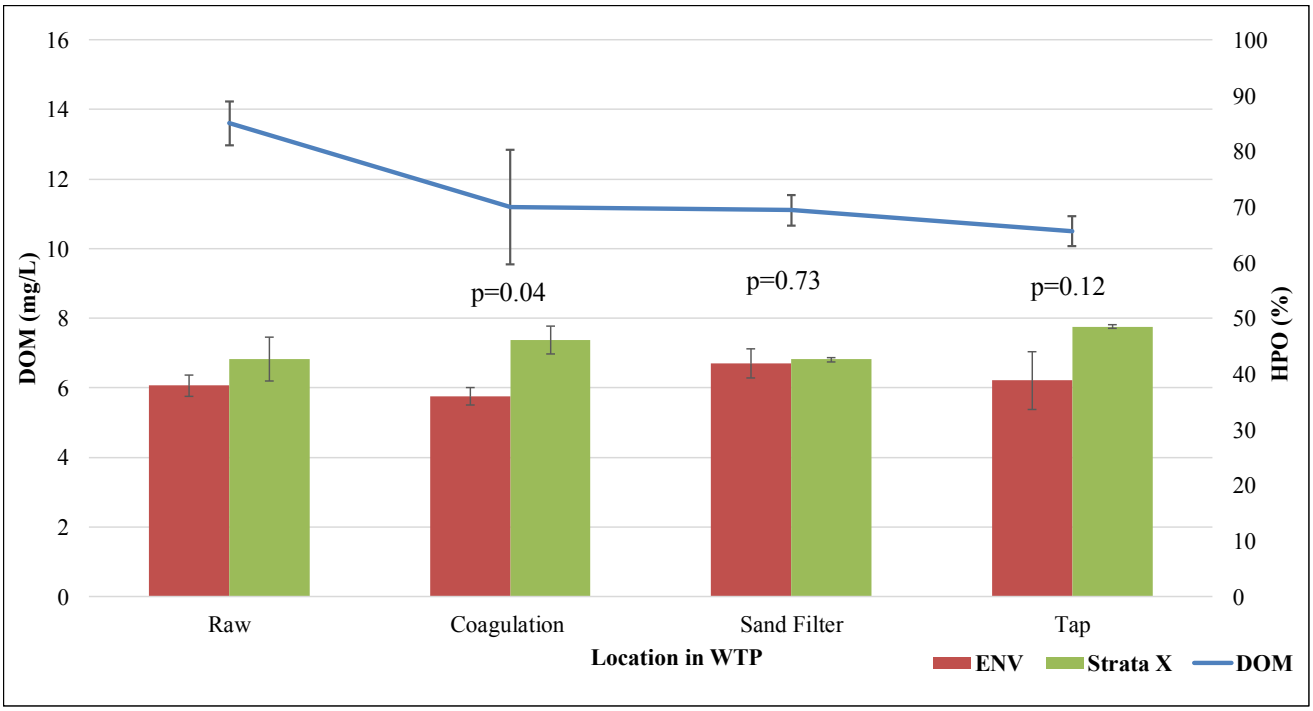


Figure 46: Changes in DOM and HPO fraction by treatment processes at the Waterhen WTP. Samples were collected on January 22, 2015. The p = value reported represents the significant difference using a t-Test at 95% confidence.

4.4.1.3 THMFP and Specific THMFP of DOM Fractions Isolated from the Waterhen River Using SPE

The THMFP of the Waterhen River on January 22 and August 19, 2015 were 128±44 µg/L and 198±19 µg/L, respectively (Table 12). There is higher formation of THMs in the raw water collected in August compared to the January sample even though there is higher HPO concentration in the January sample set. This implies there is more propensity for DOM to form THMs in the summer compared to the winter. This can be seen in the STHMFP results for each sample set. The STHMFP, presented as the concentration of total THMs formed per milligram DOM (µgTHM/mgDOM), which is 17.3±1.2 µgTHM/mgDOM in the summer, however, only 9.3±2.8 µgTHM/mgDOM in winter.

When evaluating the THMFP for the HPO fraction isolated with each SPE with that of the HPI fraction, which did not adsorb to each sorbent, the fraction that sorbed (i.e., the HPO fraction) had a significantly higher THMFP compared to the non-sorbed, or HPI fraction, for all SPEs tested (Table

12). This is expected considering the HPO fraction contains mainly humic and fulvic acids which are known to form more THMs compared to the HPI fraction (Singer, 1999). For all SPEs tested there was also a greater STHMFP for both the HPO and HPI fractions.

The results showed a significant difference in the STHMFP for the HPO fraction collected from each sorbent on January 22, 2015 ($p = 0.0002$). For this sample set, the XAD resin was found to have a significantly higher STHMFP for the HPO fraction compared to either ENV or Strata, while ENV and Strata found to have no significant difference.

For the August 2015 samples, there was no significant difference measured in the STHMFP for all four sorbents ($p = 0.54$).

The results presented here show that all three SPEs are able to isolate a fraction of DOM which has the greatest THMFP. This provides strong evidence that these SPEs can be used to monitor the changes in DOM composition, particularly the fraction of DOM (i.e., the HPO fraction) that is linked to the formation of THMs. The results further show the value of monitoring the DOM composition of surface waters when trying to gauge the propensity, and control the formation, of THMs in potable water systems such as the Waterhen Water Treatment Plant.

Table 12: THMFP and STHMFP results for DOM fractions collected the Waterhen River.

Sorbent	THMFP (µg/L)				Specific THMFP (µgTHM/mgDOM)			
	22-Jan-15		19-Aug-15		22-Jan-15		19-Aug-15	
	HPO	HPI	HPO	HPI	HPO	HPI	HPO	HPI
Raw Water	128±44		198±19		9.3±2.8		17.3±1.2	
XAD	93±11	14±7	130±5	24±4	24±2	1.6±0.3	28.3±2.0	3.5±0.5
Bond Elute ENV	60±10	15±6	86±8	21±3	18.0±0.9	1.7±0.3	30.8±2.5	2.6±0.2
Strata X	83±15	11±6	106 ± 14	18 ± 4	13.4±1.2	1.3±0.4	34.7±8.8	2.3±0.4
Bond Elute PPL	N/A	N/A	103 ± 11	13 ± 5	N/A	N/A	27.8±5.4	1.8 ± 0.7

N/A = not available

4.4.2 Seasonal Field Testing of Lake Winnipegosis Using SPE

The seasonal SPE field testing results for Duck Bay (Figure 47; Table 13) indicate that the DOM concentration was increasing from 10 mg/L (June 14, 2014) to 16.0 mg/L (January 22, 2015). The increase in DOM concentration reported here is somewhat counter intuitive in that generally increases in DOM concentration occurs following snow melt in spring, or heavy precipitation events (Sharp et al., 2006). However, during 2014 there was significant flooding and rainfall in Manitoba which likely increased the amount of water entering Lake Winnipegosis. For example, when comparing the difference in precipitation in the Interlake area (measured in Dauphin, Manitoba), there was 379 mm of total precipitation (rain and snow) recorded in 2012, 443 mm of total precipitation in 2013, and 533 mm total precipitation recorded in 2014 (Government of Manitoba, 2012-2015). Increased runoff following large rain events, or periods of flooding, would increase the overall concentration of DOM entering the lake accounting for the steady increase in DOM observed throughout the study period.

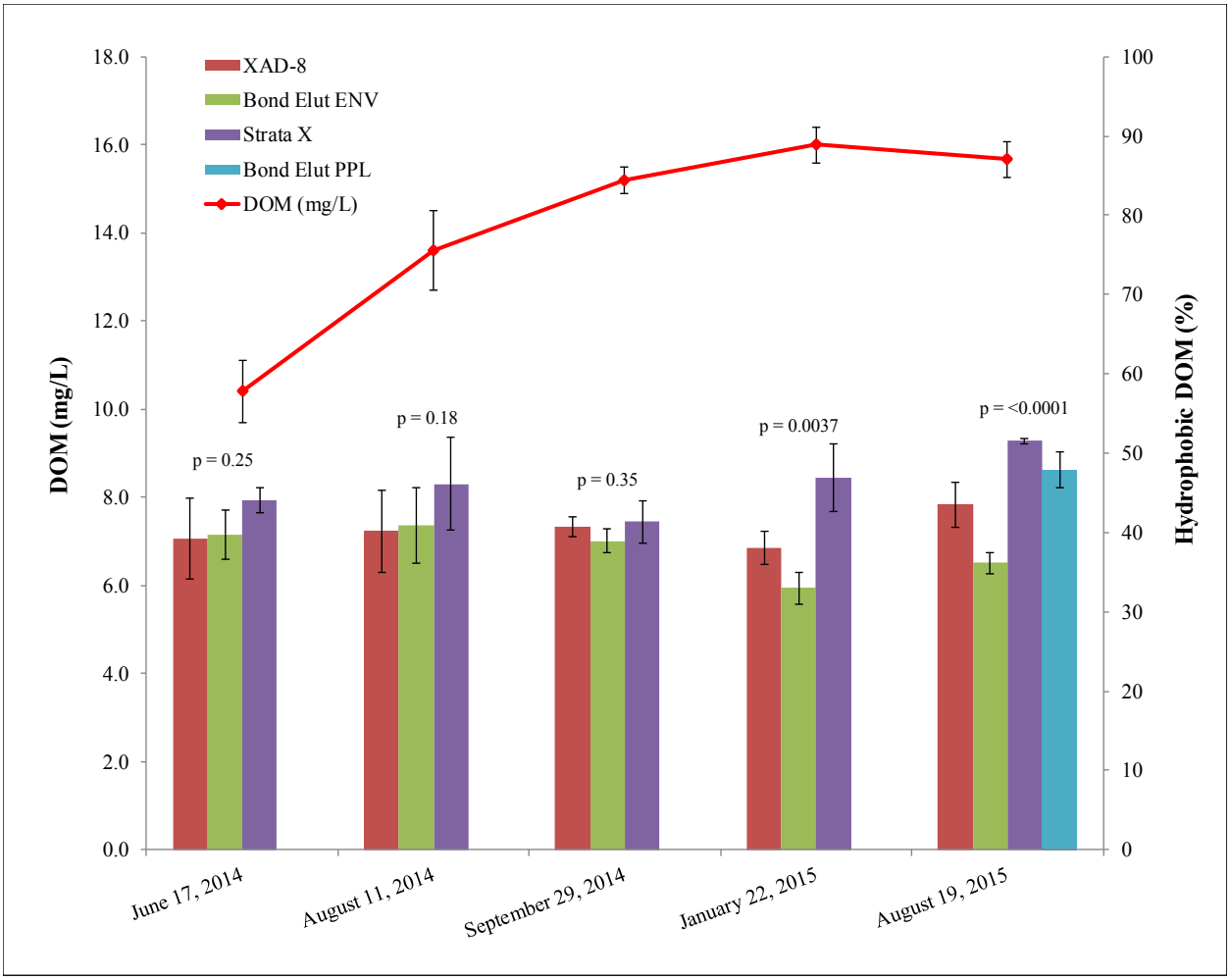


Figure 47: Seasonal DOM fractionation results showing changes in the HPO fraction in Lake Winnipegosis according to samples collected from the raw water line located in the Duck Bay Water Treatment Plant (Duck Bay, Manitoba). The p = value reported represent the significant variance measured using ANOVA.

Table 13: Seasonal DOM fractionation results showing changes in the HPO fraction in Lake Winnipegosis according to samples collected from the raw water line located in the Duck Bay Water Treatment Plant (Duck Bay, Manitoba).

Date	DOM (mg/L)	Fractionation Methods			
		XAD-8 (% HPO)	Bond Elute ENV (% HPO)	Strata X (% HPO)	Bond Elute PPL (% HPO)
June 17, 2014	10.4±0.7	39.2±5.1	39.8±3.1	44.1±1.6	Not Tested
August 11, 2014	13.6±0.9	40.2±5.2	40.9±4.8	46.1±5.8	Not Tested
September 29, 2014	15.2±0.3	40.7±1.2	39.0±1.6	41.3±2.6	Not Tested
January 22, 2015	16.0±0.4	38.0±2.1	33.0±2.0	46.9±4.3	Not Tested
August 19, 2015	15.8±0.4	43.5±2.9	36.2±1.4	51.6±0.4	47.9±2.2

Samples collected from Camperville (Figure 48: Table 14) and Pine Creek First Nation (Figure 49; Table 15) also show an increase in DOM concentration from the fall sample set (September 29, 2014) to the winter sample set (January 22, 2015). However, there was a decrease observed in total DOM concentration from summer to fall (August 11, 2014 to September 29, 2014) for the samples collected from Camperville. This decrease was not observed in the samples collected from Duck Bay which provides evidence that the DOM concentration is not consistent throughout the lake. This provides justification for monitoring multiple locations in large water body like Lake Winnipegosis to fully understand the influx, and composition, of DOM in the Lake.

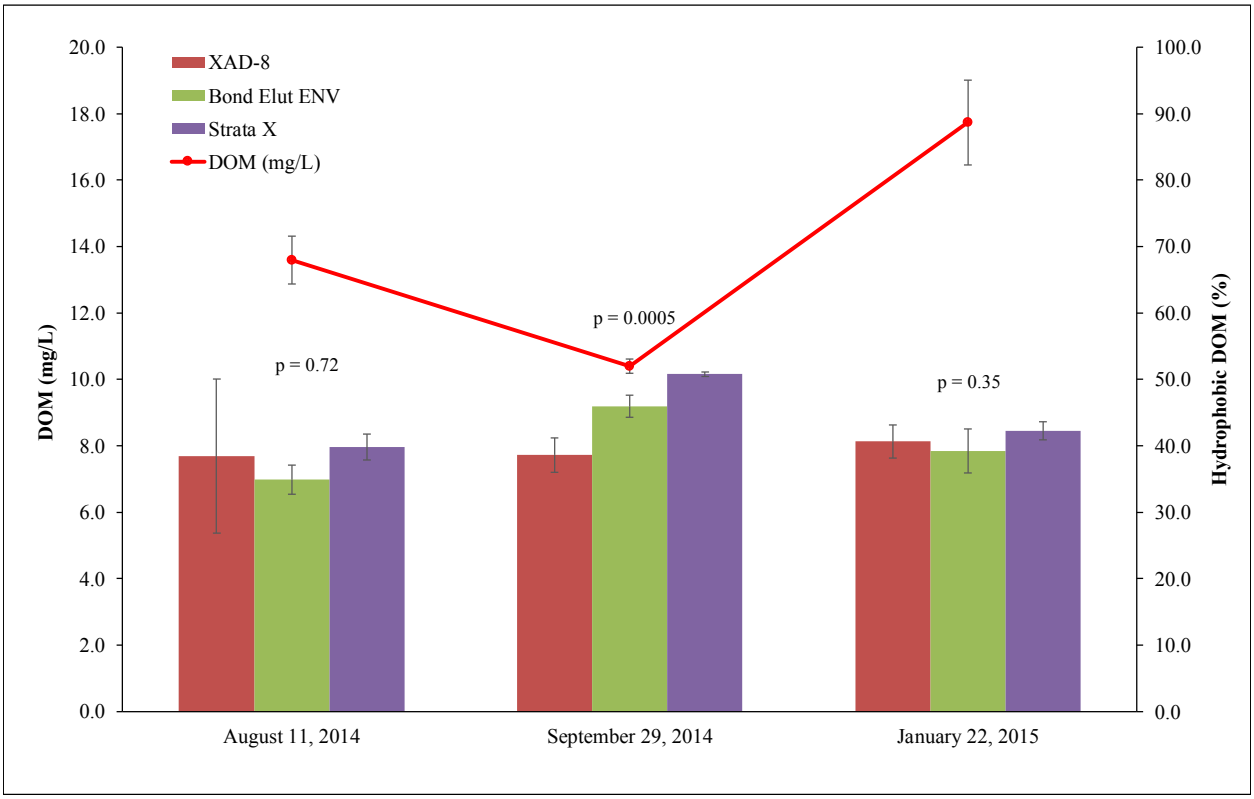


Figure 48: Seasonal DOM fractionation results showing changes in the HPO fraction in Lake Winnipegosis according to samples collected from the raw water line located in the Camperville Water Treatment Plant (Camperville, Manitoba). The p = value reported represent the significant variance measured using ANOVA.

Table 14: Seasonal DOM fractionation results showing changes in the HPO fraction in Lake Winnipegosis according to samples collected from the raw water line located in the Camperville Water Treatment Plant (Camperville, Manitoba).

Date	DOM (mg/L)	Fractionation Methods		
		XAD-8 (% HPO)	Bond Elute ENV (% HPO)	Strata X (% HPO)
August 11, 2014	13.6±0.7	38.5±11.6	34.9±2.2	39.8±2.0
September 29, 2014	10.4±0.2	38.6±2.6	45.9±1.6	50.8±0.3
January 22, 2015	17.7±1.3	40.7±2.5	39.2±3.3	42.3±1.4

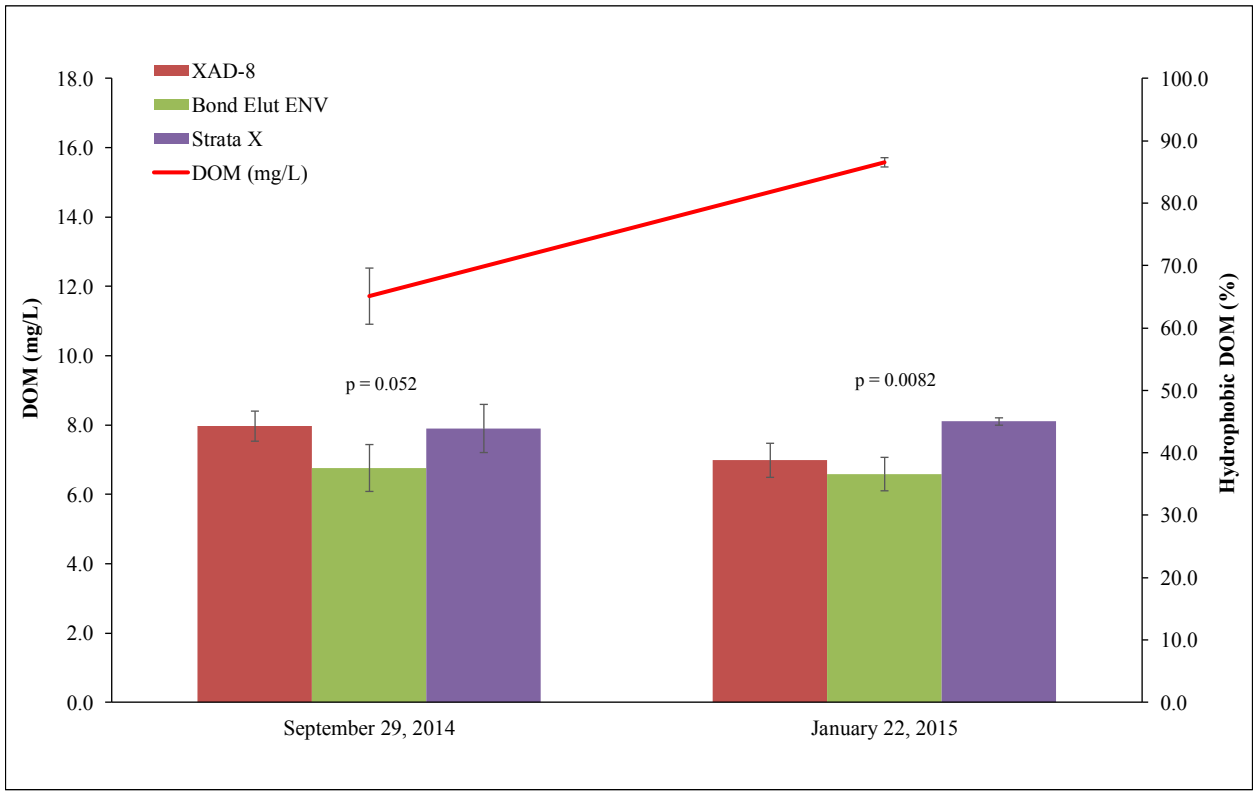


Figure 49: Seasonal DOM fractionation results showing changes in the HPO fraction in Lake Winnipegosis according to samples collected from the raw water line located in the Pine Creek First Nation Water Treatment Plant (Pine Creek First Nation, Manitoba). The p = value reported represent the significant variance measured using ANOVA.

Table 15: Seasonal DOM fractionation results showing changes in the HPO fraction in Lake Winnipegosis according to samples collected from the raw water line located in the Pine Creek First Nation Water Treatment Plant (Pine Creek First Nation, Manitoba).

Date	DOM (mg/L)	Fractionation Methods		
		XAD-8 (% HPO)	Bond Elute ENV (% HPO)	Strata X (% HPO)
September 29, 2014	11.7±0.8	44.3±2.4	37.6±3.8	43.9±3.8
January 22, 2015	15.6±0.1	38.8±2.8	36.6±2.7	45.0±0.6

When comparing the DOM composition of Lake Winnipegosis, based on SPE fractionation results, the HPO fraction was approximately 35-50% of the total DOM concentration. Furthermore, regardless of the increase in total DOM concentration in Lake Winnipegosis measured during this study, the HPO/HPI composition of the lake remained relatively constant. This suggests the composition of organic matter entering Lake Winnipegosis, either from direct runoff or from the surrounding watershed, is of similar HPO/HPI composition. This may be the result of flooding and increased rainfall in the area. Sharp et al. (2006) reported increased flux in both the HPO and HPI fractions following heavy rainfall. The increased rainfall in Manitoba during 2014 accounted for an overall increase in DOM in Lake Winnipegosis, and the DOM composition of the water entering the lake was not significantly different than the lake itself.

When comparing differences between each of the SPEs that underwent seasonal testing, the was a significant difference in the samples collected from Camperville on September 29, 2014. The Tukey HSD test results show ENV was isolating slightly less HPO DOM compared to XAD and Strata ($P<0.01$ and $P<0.05$). There was no significant difference between ENV, Strata or XAD fractionation results for samples collected from Camperville on August 11, 2014 ($p = 0.72$) or January 22, 2015 ($p = 0.35$).

Significant difference was observed between sorbents for samples collected from Duck Bay on January 22 ($p = 0.0037$) and August 19, 2015 ($p = <0.0001$). Tukey HSD test results again indicate that ENV is isolating a lower concentration of HPO DOM compared to Strata ($P<0.01$). For the samples collected from Duck Bay on August 19, 2015, PPL was also found to isolate more HPO DOM compared to ENV; however, no significant difference was found between Strata and PPL for the same sample set.

Samples collected from Pine Creek First Nation on September 29, 2014 showed no significant difference between XAD, ENV or Strata ($p = 0.051$); however, a significant difference was measured for the samples collected in January 2015 ($p = 0.0082$). The Tukey HSD test results show that ENV is isolating lower HPO DOM compared to Strata ($P<0.01$); however, was not found to be significantly different than XAD.

Overall, the fractionation results provide evidence that each SPE sorbent tested is able to isolate a fraction of DOM; however, differences in each sorbent account for minor differences in the amount, and likely type, of DOM isolated with Strata X and PPL frequently isolating more HPO DOM compared to ENV. Further explanation in the differences in DOM isolated by each sorbent is presented in *Section 4.5: Characterization of Natural Waterbodies*.

4.4.2.1 Removal of DOM Fractions by the Duck Bay and Camperville WTPs

Overall poor removal of DOM was observed for both the Duck Bay and Camperville WTPs for all sampling periods (Figures 50-55), which were similar to the results reported for DOM removal by the Waterhen WTP. This result is somewhat expected considering all three WTPs are source waters with similar water quality and using analogous treatment systems. Coagulation was found to remove on average 6-25% of the total DOM concentration at the Duck Bay WTP throughout the study period,

while the Camperville WTP removed only 4-22% of the total DOM concentration by coagulation. The overall poor removal by coagulation at the Duck Bay and Camperville WTPs can possibly be attributed to using PACl as the coagulant, as well as the fact the dominant form of DOM in Lake Winnipegosis is HPI in character, which is not well removed by chemical coagulation. The alkalinity in Lake Winnipegosis, and the Waterhen River, averages around 100 mg/L $CaCO_3$, which is considered low. Aluminum and ferric based coagulants consume more alkalinity than PACl; therefore, in situations where the raw water alkalinity is low PACl is a preferred option to prevent corrosive water from entering the distribution system (Matilainen, Vepsalainen, & Sillapaa, 2010). It has been noted that PACl does not remove DOM as effectively as alum and ferric based coagulants, although there are studies which report PACl preferentially targeted THM precursors better than aluminum or ferric salts (Matilainen, Vepsalainen, & Sillapaa, 2010; Rizzo, Belgiorno, & Meric, 2004). A preliminary investigation into the optimal coagulant dose for both the Camperville and Duck Bay WTPs was conducted in November 2012. The results found PACl had poor removal of DOM even after the optimal coagulant dose and pH was determined. At the optimal coagulant dose and pH for PACl determined in this preliminary study, only a 37% and 29% reduction in DOM was observed (Table 16). The poor removal of DOM by both systems is the cause for elevated THM formation following chlorine disinfection.

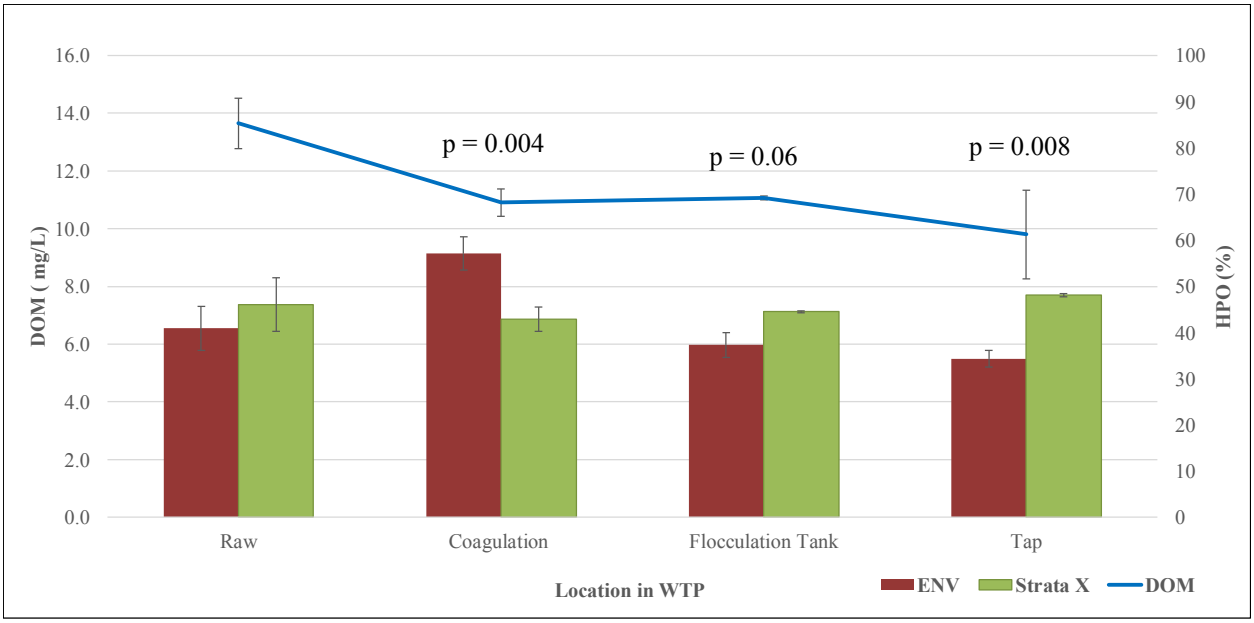


Figure 50: The change in DOM and the HPO fraction (measured using either Strata X or Bond Elute ENV) by treatment processes at the Duck Bay WTP. Samples were collected on August 11, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

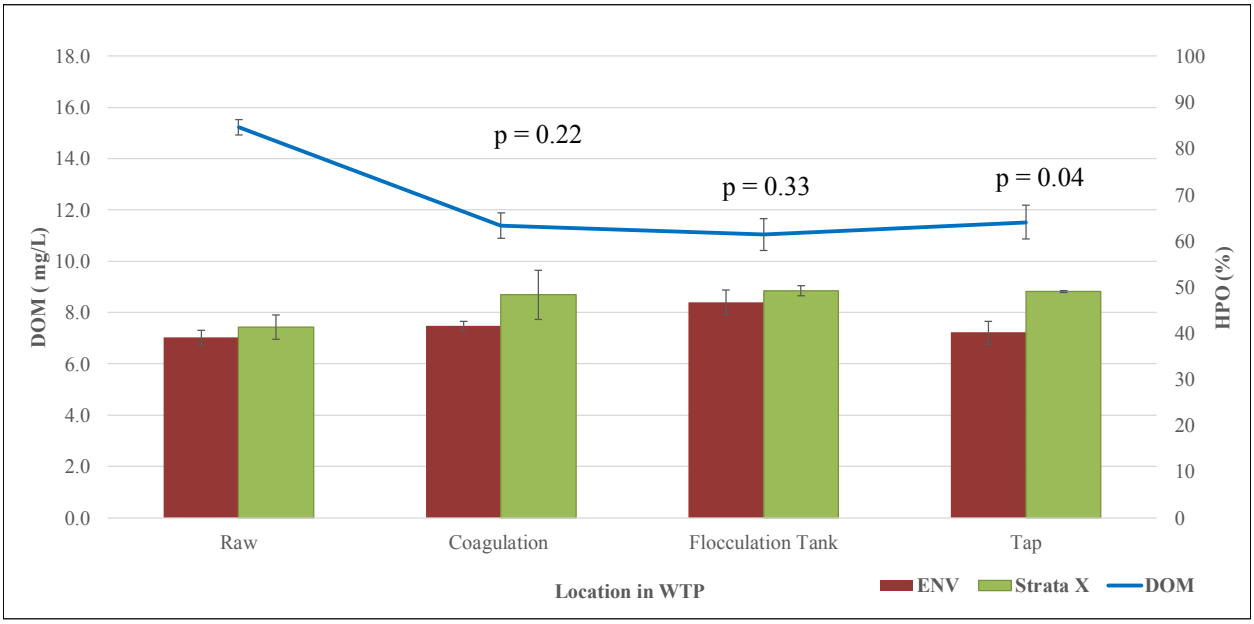


Figure 51: The change in DOM and the HPO fraction (measured using either Strata X or Bond Elute ENV) by treatment processes at the Duck Bay WTP. Samples were collected on September 29, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

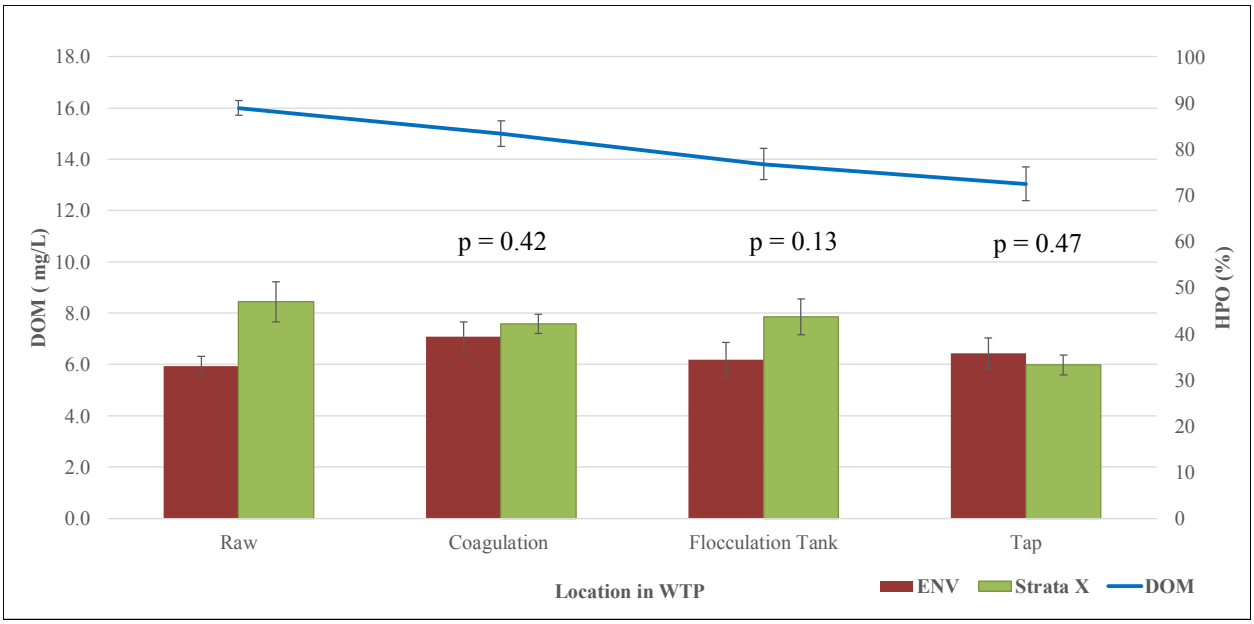


Figure 52: The change in DOM and the HPO fraction (measured using either Strata X or Bond Elute ENV) by treatment processes at the Duck Bay WTP. Samples were collected on January 22, 2015. The p = value reported represents the significant difference using a t-Test at 95% confidence.

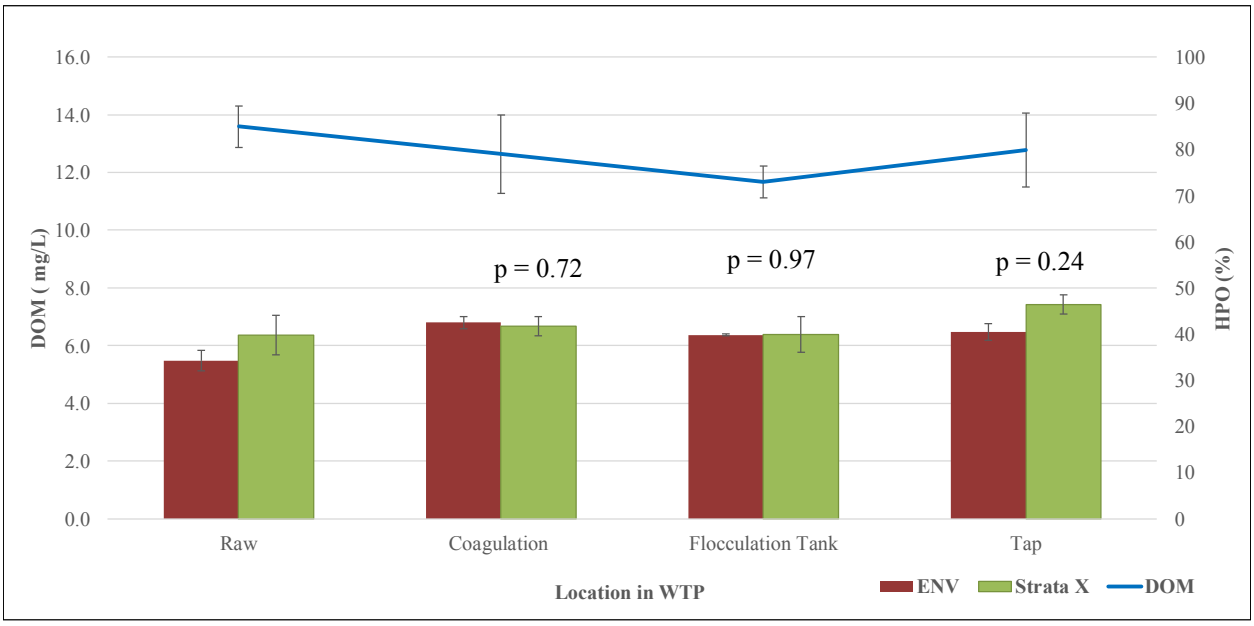


Figure 53: The change in DOM and the HPO fraction (measured using either Strata X or Bond Elute ENV) by treatment processes at the Camperville WTP. Samples were collected on August 11, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

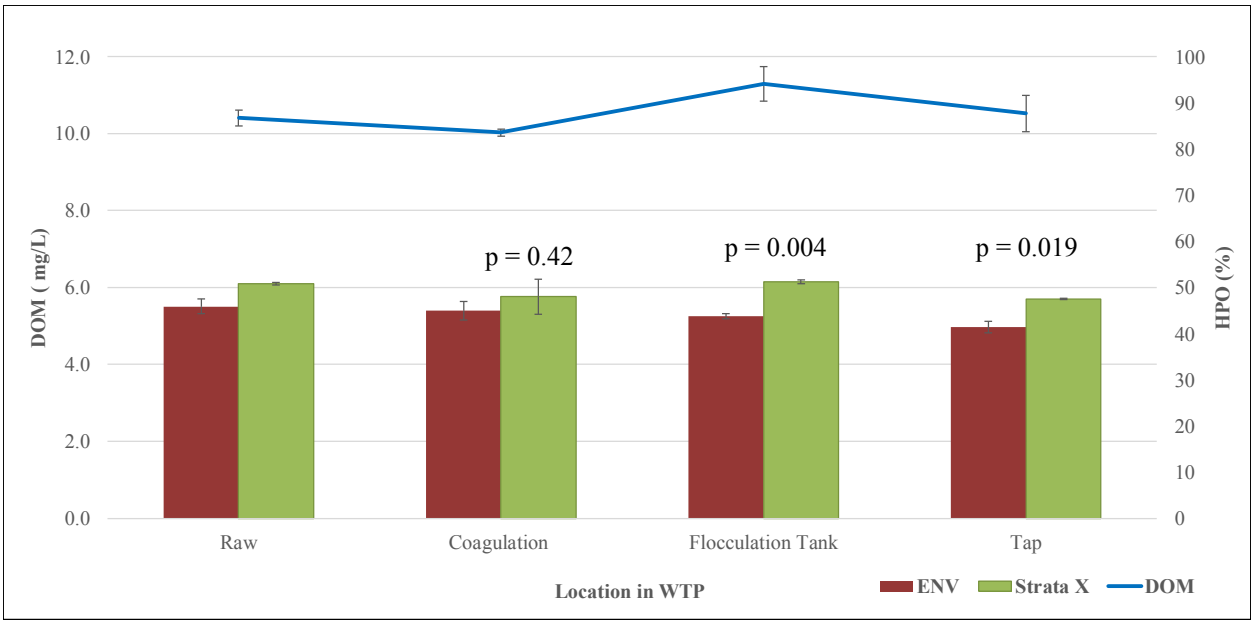


Figure 54: The change in DOM and the HPO fraction (measured using either Strata X or Bond Elute ENV) by treatment processes at the Camperville WTP. Samples were collected on September 29, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

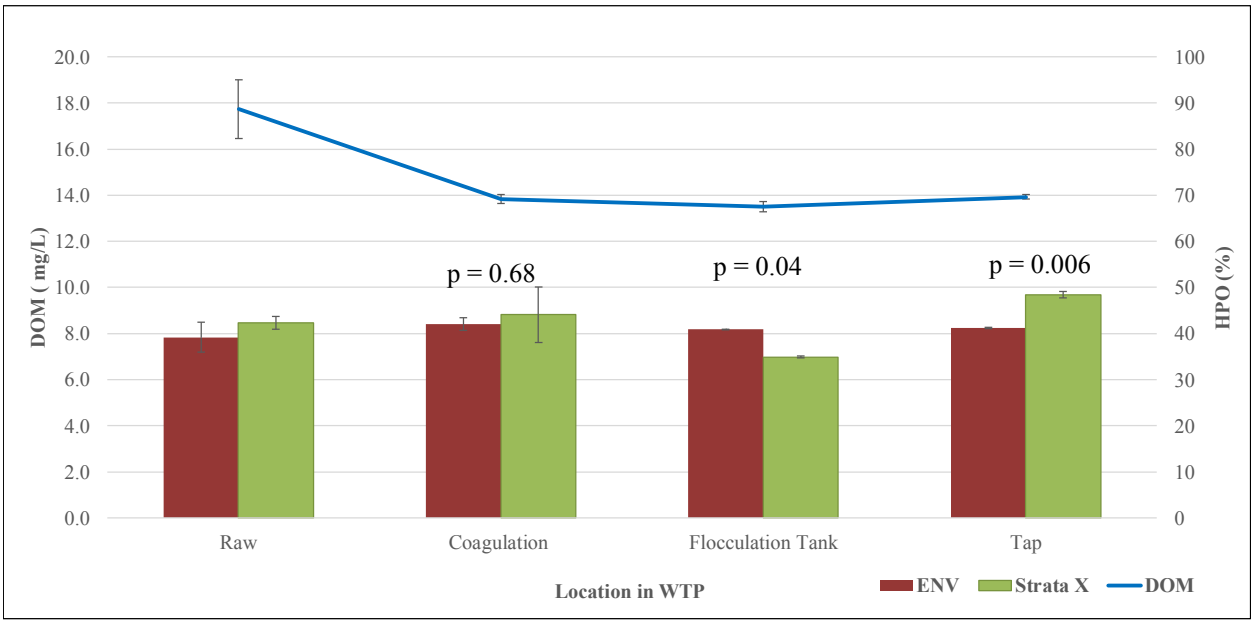


Figure 55: The change in DOM and the HPO fraction (measured using either Strata X or Bond Elute ENV) by treatment processes at the Camperville WTP. Samples were collected on January 22, 2015. The p = value reported represents the significant difference using a t-Test at 95% confidence.

Table 16: Optimization of PACl dose at the Camperville and Duck Bay WTPs. Raw water DOM concentration for both locations was 7.96 mg/L. Samples were collected on November 2, 2012.

Location	PACl (mg/L)	pH	DOM Removed (mg/L)	% DOM Removed
Camperville, MB	80	7	2.96	37.2
Duck Bay, MB	110	7	2.28	28.6

4.4.2.2 Removal of DOM Fractions by the Pine Creek First Nation WTP

Figures 56 and 57 show the removal of DOM, and DOM fractions, by the Pine Creek First Nation WTP. The raw water DOM concentration and composition was similar to the results presented for Duck Bay and Camperville for the same sampling period, with DOM ranging between 12-16 mg/L, and being approximately 35-40% HPO in character. For both sampling sets it was observed that there was no significant reduction in DOM following coagulation. DAF treatment was also ineffective at reducing DOM, or DOM fractions (January 22, 2015 dataset). The RO membrane was found to have the greatest reduction in DOM for both the September 29, 2014 and January 22, 2015 sample sets of 33% and 27%, respectively. Generally, RO membranes are able to achieve >99% reduction of DOM, provided the integrity of the membrane is intact. However, a membrane which may be heavily fouled, or has compromised integrity such as a damaged seal, may impact the membranes performance equating to low rejection rates (Pype, et al., 2013). Given the poor rejection of DOM by the RO membrane system at the Pine Creek First Nation WTP it is likely the membrane has either fouled or is in need of a chemical clean-in-place (CIP). If DOM rejection rates are not improved following a CIP it is likely the membrane has irreversible fouling or damage and requires replacement. Given there is no available background information as to how the membrane operates, it is difficult to determine the cause for poor removal of DOM by the RO membrane. For both samples there was an increase in DOM measured in the treated water collected from the cold-water tap located in the WTP. It should be

noted that there appeared to be no primary disinfection (i.e., chlorination) of the treated water during the time of collection (Figure 58). The reason for no addition of a disinfectant is unclear and no operator was available at the time of the site visit to provide insight. It is assumed the treated water is stored in an onsite reservoir; however, if the treated water is not properly disinfected prior to storage there is a strong possibility for bacterial growth in treated water storage reservoir. Note, there is no potable water distribution system in Pine Creek First Nation and drinking water is collected from the WTP in cisterns.

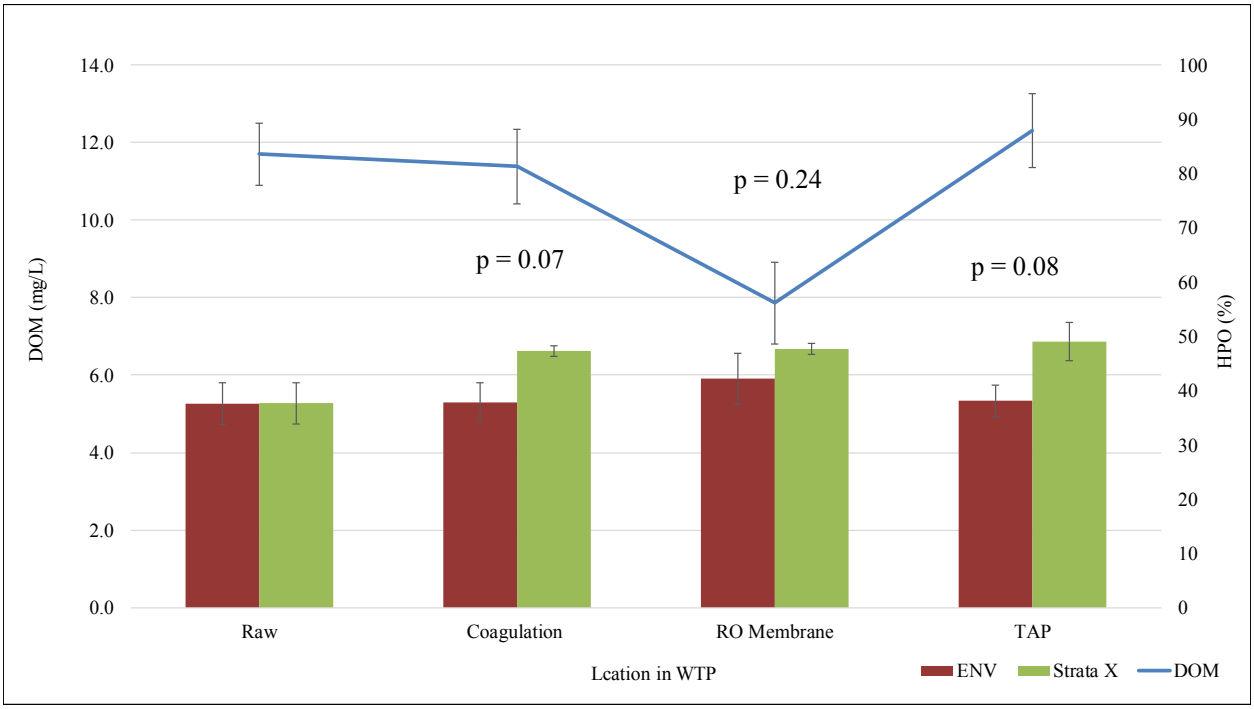


Figure 56: The change in DOM and the HPO fraction (measured using either Strata X or Bond Elute ENV) by treatment processes at the Pine Creek First Nation WTP. Samples were collected on September 29, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

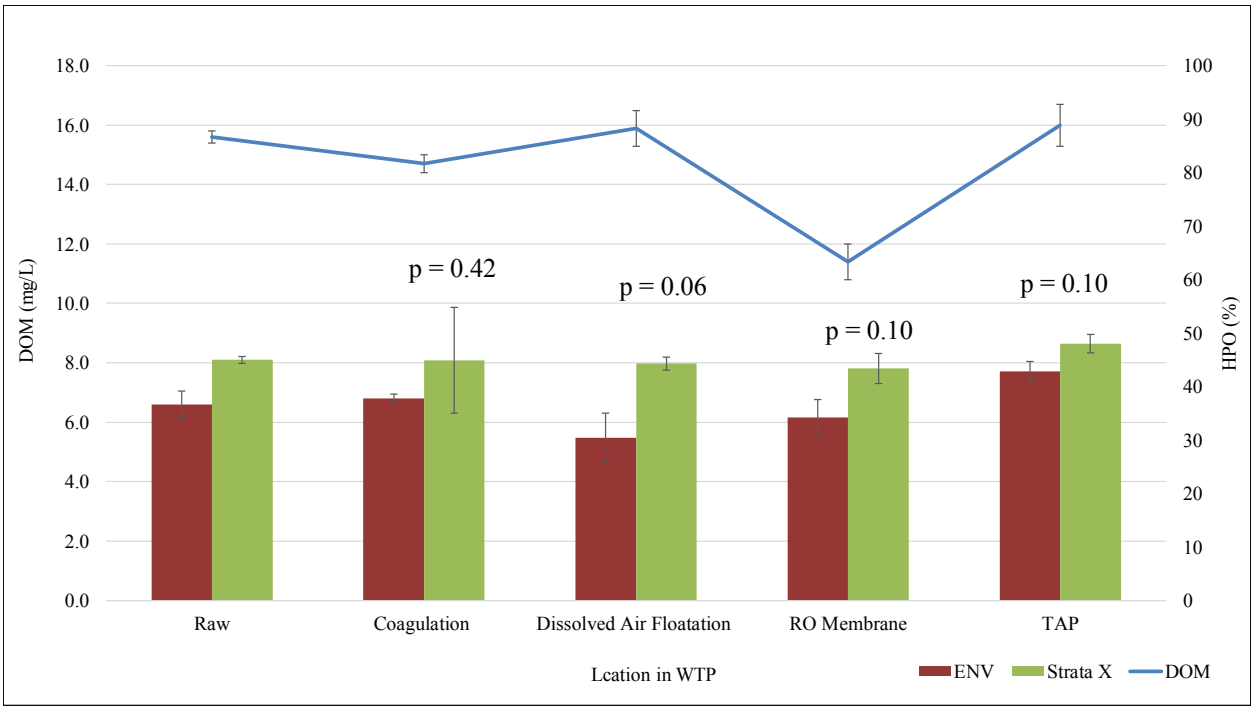


Figure 57: The change in DOM and the HPO fraction (measured using either Strata X or Bond Elute ENV) by treatment processes at the Pine Creek First Nation WTP. Samples were collected on January 22, 2015. The p = value reported represents the significant difference using a t-Test at 95% confidence.

Figure 58: Photo of RO membrane system at the Pine Creek First Nation WTP showing a note stating, "*unchlorinated water filtered only take at own risk*." Picture was taken in January 22, 2015.

4.4.2.3 THMFP and Specific THMFP of DOM Fractions Isolated from Lake Winnipegosis Using SPE

The THMFP and STHMFP results for raw water and DOM fractions collected from Duck Bay WTP, Camperville WTP and Pine Creek First Nation WTP are presented in Tables 17 to 19, respectively.

The raw water THMFP and STHMFP from Lake Winnipegosis (Tables 17-19) were similar to the results reported for the Waterhen River (Table 12) in both the winter (January) and summer (August) sample sets, where there was higher unfractionated DOM THMFP and STHMFP in the summer compared to the winter.

Comparing the THMFP and STHMFP for fractions collected from Duck Bay on January 22 and August 19, 2025 show a significant difference in the STHMFP for the HPO fraction isolated with each sorbent with $p = 0.001$ and $p= 0.035$, (See Appendix A) respectively. Similarly, with the samples collected from Pine Creek First Nation, a significant difference was found in the STHMFP results for the HPO fraction isolated with each sorbent. The Tukey HSD results found that ENV was forming more THMs pre mgHPO-DOM compared to Strata. This indicates that the ENV HPO fraction was isolating THM precursors more specifically compared to Strata, as ENV only isolated 38.8% HPO DOM compared to Strata which isolated 45% HPO DOM for this sample set.

There was not significant difference in the STHMFP results for the HPO fraction isolated with ENV, Strata, and XAD collected from Camperville on January 22, 2015.

The THMFP and STHMFP results for Lake Winnipegosis showed good comparisons with the results found for the Waterhen River. The THMFP and STHMRP results presented for the Waterhen River and Lake Winnipegosis show that the SPEs tested generate comparable results across two

different waterbodies which are part of the same system. This indicates that THMFP and STHMFP data collected from this system can be useful for other WTPs which are treating surface waters of the same system. Furthermore, the results further indicate that ENV appears to more frequently isolate an HPO DOM fraction with greater STHMFP compared to Strata and PPL.

Table 17: THMFP and STHMFP from DOM fractions collected from Lake Winnipegosis via the Duck Bay Water Treatment Plant raw water intake.

Sorbent	THMFP (µg/L)				Specific THMFP (µgTHM/mgDOM)			
	22-Jan-15		19-Aug-15		22-Jan-15		19-Aug-15	
	HPO	HPI	HPO	HPI	HPO	HPI	HPO	HPI
Raw Water	170±16		238±16		10.6±0.9		15.0±1.0	
XAD	146±9	44±5	126±7	40±5	24.3±1.2	4.5±0.4	21.1±1.3	4.6±0.6
Bond Elute ENV	119±9	14±4	104±8	23±3	22.3±0.8	1.3±0.4	21.6±2.4	2.4±0.2
Strata X	147±13	42±12	124±10	33±5	19.4±0.4	4.9±1.1	18.0±1.7	4.2±0.7
Bond Elute PPL	N/A	N/A	116±11	28±3	N/A	N/A	17.6±0.7	3.3±0.2

Table 18: THMFP and STHMFP from DOM fractions collected from Lake Winnipegosis via the Camperville Water Treatment Plant raw water intake.

Sorbent	THMFP (µg/L)		Specific THMFP (µgTHM/mgDOM)	
	22-Jan-15		22-Jan-15	
	HPO	HPI	HPO	HPI
Raw Water	225±33		12.8±2.5	
XAD	128±42	21±8	18.4±5.7	2.2±1.0
Bond Elute ENV	86±10	27±9	13.4±1.3	2.6±0.8
Strata X	101±15	75±24	12.4±0.3	6.8±2.3

Table 19: THMFP and STHMFP from DOM fractions collected from Lake Winnipegosis via the Pine Creek First Nation Water Treatment Plant raw water intake.

Sorbent	THMFP (µg/L)		Specific THMFP (µgTHM/mgDOM)	
	22-Jan-15			
	HPO	HPI	HPO	HPI
Raw Water	206±17		13.2±1.1	
XAD	161±21	41±3	26.2±1.9	4.2±0.1
Bond Elute ENV	109±12	38±7	19.1±1.3	3.9±0.6
Strata X	90±14	9±2	12.9±1.8	6.8±2.3

4.4.3 Seasonal Field Testing of the La Salle River Using SPE

The raw water DOM concentration in the La Salle River during the summer of 2014 (May to July) remained relatively constant, ranging from 12.3 mg/L to 13.0 mg/L (Figure 59). However, in March 2015 there was a significant increase in DOM measured in raw water. This is likely a combination of flooding in Manitoba in 2014, particularly the Assiniboine River, and spring melt which can increase the concentration of organic matter entering a surface water (Kusch & Owen, 2014; Mann, et al., 2012). Mann et al. (2012) showed similar increases in DOM concentration following spring melt and freshet entering the Kolyma River basin. However, the increase in DOM reported in the Mann et al. study was attributed to humic matter from the surrounding catchment. The increase in total DOM measured in March 2015 was more HPI in character which is contradictory to results reported in the Mann et al. study. The increase in the HPI fraction in the La Salle River in March 2015 can likely be attributed to higher concentrations of HPI organic matter entering the water following overland flooding as the flood waters would dissolve more HPI organic matter than HPO, thereby increasing the overall HPI character as the flood waters enter the La Salle River. A similar explanation was provided by Scott et al. (2001) who reported an increase in HPI DOM during an autumn flush following a long drought period. The Assiniboine River feeds the La Salle River via the Assiniboine-La Salle Diversion (Figure 60) (Mitchell, et al., 2012). Significant flooding of the Assiniboine River in the spring and early summer of 2014 would likely have increased the DOM concentration in the river due to increased runoff from overland flooding. The increase in DOM measured in the La Salle River starting in the fall of 2014 and continuing into 2015 is likely a result of the increase in DOM that would be expected given the extensive flooding of the Assiniboine River. It should be noted that the DOM in the Assiniboine River was not measured in this study and the increase in DOM concentration during the 2014 flood is speculative. However, samples taken in June

and July 2015 show the DOM concentration returned to historical concentrations ranging between 16 – 18 mg/L. This provides some evidence that the significant increase in DOM measured in the fall of 2014 and spring of 2015 was a result of the combination of flooding of the Assiniboine River and the intrusion of freshet following spring melt.

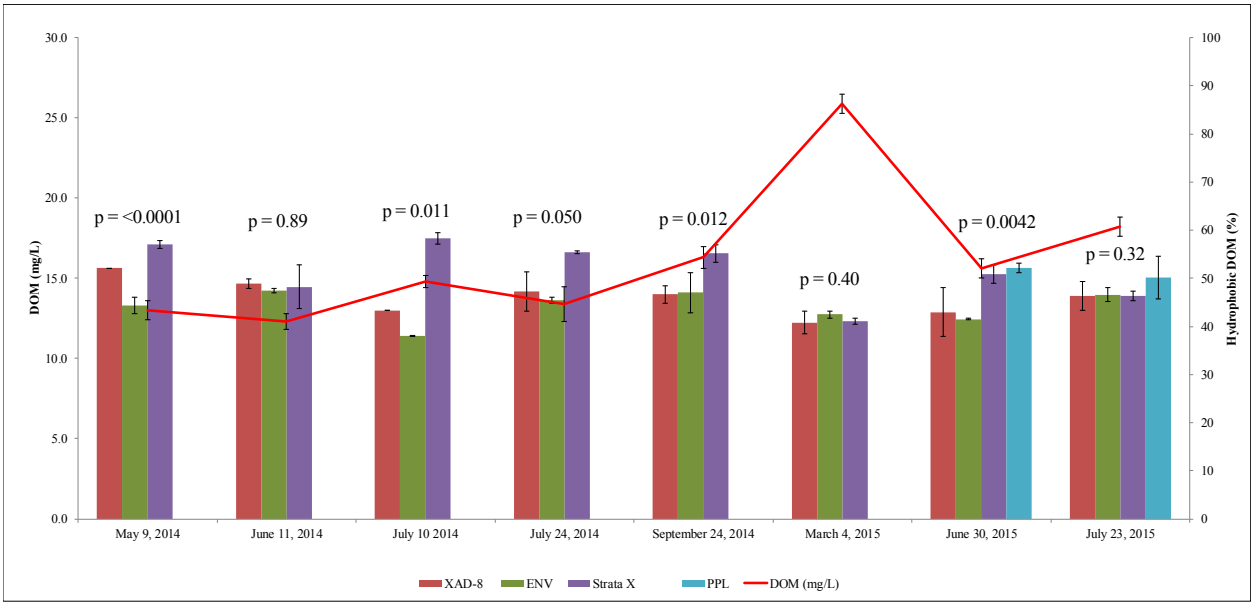


Figure 59: Seasonal DOM fractionation results showing changes in the HPO fraction in the La Salle River according to samples collected from the raw water line located in the Macdonald Water Treatment Plant (Sanford, Manitoba). The p = value reported represent the significant variance measured using ANOVA.

The seasonal DOM fractionation results for the La Salle River showed signification variation throughout the sample period. All sample sets, but those collected on June 11, 2015, March 4, 2015 and July 3, 2015, found a significant difference between the sorbents tested (Figure 59). In all sample sets where a statistical difference was measured the Tukey RSD test results indicated that ENV was isolating less HPO DOM compared to Strata in all samples ($P<0.05$). PPL was found to isolate a significantly greater fraction of HPO DOM compared to ENV for samples collected on June 30, 2015 ($P<0.05$); however, no statistical difference was measured in the fractionation results for ENV and

PPL for samples collected on July 23, 2015. No significant difference was measured between PPL and Strata for samples collected in June and July of 2015.

Overall, similar results were found for the La Salle River as was observed for the Waterhen River and Lake Winnipegosis results, where ENV appear to be isolating a lower concentration of HPO DOM compared to Strata or PPL.

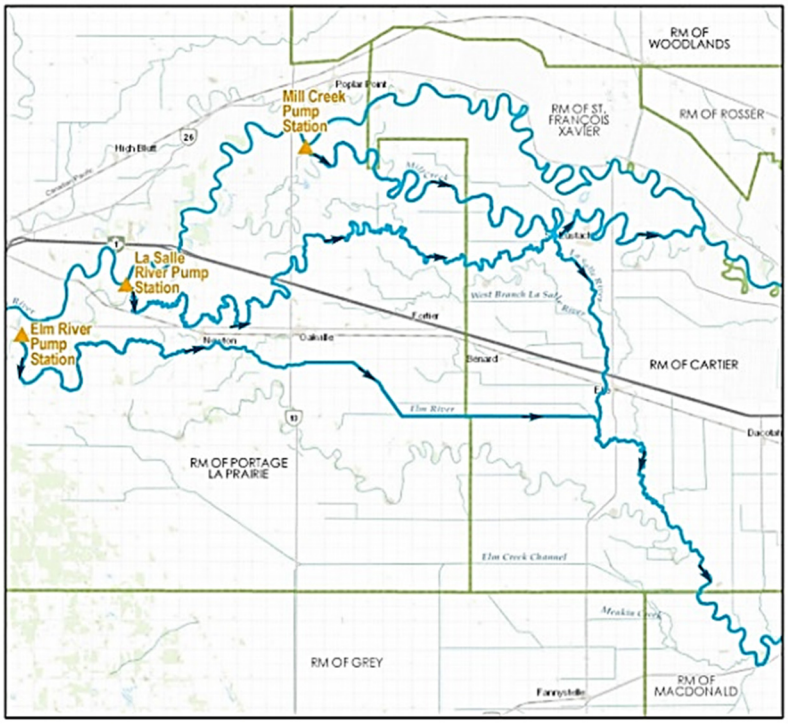


Figure 60: Locations of three pumping stations along the Assiniboine River. Figure taken from (Lupkowski, 2018). Permission provided by Golden West Broadcasting see Appendix C for permission details.

4.4.3.1 Removal of DOM Fractions by the Macdonald Water Treatment Plant

The removal of DOM by the MWTP was similar for all sample sets (Figures 61-65). There was significant variation measured in the reduction of DOM by the ultrafiltration system, which ranged from 2.5% to 23.9% during the study. The difference in removal of DOM may be related the lower reaction kinetics of metal coagulants in cool water. The poorest removal of DOM by the UF system

occurred in September 2014 (2.5% reduction) and March of 2015 (3.7% reduction), which would have cooler raw water temperatures compared to samples collected in June 2014 (19.1% and 23.9% reduction) and June 2015 (11.3% reduction). Furthermore, in the June 2014 samples, where there was the greatest measured reduction in DOM, there was also a reduction in the HPO fraction. This result is expected as metal coagulant added prior to the UF system would target the HPO fraction compared to the HPI fraction, due to the negative charge associated with carboxyl groups of HA and FA at ambient pH conditions in natural water. There is an increase in DOM measured in the treated water (Tap), ranging from 3.4 mg/L to 4.9 mg/L, due to the blend of approximately 25% UF permeate with the RO permeate. There appears to be lower HPO concentration in the blended water, according to both ENV and Strata X, in the sample collected during 2015, compared to the 2014 samples. It is unclear if there was an alteration in WTP operation that would have accounted for the differences observed in the DOM composition in the treated water during 2014 and 2015.

Similar to the previously discussed seasonal DOM composition monitoring results, the Strata X isolated a slightly higher concentration compared to the ENV in nearly all samples collected from the MWTP (i.e., $p < 0.05$). This again provides some evidence that Strata X is isolating DOM with some HPI character that does not interact with the ST-DVB polymer of the Bond Elute ENV SPE. The DOM composition was only measured using the Bond Elute PPL SPE on June 30, 2015 (Figure 65). Bond Elute PPL showed similar HPO isolation as the Strata X for the raw water ($p = 0.86$) and UF permeate ($p = 0.17$) samples, which was also observed in field testing of the Waterhen River and Lake Winnipegosis. However, Strata X and Bond Elute PPL isolated a significantly different concentration of DOM ($p = 0.008$) for the sample of treated water. It is unclear why this difference was measure in the treated (blended) water only, and given the limited data comparing the DOM composition of

treated water measured using Bond Elute PPL, further investigation into this cause for the difference observed here is warranted.

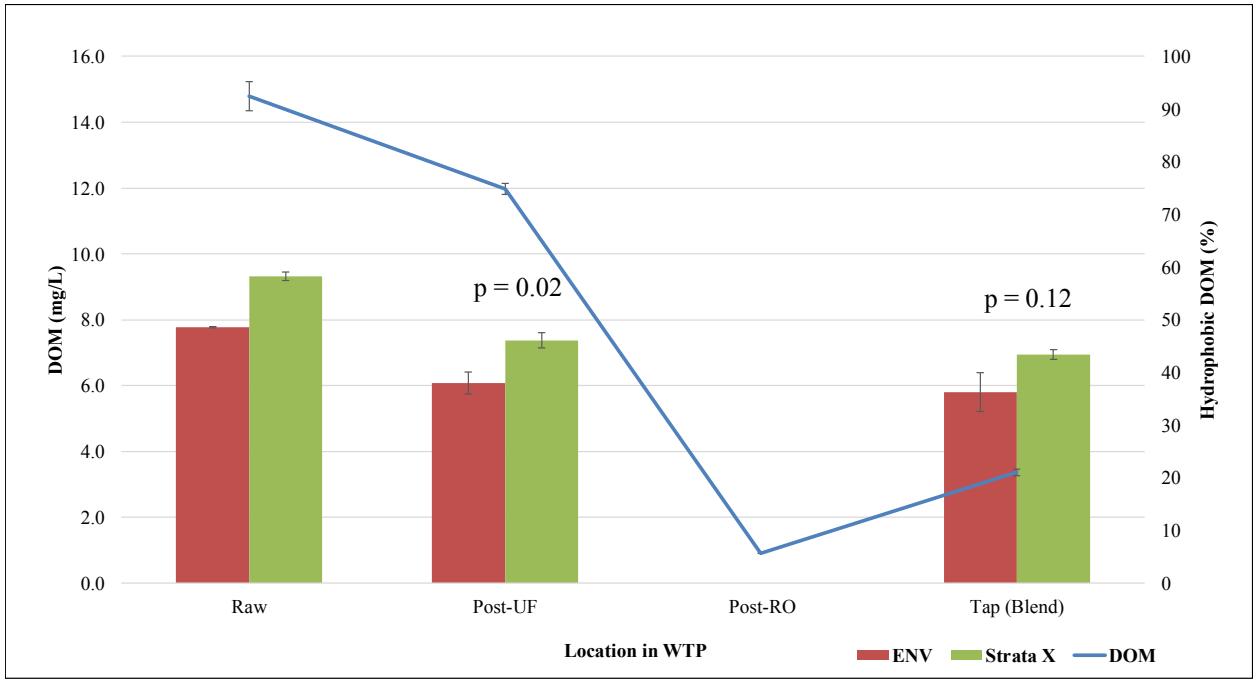


Figure 61: Removal of DOM fractions measured using Strata X and Bond Elute ENV. Samples were collected from the Macdonald Water Treatment Plant on July 10, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

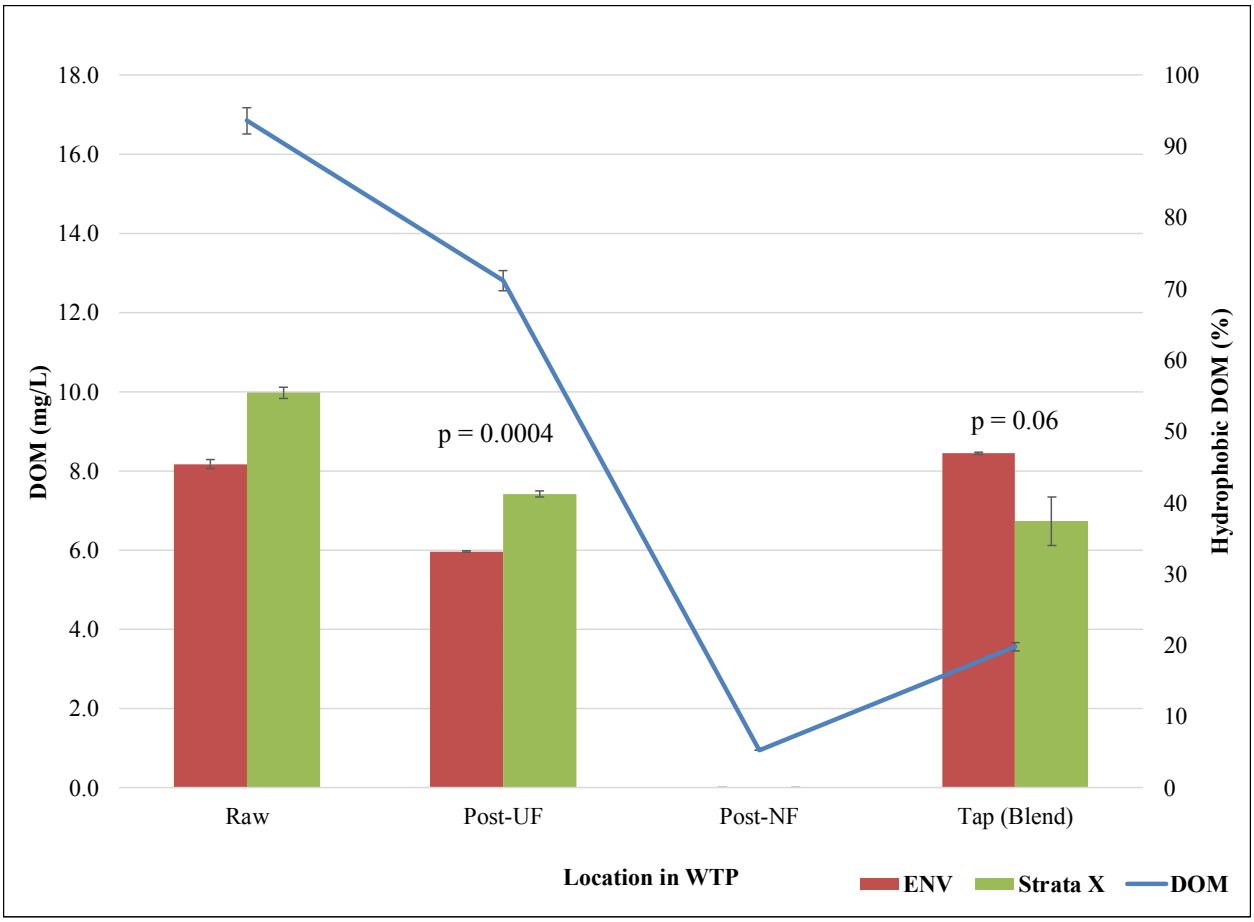


Figure 62: Removal of DOM fractions measured using Strata X and Bond Elute ENV. Samples were collected from the Macdonald Water Treatment Plant on July 24, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

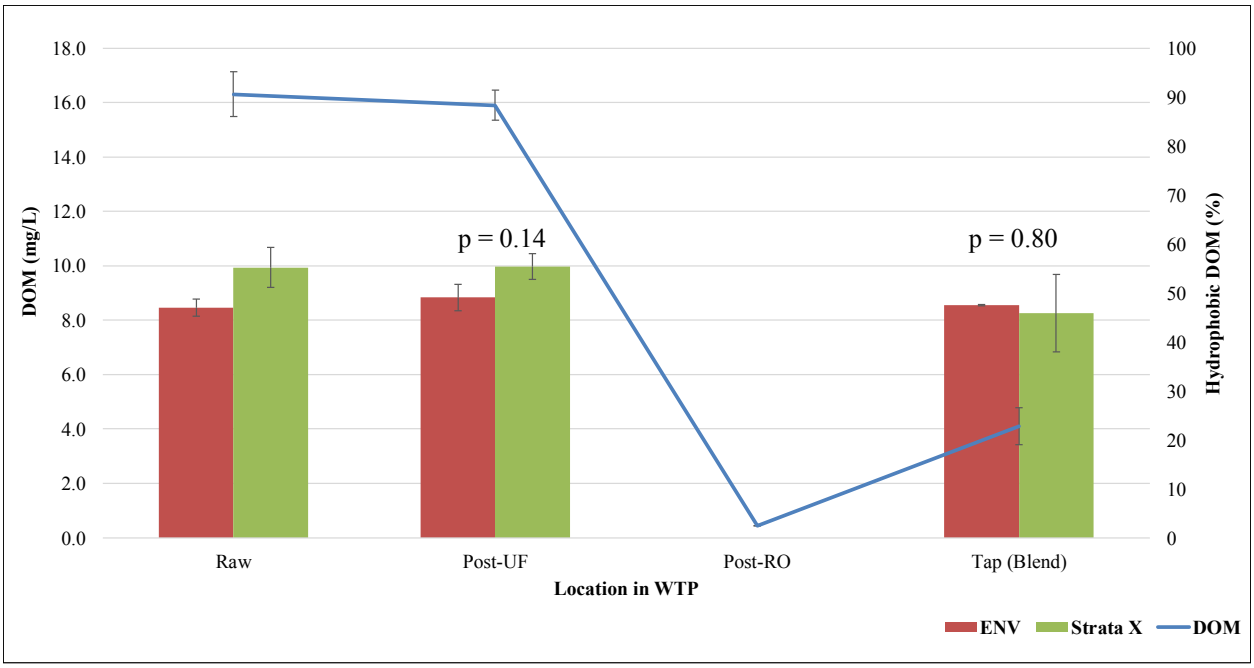


Figure 63: Removal of DOM fractions measured using Strata X and Bond Elute ENV. Samples were collected from the Macdonald Water Treatment Plant on September 24, 2014. The p = value reported represents the significant difference using a t-Test at 95% confidence.

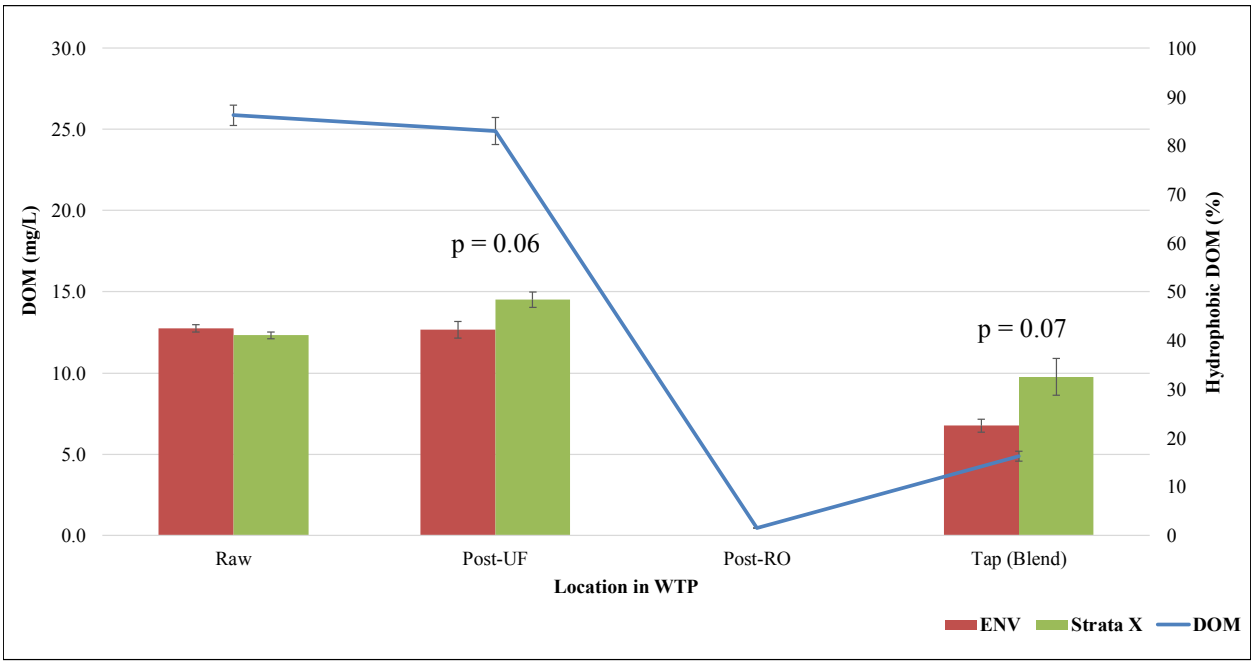


Figure 64: Removal of DOM fractions measured using Strata X and Bond Elute ENV. Samples were collected from the Macdonald Water Treatment Plant on March 4, 2015. The p = value reported represents the significant difference using a t-Test at 95% confidence.

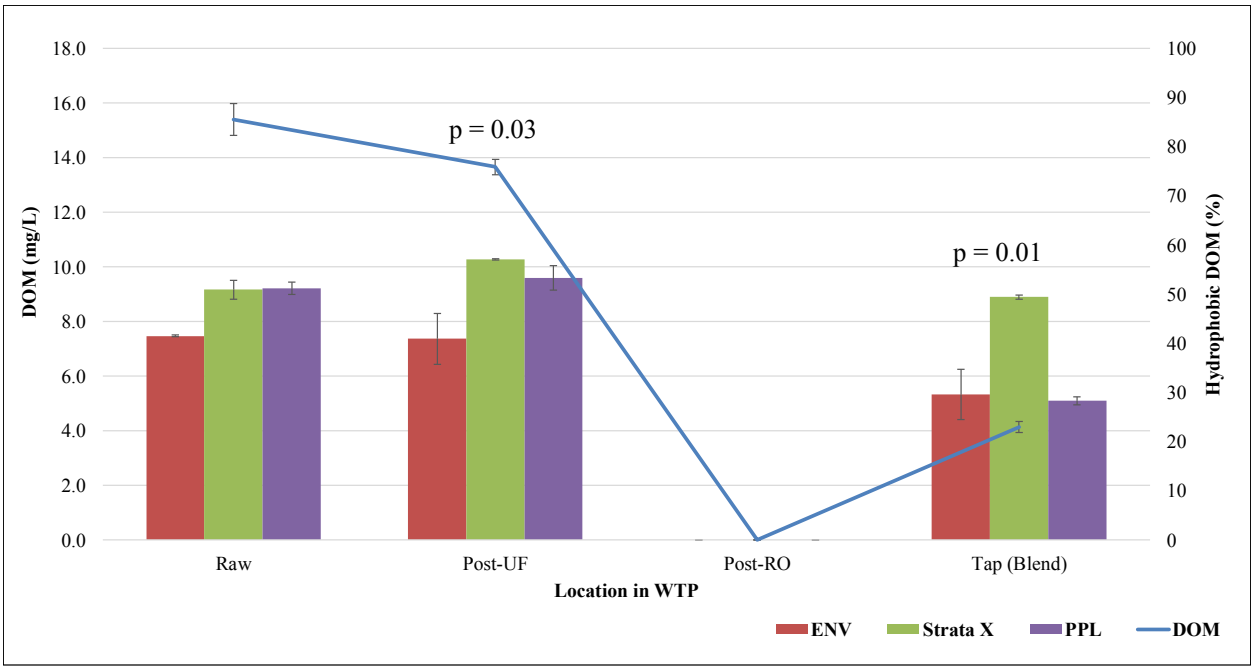


Figure 65: Removal of DOM fractions measured using Strata X, Bond Elute ENV and Bond Elute PPL. Samples were collected from the Macdonald Water Treatment Plant on June 30, 2015. The p = value reported represents the significant difference using one way ANOVA analysis.

4.4.3.2 THMFP and Specific THMFP of DOM Fractions Isolated from the La Salle River Using SPE

The THMFP results (Table 20) for the La Salle River show that there is a greater THMFP in the summer compared to the winter. This result was also noted for both the Waterhen River and Lake Winnipegosis. Also, as found with the Waterhen River and Lake Winnipegosis, the HPO fraction isolated with each sorbent had a greater THMFP and STHMFP than the HPI fraction.

When comparing the THMFP and STHMFP for the HPO fractions isolated with each sorbent the fraction isolated with ENV was found to have the lowest average THMFP. However, the STHMP results show that ENV had a higher STHMFP ($P<0.01$) (March 4, 2015 sample set) compared to Strata. This provides evidence that although ENV is isolating less HPO DOM compared to Strata X and PPL, ENV appears to be isolating a fraction that is more prone to forming THMs. This implies ENV may be more suited for onsite use by water treatment operators.

The THMFP and STHMFP results measured from the HPO fractions collected from the July 23, 2015 samples show no significant difference between the three SPE candidates.

Table 20: THMFP and STHMFP for the La Salle River.

Sorbent	THMFP (µg/L)				Specific THMFP (µgTHM/mgDOM)			
	4-Mar-15		23-Jul-15		4-Mar-15		23-Jul-15	
	HPO	HPI	HPO	HPI	HPO	HPI	HPO	HPI
Raw Water	186±17		222 ± 13		7.7±0.7		12.2 ± 0.5	
XAD	266±40	75±24	152±10	40±17	24.2±3.6	3.9±0.6	18.2±0.8	4.1±1.6
Bond Elute ENV	177±44	77±22	113±18	42±10	15.6±1.9	5.5±0.9	13.4±1.3	4.4±1.0
Strata X	284±43	81±23	125±19	39±23	25.5±1.7	6.1±1.5	15.0±2.2	4.0±2.6
Bond Elute PPL	N/A	N/A	152±17	42±9	N/A	N/A	15.9±2.2	4.5±1.1

N/A = not tested

4.5 Characterization of Natural Waterbodies

4.5.1 Total DOM, HPI- and HPO-DOM Composition of Natural Waters

Both total DOM and its HPO and HPI fractions in surface waters are heavily impacted by the local environment, since runoff from surrounding catchment soils is often the largest contributor (Graeber *et al.*, 2012). From DOC measurements on the raw water, the La Salle River was found to have the greatest concentration of total DOM (Table 21). This would be expected, given its location on the Canadian Prairies, an agricultural grassland region with rich organic, chernozemic soils. Lake Winnipegosis (measured at Duck Bay) and the Waterhen River had lower DOM, again as expected, since they are in the Canadian Shield, which is largely dominated by boreal forests on bedrock covered with thin soil.

Table 21: DOM composition (measured as DOC) of the La Salle River, Lake Winnipegosis, and the Waterhen River. Error is reported as standard deviation from the mean. Figure taken from (Goss et al., 2017).

Raw DOC (mg/L)	Sorbent	% HPI	% HPO	% HPO Recovered
La Salle River July 23, 2015 (Raw pH = 8.1)				
18.3 ± 0.7	ENV	53.4 ± 1.5	46.6 ± 1.5	88.4 ± 2.0
	PPL	49.8 ± 4.4	50.2 ± 4.4	90.8 ± 1.0
	Strata-X	53.6 ± 1.0	46.4 ± 1.0	84.5 ± 1.3
Lake Winnipegosis August 19, 2015 (Raw pH = 8.4)				
15.8 ± 0.4	ENV	63.8 ± 1.4	36.2 ± 1.4	86.6 ± 1.1
	PPL	52.1 ± 2.2	47.9 ± 2.2	86.9 ± 1.6
	Strata-X	48.5 ± 0.4	51.6 ± 0.3	83.2 ± 1.2
Waterhen River August 19, 2015 (Raw pH = 8.3)				
11.4 ± 0.5	ENV	71.5 ± 2.2	28.6 ± 2.2	86.0 ± 1.7
	PPL	62.7 ± 2.9	37.3 ± 2.8	88.4 ± 1.5
	Strata-X	68.1 ± 3.9	31.9 ± 3.9	86.1 ± 2.3

Differences in chemical character and in pore size of each sorbent could account for differences in the fraction of DOM isolated. Of the two ST-DVB polymer-based SPEs, extraction efficiency of HPO-DOM was best for the PPL, for all three waters in the study (Table 21). The greater efficiency of PPL over ENV for isolation of DOM from seawater was attributed to the smaller PPL pore size (Dittmar et al., 2008): PPL (150 Å) and ENV (450 Å). However, modifications to the ST-DVB polymer also increase the hydrophobicity to some extent. The ENV contains ST-DVB with a high degree of cross-linking (Fontanals et al., 2010); the ST-DVB polymer in PPL has a proprietary non-polar surface modification that increases the capacity for non-polar analytes. Because the modifications are proprietary, it is difficult to draw a firm conclusion about the relative importance of pore size and surface modifications. However, it seems unlikely that pore size alone could account for differences in extraction efficiency. The pore size of Strata-X (85 Å) is about half that of PPL and one fifth that of ENV. If pore size alone were the only mechanism responsible for the different

efficiencies, the Strata-X should have isolated significantly more HPO-DOM in all samples, but this was not the case.

The chemical interaction between HPO DOM and ST-DVB (ENV and PPL) is fundamentally different from that between HPO DOM and NVP (Strata). The isolation of HPO compounds by ST-DVB polymers is mediated by non-polar van der Waal's forces and π-π interactions of the aromatic benzene rings. NVP polymers can also isolate material through hydrogen bonding and dipole interactions with the amide group on the lactam ring (Fontanals et al., 2010). The hydrogen bonding capability of the NVP would assist in extraction of HPO-DOM that possesses some hydrophilic moieties, although the truly hydrophilic DOM will not be retained. The latter is reported here as % total DOM, as measured by TOC of non-sorbed material (Table 21).

The % recovery of HPO-DOM from each SPE was found to be similar among all three waters tested, ranging from 83 to 91%. A slightly higher % recovery with NaOH elution was observed for both ST-DVB SPEs compared to the NVP, which might possibly retain minor amounts of HPO-DOM to the mixed mode interactions (hydrogen bonding and dipole interactions) between DOM and NVP. The high recovery (>85%) of the HPO fraction by all three SPEs shows that they are suitable for extracting and recovering a representative fraction of HPO DOM for further characterization or reactivity testing.

4.5.2 FTIR Spectra of Natural Water HPO DOM Isolated Using SPE Candidates

FTIR spectra of the solid isolates were obtained with microATR to assess the composition and the heterogeneity of the material. The monochrome images (Figure 66, inserts) indicate variation in the integrated intensity of the band at 1709 cm^{-1}. All IR spectra in the false color images were found to have identical spectrochemical profiles. The observed variation is due only to uneven contact between

the smoothly curved ATR surface and the rough surface of the dried sample on the microscope slide, where best contact appears as white, (Figure 66). FTIR spectra of the HPO fractions isolated by each SPE from all three surface waters show a strong peak at 1709 cm^{-1}, along with a very weak shoulder at ~1640 cm^{-1}. This demonstrates that all three surface waters have an HPO fraction that is primarily FA in character.

Although the spectra of the HPO isolates are similar, some differences were evident between the three SPEs in each waterbody. The HPO fraction isolated from the La Salle River using ENV had very little polysaccharide-like character compared to both PPL and Strata. The smaller pore size of PPL and Strata, as well as chemical interactions between SPE and DOM, may play a role in isolating an HPO fraction with more polysaccharide character from this river. For the Lake Winnipegosis extracts, the peak at 1045 cm^{-1} is strongest for the Strata isolate, again indicating that Strata X is isolating DOM with more polysaccharide-like character, due to the hydrogen bonding capability of the NVP. All three SPE isolates from the Waterhen River show a similar band at 1040 cm^{-1}. This peak is slightly less well-defined for the HPO fraction isolated using ENV, providing further evidence that PPL and Strata are isolating DOM with more polysaccharide, or HPI, character compared to ENV. Further characterization of these waters by elemental analysis or ^{13}C NMR might be useful in resolving the questions raised here.

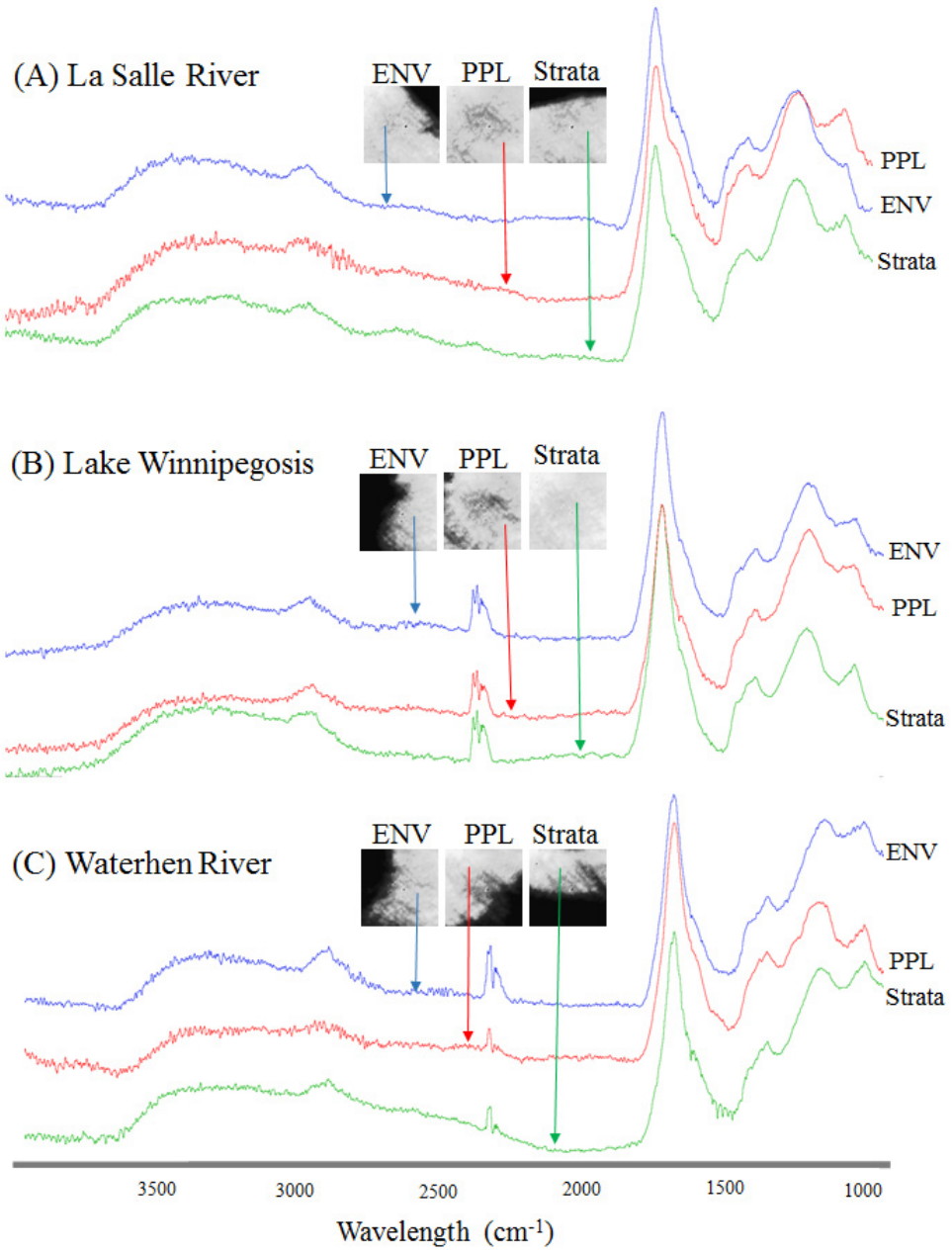


Figure 66: FTIR spectra of the HPO fraction of DOM isolated using Bond Elute ENV (Blue spectrum), Bond Elute PPL (Red spectrum) and Strata-X (Green spectrum) from three surface waters: A) the La Salle River, B) Lake Winnipegosis, and C) the Waterhen River. False color images for each SPE (grey scale inserts) shows the integrated absorbance intensity of the peak at 1709 cm^{-1}. All spectra are displayed on a common scale, offset for clarity. Figure taken from (Goss et al., 2017).

4.6 Onsite DOM Composition Monitoring by Operators at the Macdonald WTP Using SPE

The objective of this research was to determine an alternative method to the XAD resin that would provide a rapid and simple method to measure the HPO fraction of DOM, to allow WTP operators a suitable option to monitor THM precursors in source water. All three SPEs tested in this study were able to isolate the fraction of DOM similar to the XAD resin and the fraction isolated by each SPE had the greatest potential to form THMs. Furthermore, all three SPEs required less equipment and time compared to the XAD method, showing the suitability of these sorbents to be used as a simple method for WTP operators to conduct onsite. However, of the three SPEs tested in this study, the Bond Elute ENV was the most rapid compared to Strata X and Bond Elute PPL. Although the HPO fraction isolated with the Bond Elute ENV had lower THMFP compared to the HPO fraction isolated with Strata X or Bond Elute PPL, the ENV had similar, and in some cases, better STHMFP results implying this SPE may be isolating more THM precursors compared to the Strata X or Bond Elute PPL. As such, Bond Elute ENV was selected for onsite trials by WTP operators to determine if SPE was simple and rapid enough to be incorporated as a tool for WTP operators to measure and monitor changes in DOM in their source water.

The MWTP was selected as the facility to test Bond Elute ENV. The main reason for this choice was the close proximity of this WTP to the University of Manitoba. The results presented in Table 22 and Figure 67 show the total DOM concentration decreased during the summer (July – early August). From late August to early November the concentration of DOM remained constant ranging from 11.6 mg/L to 12.7 mg/L. However, when evaluating the DOM composition measured using the Bond Elute ENV, the results show there is fluctuations in the HPO/HPI character. From August 25 to October 8 there is a constant decrease in the HPO fraction from 5.6 mg/L to 4.6 mg/L. However, the sample collected on October 29 measured an increase in the HPO fraction of approximately 1.6 mg/L.

This compositional change of the DOM in the La Salle River would not have been observed by measuring the total DOM concentration, as there was not significant change in the overall DOM composition. This result supports the use of SPE, particularly the Bond Elute ENV, as a method for monitoring changes in the concentration of THM precursors in source waters, as well as during treatment.

Table 22: Fractionation results for the La Salle River obtained onsite by operators at the Macdonald Water Treatment Plant using Bond Elute ENV.

Date	DOC (mg/L)	HPI (mg/L)	HPO (mg/L)	HPI (%)	HPO (%)
July 23, 2015	16.2	11.3	4.9	69.6	30.4
August 12, 2015	15.5	9.2	6.3	59.5	40.5
August 25, 2015	12.7	7.1	5.6	55.7	44.3
September 24, 2015	12.3	6.9	5.3	56.5	43.5
October 8, 2015	11.9	7.3	4.6	61.1	38.9
October 29, 2015	11.9	5.5	6.4	46.3	53.7
November 6, 2015	11.6	7.6	4.0	65.5	34.5
November 24, 2015	13.4	8.3	5.1	62.1	37.9
January 21, 2016	12.8	8.2	4.6	63.8	36.2

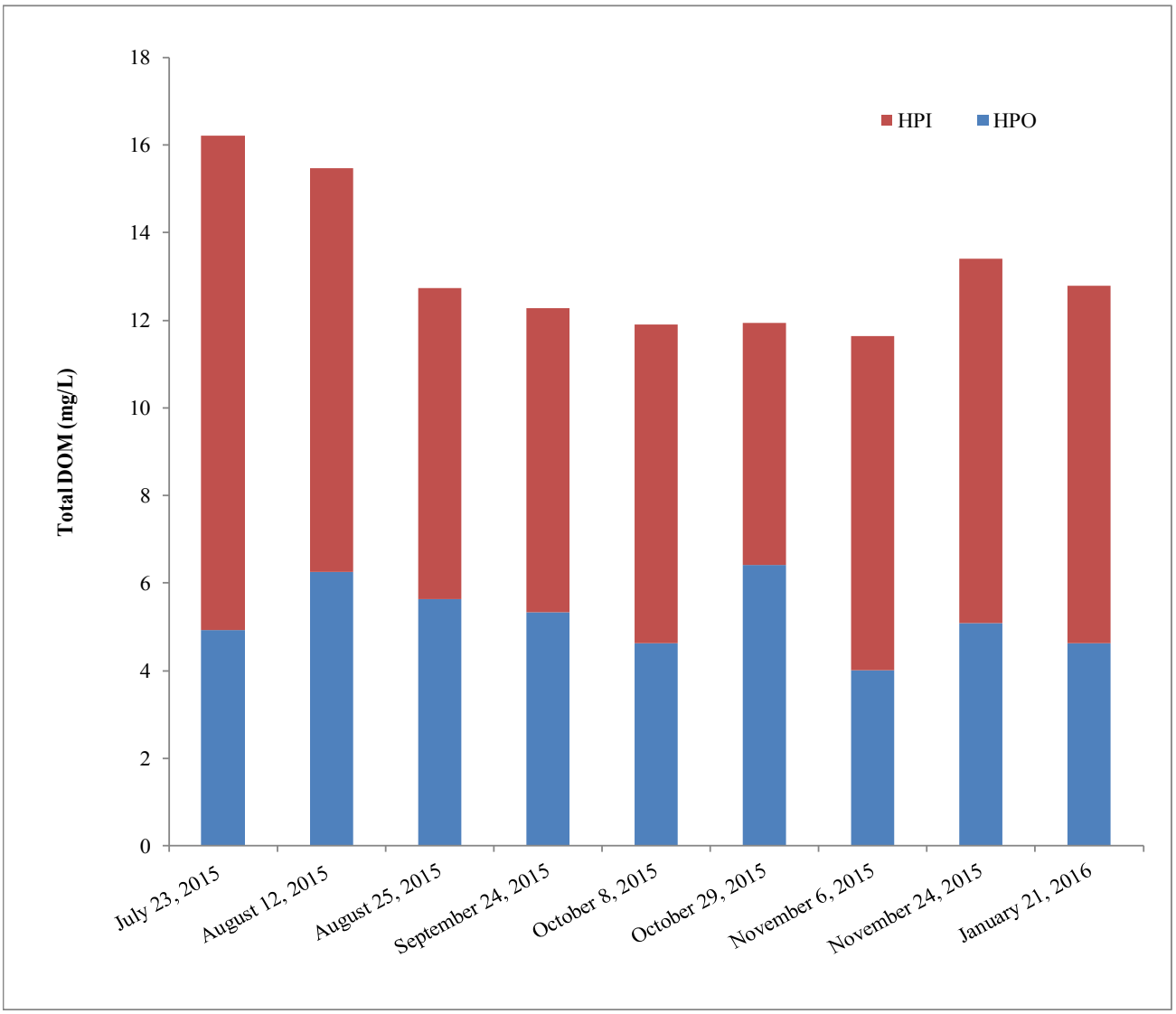


Figure 67: Fractionation results for the La Salle River obtained onsite by operators at the Macdonald Water Treatment Plant using Bond Elute ENV.

4.7 Factors Potentially Affecting SPE method for Monitoring of THMs Precursors

4.7.1 Effects of Seasonal Water Quality

When evaluating if there was any impact from seasonal changes that may have affected the SPE performance for isolating the HPO fraction from raw surface waters, there does not appear to be a clear indication that seasonal changes impacted the ability of the SPEs tested here. For example, samples collected from the Waterhen River in June and July 2014, i.e. summer water conditions, showed no significant difference between sorbents. However, samples collected from the Waterhen River in August 2015, i.e. also summer water conditions, there was a difference between sorbents ($p = 0.006$) (See Appendix A). Similar results were found for Lake Winnipegosis samples collected from Duck Bay, where the fractionation results collected in August 2014 showed no significant difference while samples collected in August 2015 showed the sorbents were significantly different in terms of isolating an HPO fraction. The HPO isolation results for seasonal raw water samples collected the La Salle River, again, did not show any correlation between the fractionation results as significant differences were measured between sorbents in all seasons.

4.7.2 Effect of Treatment Processes

Comparing the SPE fractionation results post water treatment process for the water treatment plants monitored in this study, there does not appear to be a water treatment process that impacted the performance of any of the SPEs tested. When evaluating the SPE fractionation results between ENV and Strata for monitoring the removal of the HPO fraction at the Waterhen WTP, ENV was not significantly different than Strata for samples collected after coagulation and filtration (August 12, 2014). However, there was a difference between ENV and Strata for the

treated water samples collected on August 12, 2014. On the other hand, samples collected from the Waterhen WTP in September 2014 and January 2015 found significant difference between ENV and Strata following coagulation ($p = 0.04$) and filtration ($p = 0.04$), while the treated water fractionation results we not found to be significantly different. This implies the treatment processes themselves are not impacting fractionation with either ENV or Strata. It should be noted that PPL only underwent treatment process monitoring in June 2015 at the Macdonald WTP. The results found a significant difference between the three SPEs for both the UF permeate and treated water.

4.8 Assessment of Possible Interference from Hardness to SPE Fractionation

Studies have shown that positively charged calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions, related to hardness, can cause conformational changes in the shape of humic matter (von Wandruszka, 1998). The interaction between and humic molecule and the positively charged cation could cause the molecule to coil forming a pseudomicellar structure (von Wandruszka, 1998). The coiling of the humic molecule could reduce the overall size of the humic molecule possibly affecting the interaction with hydrophobic sorbents. Although hardness was not measured on samples collected during the SPE field work, water treatment data was collected from Waterhen River and the La Salle River to provide some insight into the possible interference to SPE fractionation from positively charged cations associated with hardness. These two systems are characteristic of high hardness (180 mg/L $CaCO_3$) and extremely high hardness (>350 mg/L $CaCO_3$) waters.

Available water treatment plant data from the Waterhen WTP indicated that the hardness in the Waterhen River is approximately 200 mg/L $CaCO_3$. The Waterhen WTP does not continually monitor hardness as the concentration remains relatively stable according to the plant operator. The seasonal SPE fractionation results for the Waterhen River collected on June 17 and August 12, 2014 showed no

statistical difference between each sorbent, while the September 29, 2014, January 22, 2015 and August 19, 2015 results found ENV was isolating lower HPO DOM compared to Strata. If hardness were to affect SPE fractionation the results should have been consistent between sample sets collected from the Waterhen River if it's assumed the hardness is relatively stable; however, this was not observed.

One the other hand, the La Salle River has very high hardness ranging from 300 mg/L $CaCO_3$ to >700 mg/L $CaCO_3$ based on available plant data from 2015 (weekly records). If hardness were to impact SPE fractionation results due to a conformational change or charge interaction, the interference would have been amplified in the La Salle River. SPE fractionation results for the La Salle River collected on June 30, 2015 found that ENV was isolating less HPO DOM compared to Strata. Water quality test results from the plant taken on June 29, 2015 show the hardness to be 322 mg/L $CaCO_3$. However, SPE fractionation of the La Salle River on July 23, 2015 found no statistical difference between ENV or Strata, even though the hardness was measured by the Macdonald WTP operators the day prior to be 343 mg/L $CaCO_3$, which was similar to the hardness measured in June 2015. This implies that hardness is not affecting fractionation results. The likely reason is that SPE fractionation occurs at low pH (pH=2). At this pH metal cations like Ca^{+2} and Mg^{2+} would remain ionized while the carboxyl groups of humic acid would become protonated and would therefore not interact with charged cations.

There is limited data here to provide a definitive answer to effects of hardness on SPE fractionation, however, there does not appear to be an interference to SPE fractionation resulting from hardness, although the results presented here are limited. Further testing under controlled settings would allow for more in depth analysis to the impact's hardness may have to SPE fractionation results.

4.9 Proposed Applications of SPE in Water Treatment

Although there are differences among the THMFP and specific THMFP for each water, the results show that the SPEs are useful in measuring THM precursors in natural waters. Furthermore, the simplicity of the SPE method compared to the classic resin fractionation method using XAD (e.g., Dittmar et al., 2008) indicates the suitability of SPE as a tool for monitoring THM precursors in surface waters. SPE could also be used to monitor the removal of the HPO fraction during water treatment, allowing for treatment process optimization. This reduction of THMs in finished drinking water would enable better compliance with provincial regulations.

Although the present study was focused on ST-DVB or NVP SPEs for isolation of THM precursors in DOM, these SPEs provide further benefits. For example, Roccaro et al. (2014) reported that the HPO fraction was the major contributor to the formation of HAAs, the second most commonly regulated DBP. Furthermore, as the ST-DVB and NVP SPEs separate the HPO (sorbed) and HPI (non-sorbed) fractions, these SPEs could also be used to monitor the formation of DBP by the HPI fraction, such as haloacetonitriles and dichloroacetamide (Roccaro et al., 2014; Chu et al., 2010). This shows that these SPEs could easily be used to measure precursors of several important DBPs formed in the HPO and HPI DOM fractions

Chapter 5: Future Work and Engineering Significance

5.1 Future Work

The focus if this research was to investigate SPE with the intent that the method could be used by WTP operators to monitor the HPO fraction of DOM in surface waters to gauge changes in the potential to form THMs (i.e., changes in the concentration of THM precursors). This study also evaluated the use of SPE to follow changes in the DOM character following treatment with the idea

that SPE could be applied to identify treatment processes which were not effectively removing DOM or DOM fractions. The results of this study concluded that SPE was applicable for both seasonal monitoring and for identifying treatment processes which were not reducing DOM or the HPO fraction. However, there were some limitations to this study that require further investigation.

Although this study successfully measured the concentration of DOM fractions in surface waters with different water qualities (i.e., pH, alkalinity, hardness, and total dissolved solids) the impact of specific water quality characteristics on the fractionation results was not investigated. Water pH and alkalinity are not expected to impact the results since the sample is reduced to pH of 2 prior to conducting SPE fractionation. The reduction in pH should negate the effect of raw water pH and alkalinity, aside from altering the amount of acid required to reduce the pH. Total dissolved solids are composed of inorganic salts such as magnesium, calcium, carbonate and bicarbonates. The literature review portion of this work presented evidence that ions can impact the physical shape of organic matter through the static interaction of negatively charged DOM functional groups and the positively charged metal ion (e.g., Mg^{2+}). This could impact fractionation methods based on size (i.e., size exclusion chromatography); however, at low pH the charged functional groups on DOM would be protonated and therefore would not interact with metal ions. That being said, there are pore size differences between the SPEs used in this study giving rise to the potential for the size of DOM molecules to impact results. Dittmar et al. (2008) showed the applicability to isolate DOM from seawater using Bond Elute PPL and ENV. The Dittmar et al. method applied an extra step to the SPE fractionation procedure by washing the cartridge, following fractionation but prior to elution, with 0.01N HCl to remove salt, likely a result of the high salt and total dissolved solid content of seawater. Dittmar et al. removed salt content to avoid impact on subsequent molecular analysis of the isolates. This step was not applied in this current study as the salt content of surface waters was assumed to be

low in comparison to seawater, and the presence of salts did not impact qualitative and quantitative measurements (TOC analysis and FTIR imaging). However, given there are limited studies investigating the impact of various water quality characteristics on SPE fractionation of surface water DOM, further research in this area is warranted.

This study demonstrated SPE can be useful in following the changes in DOM character following drinking water treatment processes. However, the study did not attempt to optimize the removal of DOM, and more specifically the HPO fraction, by water treatment processes, such as coagulation. Future work could apply SPE fractionation into the optimization of various water treatment processes aimed to remove HPO DOM, particularly coagulation and ion exchange. Enhanced coagulation aims to reduce overall DOM concentration to control THM formation; however, if SPE was used along with enhanced coagulation the operational parameters for coagulation could be adjusted to improve the removal of HPO DOM, and not bulk DOM. This could equate to better overall control of THMs in finished drinking water.

This study was limited to investigating the most commonly found, and regulated, DBP in public water systems, THMs. However, as presented in the literature section of this thesis, THMs may not be as toxic as once proposed and that other DBPs may in fact have a greater impact to human health than THMs. Therefore, the SPE fractionation work presented in this study could be extended to include other DBPs which may be emerging or are suspected or known to be more toxic than THMs. Better understanding of what fraction of DOM contains precursors to emerging DBPs could be applied to future optimization of water systems to better control the presence of emerging DBPs which are likely to be regulated in the future.

The HPO fraction of DOM, and the impact this fraction can have on THM formation, was a major focus of this research. The SPE fractionation presented in this study not only isolated the HPO fraction but also isolated the HPI fraction of DOM. The HPI fraction has been shown to impact water treatment processes, particularly with regards to membrane fouling (Kweon & Lawler, 2005). The SPE method here could be applied to show the impact of the HPI fraction on membrane fouling, as well as in the design of pretreatment options to reduce this fraction and prevent the fouling of membranes.

Lastly, FTIR imaging was used to characterize the HPO fraction isolated from the SPEs tested. The results presented some evidence to suggest the differences in HPO fraction isolated by PPL, ENV and Strata X was a result of sorption of DOM with some HPI character. Additional qualitative measurements, such as ^{13}C-NMR, parallel factor analysis of fluorescence excitation/emission spectroscopy or elemental analysis, could provide more evidence of the differences in the fractions isolated by each SPE.

5.2 Engineering Significance

Since the discovery of chlorinated DBPs, mainly THMs, in public drinking water supplies by Rook and Bellar et al. in the 1970s, there has been increased regulatory pressure to limit the presence of these compounds in water. The formation of DBPs, such as THMs, is largely a result of a reaction between DOM and free chlorine during the disinfection process. There is strong evidence in the literature that indicates the fraction of DOM containing hydrophobic organic matter, mainly humic and fulvic acids, are the main precursors to THMs. Logically, drinking water treatment plants aim to reduce the presence of THM precursors in drinking water. However, there is no currently applicable tool, or test, for operators to accurately (i.e., quantitatively), and rapidly, measure the composition of DOM in potable water sources to gauge the propensity for the source water to form THMs. As a result, WTPs monitor the removal of total or dissolved organic matter as a blanket approach to measure the removal of THM precursors prior to disinfection. This approach can be problematic considering there is lower THM formation potential with the HPI fraction of DOM. The development of a rapid SPE method for measuring the HPO/HPI composition of DOM, such as the methods presented in this work, provides a tool for WTP operators to not only measure total DOM, but allows for a quantitative method for measuring the main precursors to THMs. The rapid SPE methods presented in this study can be used to monitor changes in the HPO fraction during treatment, which can be used to identify treatment processes that require optimization to improve not only the removal of DOM, but the removal of the HPO fraction. Furthermore, since the SPE method is considerably more rapid, and simple, than other DOM fractionation methods (i.e., XAD Method), the SPE method can be used to continually monitor seasonal changes in the DOM composition of the source water. This provides greater insight into the seasonal changes in DOM composition, allowing WTP operators to adjust plant operations (e.g., coagulant dose) to address the changes in DOM composition.

The rapid SPE method presented in this study is also valuable from an engineering and water treatment plant design perspective. Water treatment systems that are designed for treatment of surface waters high in DOM, such as many of the systems found in Manitoba, largely focus on the removal of DOM through such treatment process as chemical coagulation and various filtration methods. However, these processes are generally selected, and subsequently optimized, for the removal of total DOM, and are not optimized to remove HPO fraction of DOM. If the DOM composition of the surface water source was characterized prior to the design phase, process design engineers may be able to use the DOM composition data to select more appropriate treatment processes that will target the fraction of DOM that is generating THMs. Furthermore, during the WTP optimization and commissioning phase, the SPE method could be used to further target the removal of the HPO fraction, thereby limiting the formation of THMs, and other DBPs that can form following disinfection with chlorine. The selection of appropriate treatment processes and advanced, or targeted, optimization of these process can equate to economical, and regulatory, benefits for the WTP, by eliminating redundant treatment processes from the design, by improving treatment processes selected to remove DOM, and by further limiting the presence of THMs in the treated water.

Much of this study focused on measuring the HPO fraction to monitor the changes in THM precursors; however, the SPE methods investigated in this study also quantitatively measure the HPI fraction, which can also be beneficial to engineers designing water treatment plant. There has been a large transition recently to using membrane technology to treat surface water for potable use. Research has found that the to some membranes the HPI fraction, containing proteins and polysaccharides, causes more severe irreversible membrane fouling compared to the HPO fraction (Yamamura et al., 2014; Kweon & Lawler, 2005). Therefore, if the DOM composition of the source water is known prior to selecting a particular membrane, a membrane that will be resistant to organic fouling by a particular

fraction of DOM can be implemented. Furthermore, if a membrane is known to be more prone to fouling from a particular DOM fraction, the SPE method can be used to optimize membrane pretreatment processes for targeted removal of the major DOM fouling fraction prior to membrane filtration. This can have a positive economical impact by preventing the replacement of membranes that have suffered irreversible organic fouling.

Utilization of the SPE method developed in this study can be applied by both WTP operators and civil engineers to design water treatment plants that produce better quality water, as well as provide cost savings in both the design and operation of WTPs using surface waters for potable water sources.

Chapter 6: Conclusion

Phase I of this study successfully evaluated seven prepackaged SPE cartridges for their ability to rapidly isolate the HPO fraction of DOM. Initially, the HPO fraction isolated by each of the seven SPE candidates were tested both quantitatively (i.e., DOC analysis) and qualitatively (i.e., FTIR-FPA Spectrochemical Imaging) to the currently accepted DOM fractionation method, the XAD method. Given the XAD method is long and tedious, the SPE candidates were also evaluated for their simplicity and usability within a water treatment plant, as a simple method for measuring and monitoring the DOM composition in the source water. The initial phase of testing identified two SPEs, Bond Elute ENV and Bond Elute PPL that were comparable, both quantitatively and qualitatively, to the XAD method which were selected to undergo field testing in natural water. Strata X was found to isolate an HPO fraction that was comparable qualitatively (i.e., FTIR imaging); however, this sorbent was found to isolate a DOM fraction that quantitively exceeded that of the XAD resin. Although the Strata X was not found to be comparable quantitatively to the XAD resin, Strata X isolated the most DOM of all SPEs tested suggesting there was warrant in field testing Strata X.

Phase II of this study focused on the monitoring the DOM composition in natural surface waters. Phase II consisted of a13-month study which successfully tested the three SPEs in three surface waters located in two distinct regions of Manitoba (i.e., the Canadian Shield and the Prairies). The results found that all three SPEs tested were able characterize, and monitor, the seasonal changes in DOM composition. The results generally found that the Strata X isolated more DOM compared to the Bond Elute ENV or Bond Elute PPL. The reason was attributed to the Strata X sorbent, n-vinylpyrrolidone, attracting DOM with some HPI character.

Along with seasonal monitoring of surface waters using the SPE candidates, each SPE was tested to determine if it could also monitor the removal of DOM fractions following potable water treatment. Five water treatment plants were monitored for the removal of DOM fractions using each SPE. The results found that all three SPEs were successful in monitoring changes in the DOM composition following treatment processes. Generally, it was observed that Strata, and to some extent PPL, isolated more HPO DOM compared to ENV.

Trihalomethane formation potential results indicated that the fraction of DOM isolated by each SPE candidate was the fraction most prone to form THMs, signifying the suitability of the SPE candidates to monitor organic precursors to THMs in surface waters. Of the three candidate SPEs tested, the HPO fraction isolated using Bond Elute ENV was found to have, in some cases, a statistically lower THMFP compared to both the Bond Elute PPL and Strata X; however, when the THMFP of each fraction is normalized to the DOC concentration of the DOM fraction (i.e., Specific THMFP), Bond Elute ENV was found to be similar, or even exceeding, that of Strata X and Bond Elute PPL. This suggests that although there is less HPO DOM isolated with Bond Elute ENV, and that the HPO fraction has lower THMFP, Bond Elute ENV is likely isolating an HPO-DOM that is more prone to THM formation. This, along with the shorter run-times for Bond Elute ENV, compared to Strata X and Bond Elute PPL, the results from Phase II identified Bond Elute ENV as the most appropriate SPE method to be tested by WTP operators.

The objective of Phase III of this study was to have operators in an operational water treatment plant test Bond Elute ENV themselves to gauge if the method was simple and rapid enough to be incorporated into the standard set of water quality tests currently measured by the operators. For an approximate 6-month period from July 2015 to January 2016, operators at the Macdonald Water

Treatment Plant measured the composition of the La Salle River, onsite, using Bond Elute ENV. The results found that Bond Elute ENV was valuable in identifying compositional changes in DOM, which would have gone unaccounted for if only total DOM was measured.

Overall, the results presented in this study show that SPE, particularly Bond Elute ENV, can be a valuable tool for water treatment plant operators, and engineers, to easily measure and monitor changes in DOM composition in potable water sources to better gauge, and in turn control, the formation of regulated disinfection by-products such as THMs.

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Appendix A – Raw Data

1. International Humic Substance Society Suwannee River Fractionation

Table 1-A: Raw fractionation of Suwannee River DOM standard.

Sorbent	Raw DOC (mg/L)	Hydrophilic (mg/L)	Hydrophilic (%)	Hydrophobic (mg/L)	Hydrophobic (%)
XAD-8 (DAX-8) - T1	10.1	2.2	21.6	7.9	78.4
XAD-8 (DAX-8) - T2	8.6	2.2	26.1	6.3	73.9
XAD-8 (DAX-8) - T3	11.2	2.7	23.7	8.6	76.3
Bond Elute ENV - T1	10.8	2.7	25.0	8.1	75.0
Bond Elute ENV - T2	10.9	2.7	24.7	8.2	75.3
Bond Elute ENV - T3	10.8	2.6	24.4	8.2	75.6
Bond Elute ENV - T4	9.9	2.5	24.7	7.5	75.3
Bond Elute ENV - T5	10.4	2.5	24.1	7.9	75.9
Bond Elute ENV - T6	10.3	2.5	24.0	7.8	76.0
Bond Elute Plexa - T1	10.6	3.7	34.6	6.9	65.4
Bond Elute Plexa - T2	10.4	3.7	35.4	6.7	64.6
Bond Elute Plexa - T3	10.4	3.9	37.8	6.5	62.2
Bond Elute Plexa - T4	6.3	2.3	36.6	4.0	63.4
Bond Elute Plexa - T5	6.3	2.5	38.7	3.9	61.3
Bond Elute Plexa - T6	6.3	2.6	40.6	3.8	59.4
Strata X - T1	11.1	2.1	9.1	81.3	81.3
Strata X - T2	11.1	2.1	9.0	81.2	81.2
Strata X - T3	11.1	2.1	9.0	81.1	81.1
Strata X - T4	8.0	1.4	6.7	82.9	82.9
Strata X - T5	8.3	1.4	6.9	83.2	83.2
Strata X - T6	8.5	1.4	7.1	83.6	83.6
C18-U - T1	9.4	5.2	55.4	4.2	44.6
C18-U - T2	10.9	6.4	59.0	4.5	41.0
C18-U - T3	11.3	6.3	55.9	5.0	44.1
C18-U - T4	8.6	5.0	58.5	3.6	41.5
C18-U - T5	8.5	5.5	64.9	3.0	35.1
C18-U - T6*	5.4	5.3	99.0	0.1	1.0
C18E - T1	9.2	7.8	84.2	1.5	15.8
C18E - T2	9.6	8.1	83.7	1.6	16.3
C18E - T3	9.7	8.3	86.3	1.3	13.7
C18E - T4*	9.2	9.8	106.6	-0.6	-6.6
C18E - T5*	9.3	9.1	97.8	0.2	2.2
C18E - T6*	9.3	9.1	97.8	0.2	2.2
Oasis HLB - T1	10.0	5.1	51.0	4.9	49.0
Oasis HLB - T2	9.7	5.2	53.4	4.5	46.6
Oasis HLB - T3	9.3	5.2	55.6	4.1	44.4
Oasis HLB - T4	7.0	2.9	42.3	4.0	57.7
Oasis HLB - T5	7.4	2.9	39.9	4.4	60.1
Oasis HLB - T6	7.1	3.2	45.2	3.9	54.8
Bond Elute PPL - T1	10.5	3.0	28.3	7.5	71.7
Bond Elute PPL - T2	10.7	2.6	24.5	8.0	75.5
Bond Elute PPL - T3	10.4	2.7	26.5	7.6	73.5
Bond Elute PPL - T4	10.1	2.8	27.5	7.4	72.5
Bond Elute PPL - T5	11.5	3.4	29.2	8.1	70.8
Bond Elute PPL - T6	11.0	2.8	25.4	8.2	74.6

* Trial not used in combined data analysis due to error in sample.

The raw water sample was found to have an error for C18U – T6 trial as the raw water sample used in T6 was also used for T4 and T5 (i.e. 3L of sample was prepared from stock solution); therefore, the raw water DOC should have been approximately 8.5 mg/L. It was unclear as to what caused the error in the DOC measurement for this trial

The C18-E T4 to T6 trials were excluded as there appeared to be contamination of the hydrophilic fraction possibly due to bleeding from the column or incomplete DI rinsing prior to running the DOM sample. Given the overall poor performance of the C18E column trials T1 to T3, the trials were not repeated and only T1 to T3 trials were used in the combined data set analysis.

Table 2-A: Percent recovery for SPE candidates using Suwanee River standard.

Sorbent	Raw DOC (mg/L)	Hydrophilic (mg/L)	Hydrophilic (%)	Hydrophobic (mg/L)	Hydrophobic (%)	Hydrophobic Recovered (mg/L)	Hydrophobic Recovered (%)
Strata X T1	12.7	2.7	21.6	10.0	78.4	8.3	83.0
Strata X T2	12.5	2.7	21.4	9.8	78.6	9.0	92.0
Strata X T3	12.2	2.5	20.4	9.7	79.6	9.0	92.6
ENV T1	11.5	3.6	31.5	7.9	68.5	6.2	79.0
ENV T2	12.9	4.6	35.6	8.3	64.4	7.2	86.7
ENV T3	12.0	3.8	31.9	8.2	68.2	7.4	90.4
Plexa T1	12.3	6.7	54.3	5.6	45.7	5.0	87.8
Plexa T2	12.4	7.0	56.5	5.4	43.5	5.6	103.6
Plexa T3	13.0	6.5	50.3	6.5	49.7	6.3	97.3
C18-E T1	5.3	3.5	66.5	1.8	33.6	3.0	169.1
C18-E T2	5.0	3.5	70.1	1.5	29.9	3.1	203.3
C18-E T3	5.2	3.6	68.5	1.7	31.5	3.4	204.2
C18-U T1	9.4	6.7	71.8	2.6	28.2	3.6	137.1
C18-U T2	9.4	7.3	78.2	2.0	21.8	3.1	151.9
C18-U T3	9.5	6.4	68.0	3.0	32.0	3.6	118.6
Oasis-T1	5.7	3.1	54.8	2.6	45.2	3.9	149.5
Oasis-T2	5.9	3.4	57.0	2.5	43.0	3.3	131.3
Oasis-T3	5.7	2.8	48.9	2.9	51.1	3.9	133.4
PPL - T1	10.5	3.0	28.3	7.5	71.7	7.3	96.3
PPL - T2	10.7	2.6	24.5	8.0	75.5	7.2	89.1
PPL - T3	10.4	2.7	26.5	7.6	73.5	6.9	91.2
PPL - T4	10.1	2.8	27.5	7.4	72.5	6.8	92.9
PPL - T5	11.5	3.4	29.2	8.1	70.8	7.3	89.7
PPL - T6	11.0	2.8	25.4	8.2	74.6	7.5	90.7
XAD-8 (DAX-8)	10.1	2.2	21.6	7.9	78.4	6.7	83.9
XAD-8 (DAX-8)	8.6	2.2	26.1	6.3	73.9	5.6	88.2
XAD-8 (DAX-8)	11.2	2.7	23.7	8.6	76.3	8.0	92.8

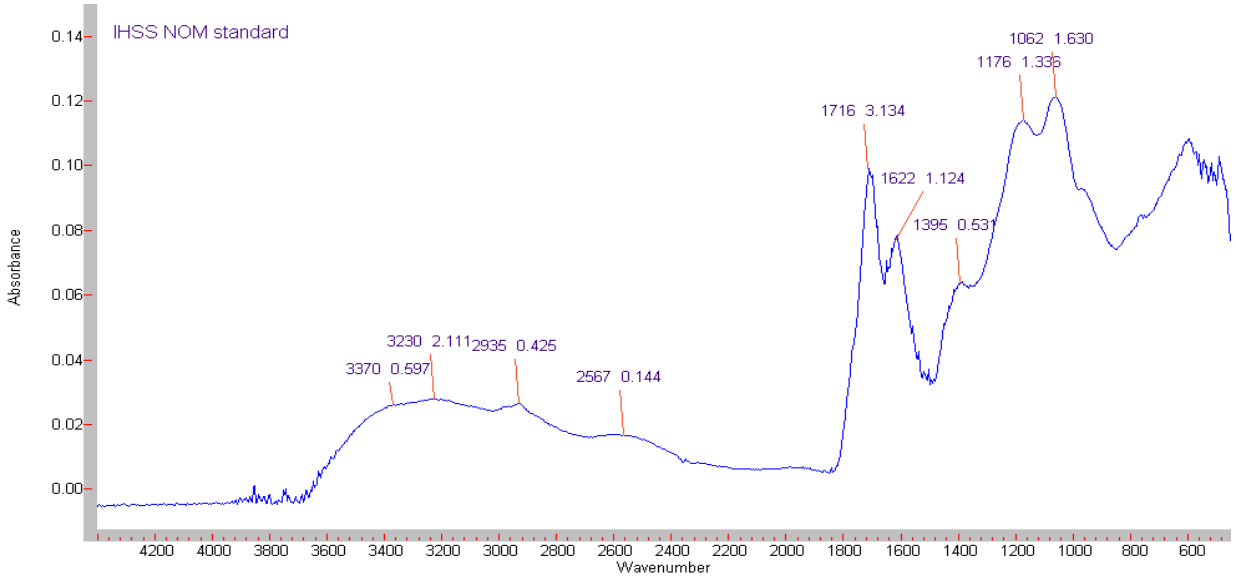

Figure 1-A: Single element FTIR spectrum of Suwannee River Natural Organic Matter standard 1R101N. Spectra was collected using Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

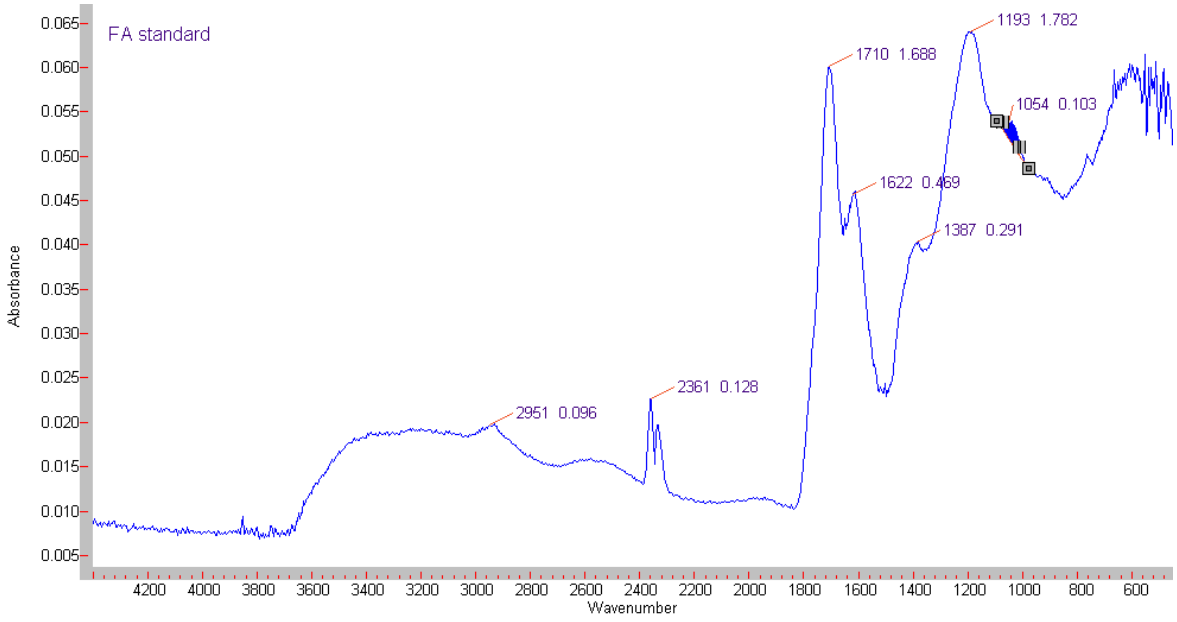

Figure 2-A: Single element FTIR spectrum of Suwannee River Fulvic Acid standard 1S101F. Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

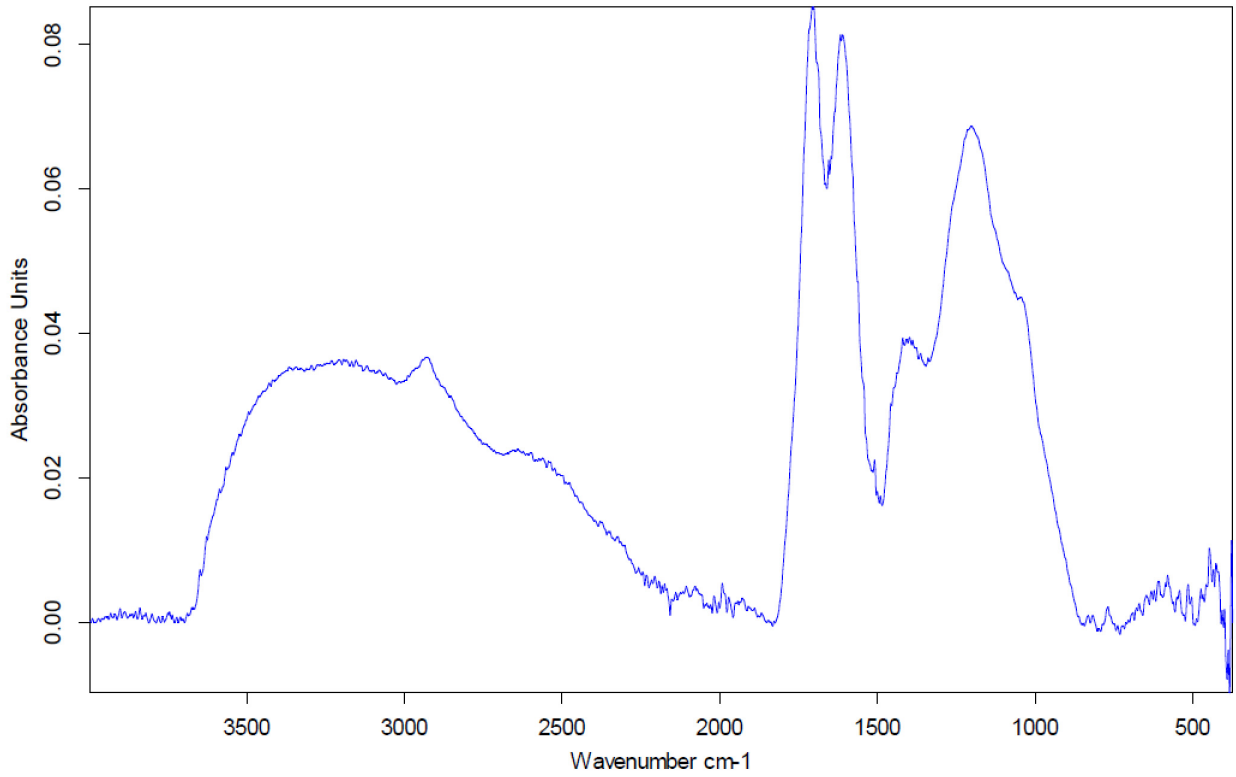

Figure 3-A: Single element FTIR spectrum of Suwannee River Humic Acid standard 1S101H. Bruker Alpha FTIR spectrometer, equipped with ALPHA's Platinum ATR, single reflection diamond ATR module, in the 4000–500 cm^{-1} region.

The single element humic acid standard spectra was not collected using the Agilent Cary 670 FTIR spectrometer as the single element spectra for the standard was already collected using a similar FTIR spectrometer equipped with an ATR. Given FTIR FPA spectrochemical imaging results for humic acid (Figure 5A) standard yielded similar spectra, it was determined there was no need to repeat the test using the Agilent FTIR spectrometer.

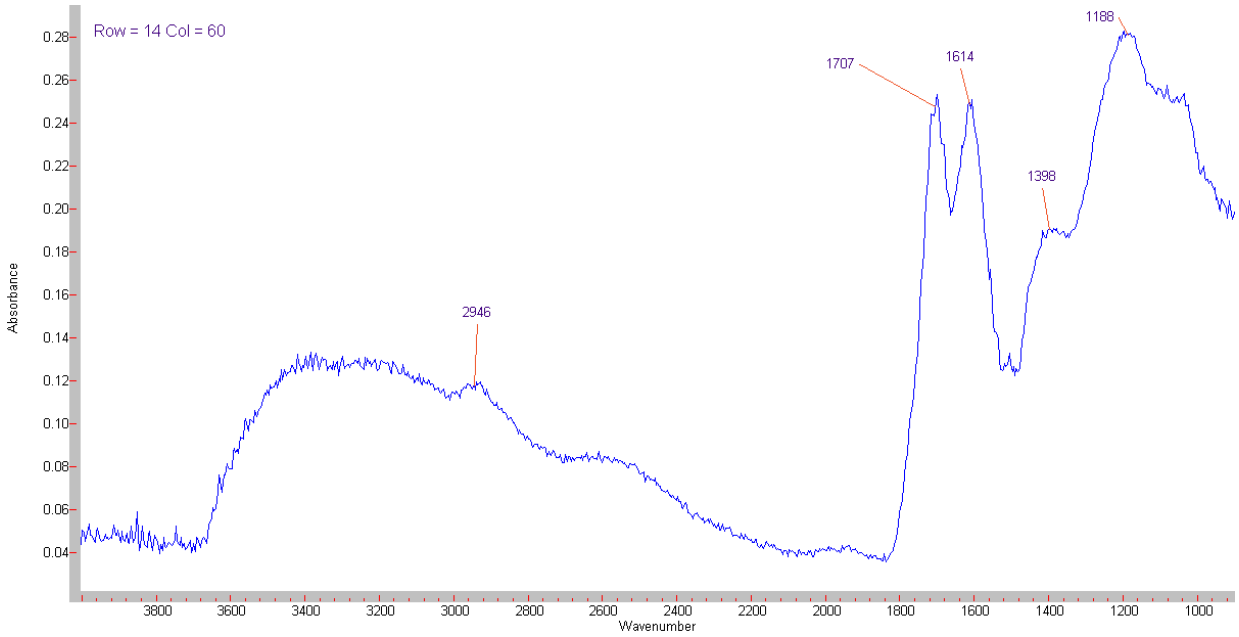

Figure 4-A: FTIR spectrum of Suwannee River Humic Acid standard 1S101H using the Agilent Cary 670 FTIR spectrometer.

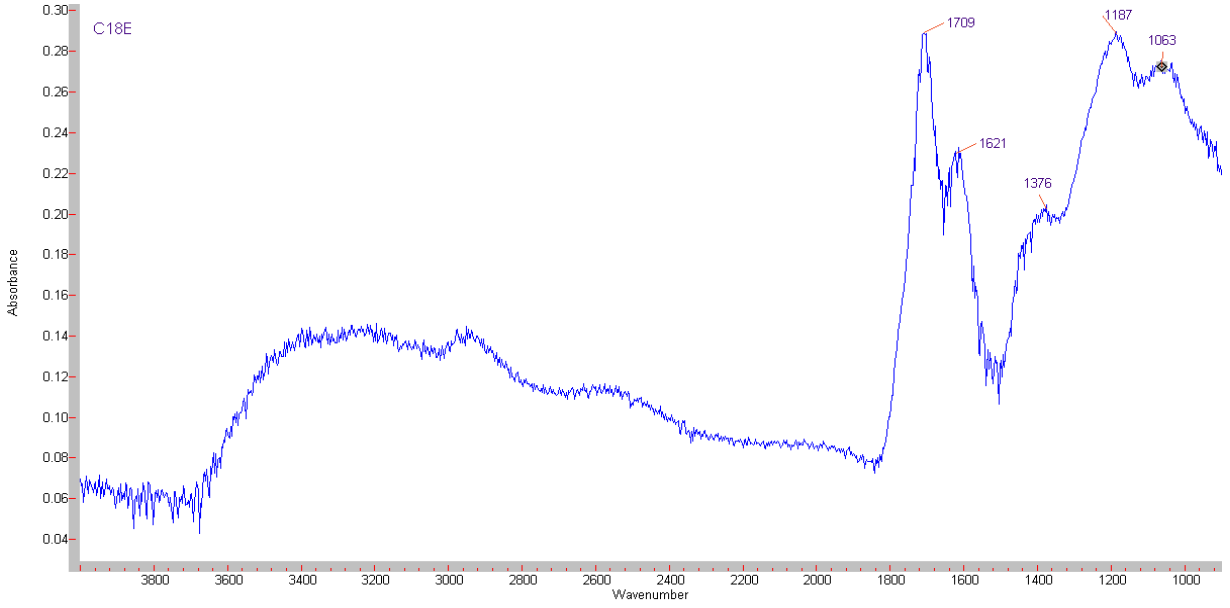

Figure 5-A: C18E extract from Suwannee River NOM standard. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

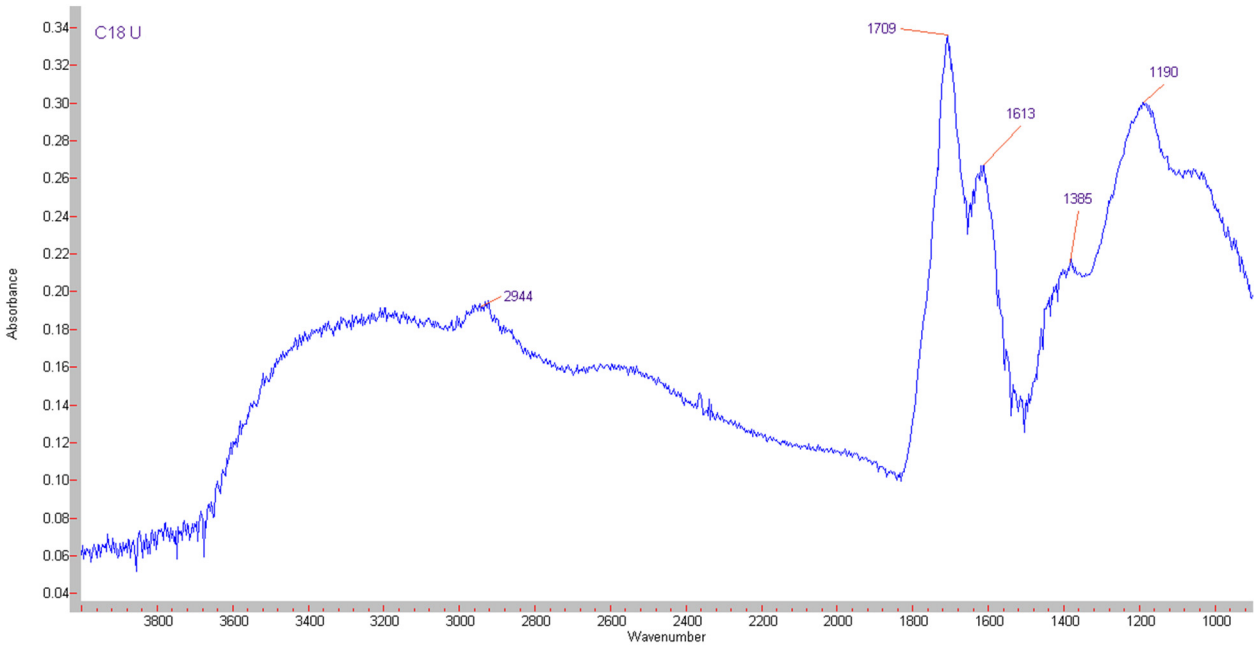

Figure 6-A: C18U extract from Suwannee River NOM standard. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

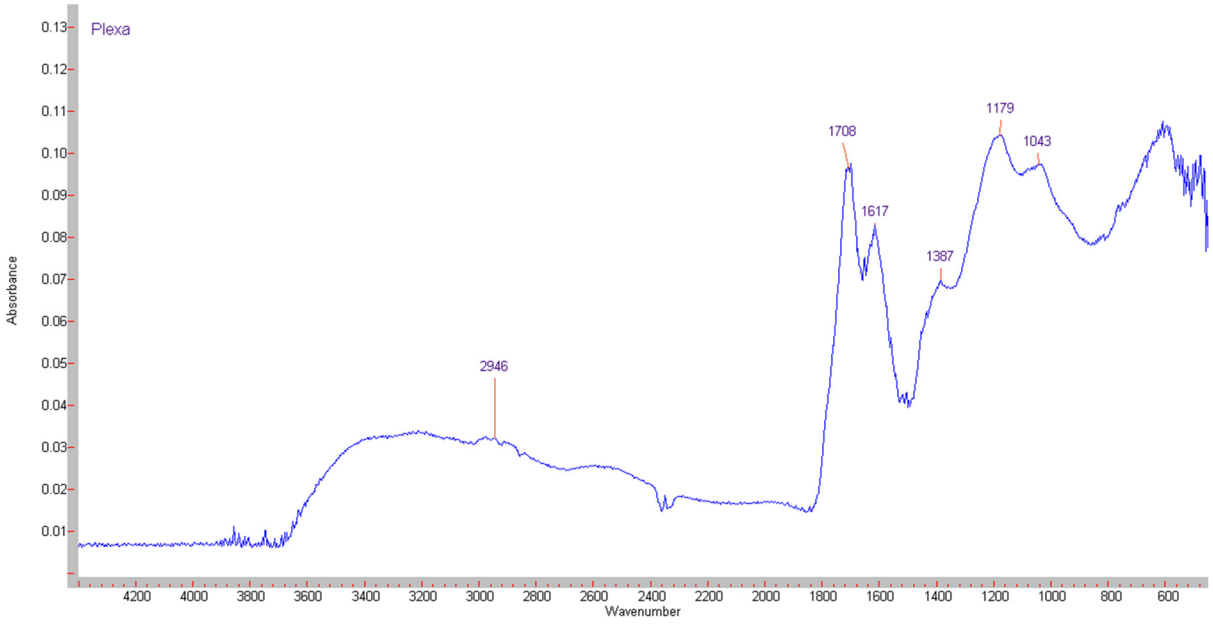

Figure 7-A: Bond Elute Plexa extract from Suwannee River NOM standard. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

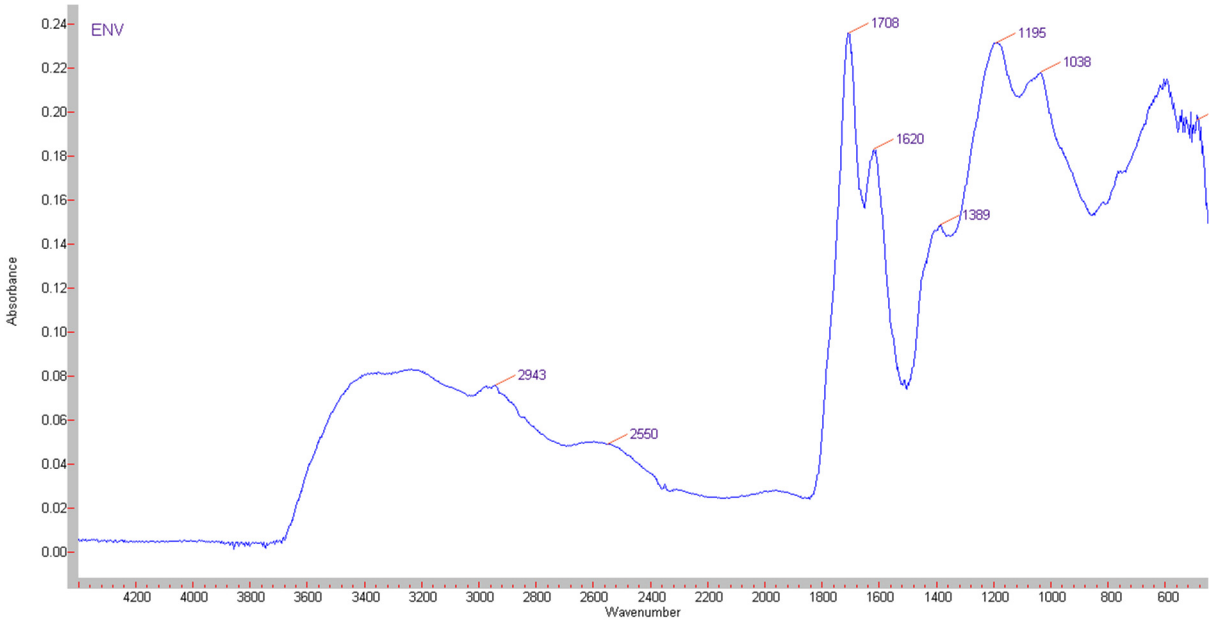

Figure 8-A: Bond Elute ENV extract from Suwannee River NOM standard. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

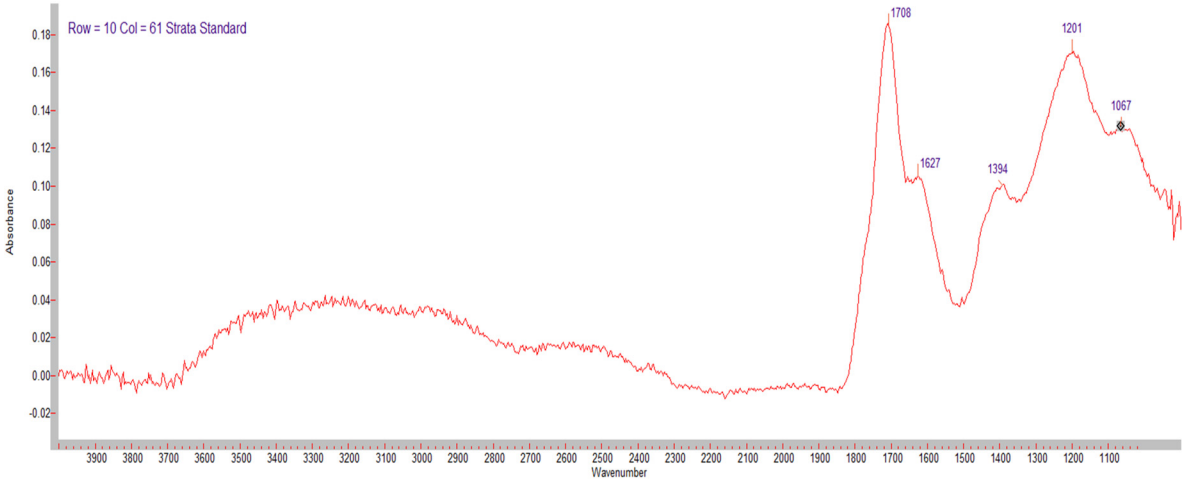

Figure 9-A: Strata X extract from Suwannee River NOM standard. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

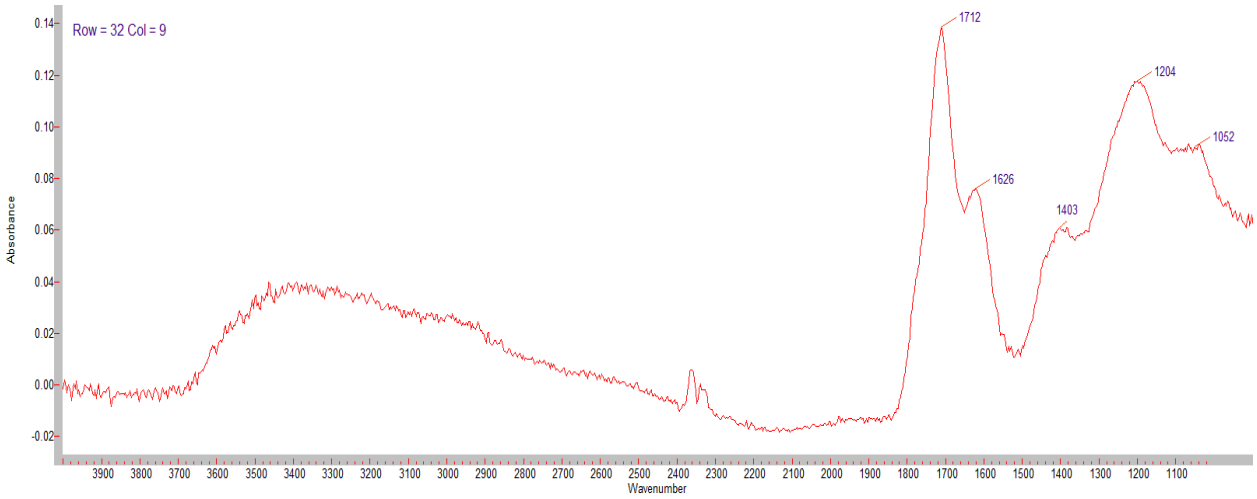

Figure 10-A: Bond Elute PPL extract from Suwannee River NOM standard. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

Figure 11-A: Oasis HLB extract from Suwannee River NOM standard. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

2. Waterhen Data

Table 3-A: Seasonal SPE fractionation data collected from the Waterhen River.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	One-way Variance (ANOVA)	Tukey HSD Test Results
June 17, 2014	Raw	ENV	8.2	5.0	3.2	39.3	39.9	1.3	0.23521	
June 17, 2014	Raw	ENV	8.2	5.0	3.2	39.0				
June 17, 2014	Raw	ENV	8.2	4.8	3.4	41.4				
June 17, 2014	Raw	Strata	8.2	4.8	3.3	40.7	43.4	2.4		
June 17, 2014	Raw	Strata	8.3	4.6	3.6	44.0				
June 17, 2014	Raw	Strata	8.4	4.6	3.8	45.4				
June 17, 2014	Raw	XAD	8.1	5.2	2.9	35.4	39.2	4.0		
June 17, 2014	Raw	XAD	8.2	4.6	3.5	43.4				
June 17, 2014	Raw	XAD	8.0	4.9	3.1	38.9				
August 12, 2014	Raw	Strata	11.4	7.6	3.9	33.9	34.5	1.0	0.629738	
August 12, 2014	Raw	Strata	11.4	7.4	4.1	35.6				
August 13, 2014	Raw	Strata	11.5	7.6	3.9	33.9				
August 12, 2014	Raw	ENV	11.3	7.4	3.8	33.9	35.2	1.7		
August 12, 2014	Raw	ENV	11.2	7.1	4.2	37.1				
August 12, 2014	Raw	ENV	10.6	6.9	3.7	34.6				
August 12, 2014	Raw	XAD	11.2	8.6	2.7	23.7	30.6	10.3		
August 12, 2014	Raw	XAD	11.5	6.6	4.9	42.5				
August 12, 2014	Raw	XAD	11.2	8.3	2.9	25.7				
September 29, 2014	Raw	ENV	10.3	6.1	4.2	40.6	40.8	0.3	0.001625	HSD[.05]=3.67 HSD[.01]=5.88 ENV vs Strata P<.01 ENV vs XAD nonsignificant Strata vs XAD P<.01
September 29, 2014	Raw	ENV	9.9	5.8	4.1	41.0				
September 29, 2014	Raw	Strata	9.6	5.1	4.5	47.0	48.0	1.4		
September 29, 2014	Raw	Strata	9.6	4.9	4.7	49.1				
September 29, 2014	Raw	XAD	10.4	6.4	4.0	38.3	38.4	1.1		
September 29, 2014	Raw	XAD	10.1	6.1	4.0	39.6				
September 29, 2014	Raw	XAD	9.9	6.2	3.7	37.3				
January 22, 2015	Raw	ENV	13.9	8.4	5.5	39.6	37.9	1.9	0.002945	HSD[.05]=6.45 HSD[.01]=9.4 ENV vs Strata P<.01 ENV vs XAD P<.05 Strata vs XAD nonsignificant
January 22, 2015	Raw	ENV	13.6	8.4	5.2	38.4				
January 22, 2015	Raw	ENV	13.1	8.4	4.7	35.8				
January 22, 2015	Raw	Strata	14.3	7.8	6.6	45.8	42.7	3.9		
January 22, 2015	Raw	Strata	14.2	8.8	5.4	38.3				
January 22, 2015	Raw	Strata	14.5	8.1	6.4	44.1				
January 22, 2015	Raw	XAD	12.9	9.1	3.8	29.6	30.3	0.9		
January 22, 2015	Raw	XAD	13.0	9.1	3.9	29.9				
January 22, 2015	Raw	XAD	13.0	9.0	4.1	31.3				
August 19, 2015	Raw	ENV	11.1	8.1	3.0	26.8	28.5	2.2	0.006384	HSD[.05]=7.95 HSD[.01]=10.87 ENV vs Strata nonsignificant ENV vs PPL P<.05 ENV vs XAD P<.01 Strata vs PPL nonsignificant Strata vs XAD P<.05 PPL vs XAD nonsignificant
August 19, 2015	Raw	ENV	12.3	8.5	3.8	31.1				
August 19, 2015	Raw	ENV	10.9	7.9	3.0	27.7				
August 19, 2015	Raw	Strata	11.4	8.2	3.1	27.6	31.9	3.9		
August 19, 2015	Raw	Strata	11.6	7.5	4.1	35.3				
August 19, 2015	Raw	Strata	11.2	7.5	3.7	32.8				
August 19, 2015	Raw	PPL	11.0	6.9	4.0	37.0	37.3	2.9		
August 19, 2015	Raw	PPL	12.0	7.1	4.8	40.4				
August 19, 2015	Raw	PPL	11.3	7.4	3.9	34.6				
August 19, 2015	Raw	XAD	11.0	7.0	4.1	36.9	40.1	2.8		
August 19, 2015	Raw	XAD	12.1	7.1	4.9	40.9				
August 19, 2015	Raw	XAD	11.6	6.7	4.9	42.4				

Table 4-A: Combined seasonal removal of DOM fractions following treatment at the Waterhen Water Treatment Plant.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	t-Test for HPO Fraction
August 12, 2014	Top Coagulation	ENV	7.1	4.7	2.4	33.7	36.5	4.0	0.30
August 12, 2014	Top Coagulation	ENV	7.1	4.3	2.8	39.4			
August 12, 2014	Top Coagulation	Strata	11.0	4.7	6.3	57.3	44.7	10.9	
August 12, 2014	Top Coagulation	Strata	10.9	6.7	4.2	38.7			
August 12, 2014	Top Coagulation	Strata	11.0	6.8	4.2	38.1			
August 12, 2014	Top Sand Filter	ENV	8.7	5.4	3.3	37.8	37.5	2.0	0.09
August 12, 2014	Top Sand Filter	ENV	8.7	5.3	3.4	38.8			
August 12, 2014	Top Sand Filter	ENV	8.7	5.3	3.4	38.8			
August 12, 2014	Top Sand Filter	ENV	8.2	5.4	2.9	34.7			
August 12, 2014	Top Sand Filter	Strata	8.9	6.0	2.8	31.9	30.4	2.1	
August 12, 2014	Top Sand Filter	Strata	8.6	6.1	2.5	28.9			
August 12, 2014	Treated	Strata	11.2	5.6	5.5	49.5	49.3	2.3	0.04
August 12, 2014	Treated	Strata	11.1	5.4	5.7	51.5			
August 12, 2014	Treated	Strata	11.1	5.9	5.2	47.0			
August 12, 2014	Treated	ENV	10.1	4.3	5.8	57.7	59.8	2.1	
August 12, 2014	Treated	ENV	10.1	4.0	6.0	59.9			
August 12, 2014	Treated	ENV	10.7	4.1	6.6	61.8			
September 29, 2014	Top Coagulation	ENV	8.0	5.9	2.1	26.2	28.9	3.9	0.07
September 29, 2014	Top Coagulation	ENV	8.3	5.7	2.6	31.7			
September 29, 2014	Top Coagulation	Strata	8.5	4.1	4.4	51.4	47.2	6.0	
September 29, 2014	Top Coagulation	Strata	7.9	4.5	3.4	43.0			
September 29, 2014	Top Sand Filter	ENV	11.1	4.9	6.2	55.7	54.9	1.1	0.04
September 29, 2014	Top Sand Filter	ENV	9.8	4.5	5.3	54.2			
September 29, 2014	Top Sand Filter	Strata	7.9	4.2	3.7	47.4	45.6	2.6	
September 29, 2014	Top Sand Filter	Strata	7.9	4.4	3.5	43.8			
September 29, 2014	Treated	ENV	7.4	4.3	3.1	41.7	40.1	2.4	0.01
September 29, 2014	Treated	ENV	7.0	4.4	2.6	36.5			
September 29, 2014	Treated	ENV	6.8	4.0	2.8	40.8			
September 29, 2014	Treated	ENV	6.5	3.8	2.7	41.2			
September 29, 2014	Treated	Strata	6.6	3.4	3.2	49.0	50.2	1.7	
September 29, 2014	Treated	Strata	6.6	3.2	3.4	51.4			
January 22, 2015	Top Coagulation	ENV	10.0	6.5	3.5	34.9	36.0	1.6	0.04
January 22, 2015	Top Coagulation	ENV	10.2	6.4	3.8	37.1			
January 22, 2015	Top Coagulation	Strata	13.6	7.1	6.5	47.9	46.1	2.5	
January 22, 2015	Top Coagulation	Strata	10.9	6.1	4.8	44.3			
January 22, 2015	Top Sand Filter	ENV	10.7	6.0	4.7	43.8	41.9	2.6	0.73
January 22, 2015	Top Sand Filter	ENV	10.8	6.4	4.3	40.1			
January 22, 2015	Top Sand Filter	Strata	11.7	6.7	4.9	42.3	42.6	0.4	
January 22, 2015	Top Sand Filter	Strata	11.1	6.4	4.8	42.9			
January 22, 2015	Treated	ENV	10.3	6.7	3.6	35.1	38.8	5.2	0.12
January 22, 2015	Treated	ENV	10.0	5.7	4.2	42.5			
January 22, 2015	Treated	Strata	11.0	5.7	5.3	48.3	48.5	0.3	
January 22, 2015	Treated	Strata	10.6	5.5	5.2	48.6			

Table 5-A: Seasonal THM formation potential measured for raw water and SPE fractions collected from the Waterhen Water on January 22, 2015.

Sample	Chloroform (µg/L)	Bromodichloromethane (µg/L)	Dibromochloromethane (µg/L)	Bromoform (µg/L)	Total THMs (µg/L)	Average THM (µg/L)	Standard Deviation
Raw T1	70.78	7.83	0	0	78.61	128	44
Raw T2	120.98	31.68	8.12	0	160.78		
Raw T3	108.69	28.73	7.26	0	144.68		
ENV HPO T1	62.3	8.68	0	0	70.98	60	10
ENV HPO T2	45.02	6.83	0	0	51.85		
ENV HPO T3	51.21	7.22	0	0	58.43		
Strata HPO T1	73.18	10.04	1.9	0	85.12	83	15
Strata HPO T2	56.53	8.25	1.75	0	66.53		
Strata HPO T3	84.98	10.94	0	0	95.92		
XAD HPO T1	79.58	12.83	2.33	0	94.74	93	12
XAD HPO T2	66.32	11.28	2.36	0	79.96		
XAD HPO T3	87.15	14.15	2.45	0	103.75		
ENV HPI T1	0.88	4.14	2.59	0	7.61	10	3
ENV HPI T2	4.05	5.11	3.7	0	12.86		
ENV HPI T3	2.96	4.69	2.55	0	10.2		
Strata HPI T1	0.214	4.11	2.77	0	7.094	11	3
Strata HPI T2	3.81	5.35	3.57	0	12.73		
Strata HPI T3	3.65	5.65	3.81	0	13.11		
XAD HPI T1	7.73	5.85	3.17	0	16.75	14	3
XAD HPI T2	5.37	5.48	3.09	0	13.94		
XAD HPI T3	3.24	4.91	3.5	0	11.65		

Table 6-A: Seasonal THM formation potential measured for raw water and SPE fractions collected from the Waterhen Water on August 19, 2015.

Sample	Chloroform (µg/L)	Bromodichloromethane (µg/L)	Dibromochloromethane (µg/L)	Bromoform (µg/L)	Total THMs (µg/L)	Average THM (µg/L)	Standard Deviation
Raw T1	146.0	23.7	8.1	0.0	177.8	198.0	18.5
Raw T2	161.9	31.2	9.0	0.0	202.1		
Raw T3	170.1	33.4	10.6	0.0	214.0		
ENV HPO T1	76.4	8.5	0.0	0.0	85.0	86.1	8.2
ENV HPO T2	83.9	10.9	0.0	0.0	94.8		
ENV HPO T3	69.6	9.0	0.0	0.0	78.6		
PPL HPO T1	92.2	10.8	0.0	0.0	103.0	103.1	11.1
PPL HPO T2	79.7	12.4	0.0	0.0	92.1		
PPL HPO T3	98.1	16.3	0.0	0.0	114.4		
Strata HPO T1	111.0	11.5	0.0	0.0	122.4	106.2	14.1
Strata HPO T2	89.8	7.0	0.0	0.0	96.7		
Strata HPO T3	91.9	7.5	0.0	0.0	99.4		
XAD HPO T1	110.9	13.7	0.0	0.0	124.6	130.4	5.1
XAD HPO T2	115.7	16.6	0.0	0.0	132.3		
XAD HPO T3	117.1	17.1	0.0	0.0	134.2		
ENV HPI T1	13.0	6.7	0.0	0.0	19.8	20.8	2.7
ENV HPI T2	11.1	7.8	0.0	0.0	18.9		
ENV HPI T3	14.9	9.0	0.0	0.0	23.9		
PPL HPI T1	7.2	0.0	0.0	0.0	7.2	13.1	5.1
PPL HPI T2	10.3	5.0	0.0	0.0	15.3		
PPL HPI T3	9.7	7.1	0.0	0.0	16.8		
Strata HPI T1	13.5	8.7	0.0	0.0	22.1	18.1	4.0
Strata HPI T2	10.9	7.1	0.0	0.0	18.0		
Strata HPI T3	7.7	6.5	0.0	0.0	14.2		
XAD HPI T1	21.2	7.0	0.0	0.0	28.2	24.1	4.0
XAD HPI T2	17.7	6.0	0.0	0.0	23.7		
XAD HPI T3	13.2	7.1	0.0	0.0	20.3		

Table 7-A: ANOVA analysis for STHMFP for HPO fractions collected from the Waterhen River.

Sample Date	Sample Location	Fractionation Type	Specific THMFP HPI-DOM (µgTHM/mgDOC)	Specific THMFP HPO-DOM (µgTHM/mgDOC)	One-way Variance for HPO STHMFP (ANOVA)	Tukey HSD Test Results for HPO STHMFP
January 22, 2015	Raw	ENV	1.2	12.9	0.000233	HSD[.05]=4.13 HSD[.01]=6 ENV vs Strata nonsignificant ENV vs XAD P<.01 Strata vs XAD P<.01
January 22, 2015	Raw	ENV	0.8	11.2		
January 22, 2015	Raw	ENV	1.5	11.0		
January 22, 2015	Raw	Strata	0.9	14.6		
January 22, 2015	Raw	Strata	1.5	12.2		
January 22, 2015	Raw	Strata	1.6	13.3		
January 22, 2015	Raw	XAD	1.5	20.9		
January 22, 2015	Raw	XAD	1.8	24.4		
January 22, 2015	Raw	XAD	1.3	25.4		
August 19, 2015	Raw	ENV	2.4	33.2	0.542393	
August 19, 2015	Raw	ENV	2.4	28.3		
August 19, 2015	Raw	ENV	2.8	30.9		
August 19, 2015	Raw	Strata	2.7	44.8		
August 19, 2015	Raw	Strata	2.4	28.3		
August 19, 2015	Raw	Strata	1.9	31.0		
August 19, 2015	Raw	PPL	1.0	28.8		
August 19, 2015	Raw	PPL	2.1	21.9		
August 19, 2015	Raw	PPL	2.3	32.6		
August 19, 2015	Raw	XAD	4.1	36.0		
August 19, 2015	Raw	XAD	3.3	29.2		
August 19, 2015	Raw	XAD	3.1	30.1		

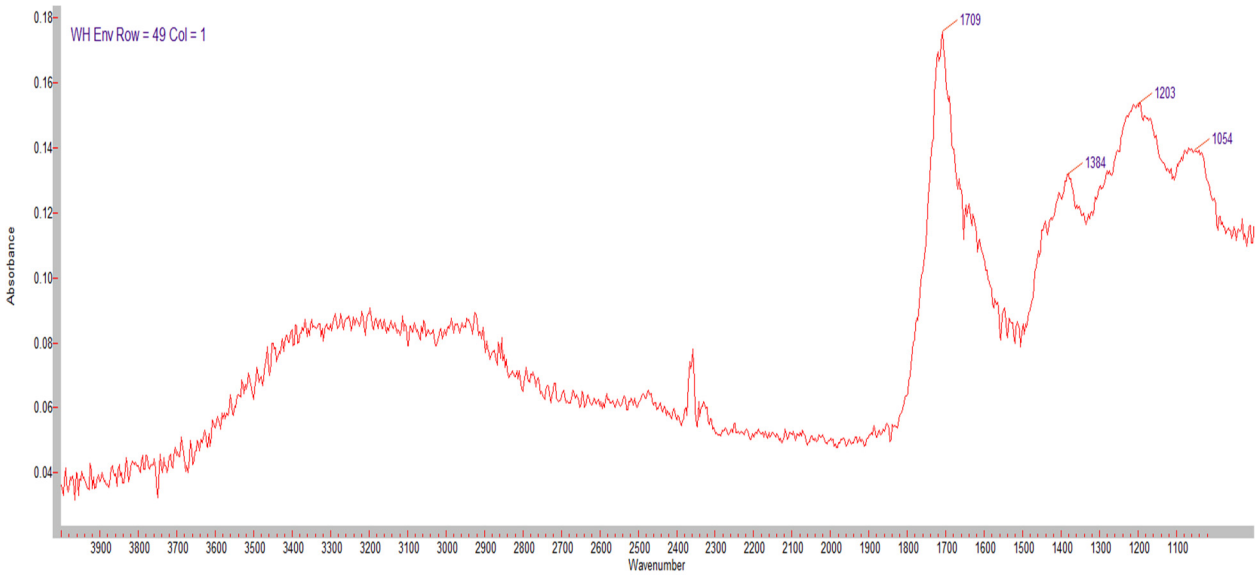

Figure 12-A: FITR spectrum of the hydrophobic fraction isolated from the Waterhen River sample using Bond Elute ENV. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

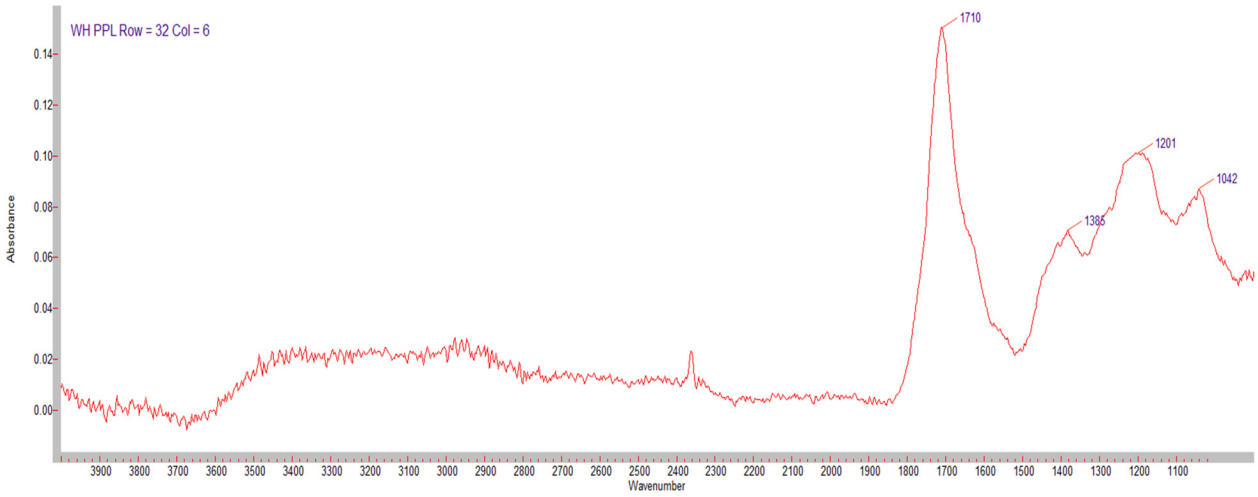

Figure 13-A: FITR spectrum of the hydrophobic fraction isolated from the Waterhen River sample using Bond Elute PPL. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

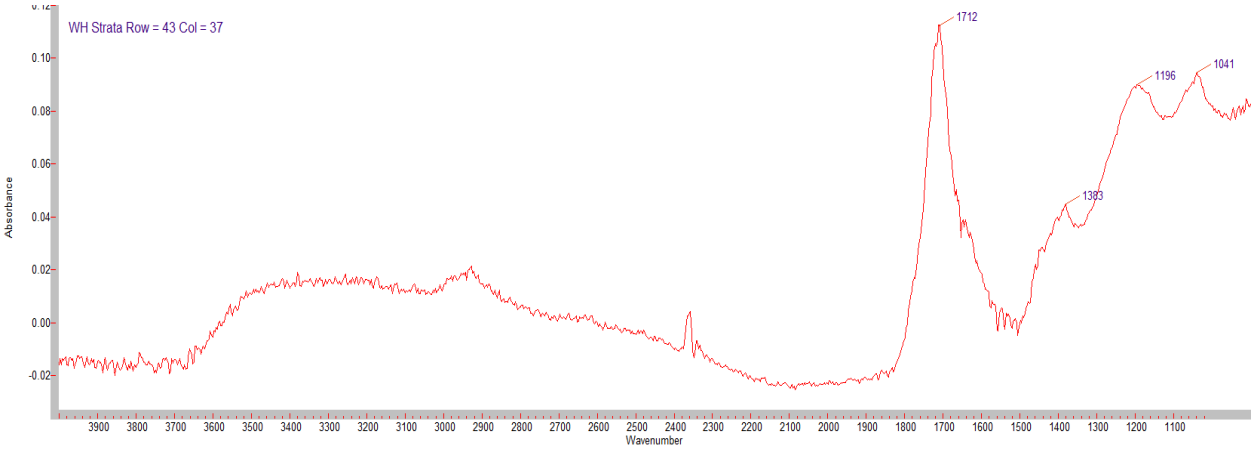

Figure 14-A: FITR spectrum of the hydrophobic fraction isolated from the Waterhen River sample using Strata X. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

3. Camperville Data

Table 8-A: Seasonal SPE fractionation data collected from Lake Winnipegosis via the Camperville Water Treatment Plant raw water intake.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	One-way Variance (ANOVA)	Tukey HSD Test Results
August 11, 2014	Raw Water	XAD	14.5	7.7	6.8	46.7	38.4	11.6	0.724215	
August 11, 2014	Raw Water	XAD	13.7	9.5	4.1	30.2				
August 11, 2014	Raw Water	ENV	13.7	9.2	4.5	32.7	34.2	2.2		
August 11, 2014	Raw Water	ENV	13.7	8.8	4.9	35.7				
August 11, 2014	Raw Water	Strata	13.7	8.1	5.6	41.2	39.8	2.0		
August 11, 2014	Raw Water	Strata	12.3	7.6	4.7	38.4				
September 29, 2014	Raw Water	XAD	10.5	6.3	4.2	40.1	38.6	2.6	0.000466	HSD[.05]=4.46 HSD[.01]=6.49 ENV vs Strata P<.05 ENV vs XAD P<.01 Strata vs XAD P<.01
September 29, 2014	Raw Water	XAD	10.5	6.3	4.2	40.1				
September 29, 2014	Raw Water	XAD	10.6	6.8	3.8	35.6				
September 29, 2014	Raw Water	ENV	10.1	5.6	4.5	44.6	45.9	1.6		
September 29, 2014	Raw Water	ENV	10.3	5.6	4.7	45.4				
September 29, 2014	Raw Water	ENV	10.8	5.6	5.1	47.8				
September 29, 2014	Raw Water	Strata	10.4	5.1	5.3	50.6	50.8	0.3		
September 29, 2014	Raw Water	Strata	10.2	5.0	5.2	51.2				
September 29, 2014	Raw Water	Strata	10.2	5.0	5.2	50.6				
January 22, 2015	Raw Water	XAD	16.5	9.3	7.1	43.5	40.7	2.5	0.347897	
January 22, 2015	Raw Water	XAD	17.1	10.3	6.8	39.9				
January 22, 2015	Raw Water	XAD	18.0	11.0	6.9	38.6				
January 22, 2015	Raw Water	ENV	16.4	10.1	6.2	38.0	39.2	3.3		
January 22, 2015	Raw Water	ENV	17.3	10.7	6.6	38.1				
January 22, 2015	Raw Water	ENV	16.3	10.5	5.8	35.4				
January 22, 2015	Raw Water	ENV	19.8	10.8	9.0	45.4				
January 22, 2015	Raw Water	ENV	17.0	10.2	6.7	39.6				
January 22, 2015	Raw Water	ENV	17.0	10.4	6.6	38.8				
January 22, 2015	Raw Water	Strata	19.4	11.5	7.9	40.6	42.3	1.4		
January 22, 2015	Raw Water	Strata	19.0	10.8	8.2	43.1				
January 22, 2015	Raw Water	Strata	19.1	10.9	8.2	43.0				

Table 9-A: Combined seasonal removal of DOM fractions following treatment at the Camperville Water Treatment Plant.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	t-Test for HPO Fraction
August 11, 2014	Top Coagulation	ENV	11.1	6.6	4.5	40.6	42.5	1.2	0.72
August 11, 2014	Top Coagulation	ENV	11.3	6.4	4.9	43.0			
August 11, 2014	Top Coagulation	ENV	14.0	8.0	6.0	43.1			
August 11, 2014	Top Coagulation	ENV	14.3	8.1	6.2	43.2			
August 11, 2014	Top Coagulation	Strata	11.3	6.1	5.3	46.4	41.7	4.1	
August 11, 2014	Top Coagulation	Strata	13.0	8.0	5.0	38.8			
August 11, 2014	Top Coagulation	Strata	13.3	8.0	5.3	39.8			
August 11, 2014	Top Sand Filter	ENV	11.2	6.8	4.5	40.0	39.8	0.2	0.97
August 11, 2014	Top Sand Filter	ENV	11.0	6.7	4.4	39.6			
August 11, 2014	Top Sand Filter	Strata	12.0	7.4	4.6	38.5	39.9	3.5	
August 11, 2014	Top Sand Filter	Strata	11.6	6.9	4.7	40.5			
August 11, 2014	Top Sand Filter	Strata	12.6	8.0	4.6	36.2			
August 11, 2014	Top Sand Filter	Strata	11.5	6.4	5.1	44.5			
August 11, 2014	Treated	ENV	12.0	7.0	5.0	41.7	40.4	1.8	0.24
August 11, 2014	Treated	ENV	11.3	6.9	4.4	39.1			
August 11, 2014	Treated	Strata	14.1	7.5	6.5	46.4	46.4	5.7	
August 11, 2014	Treated	Strata	12.6	5.9	6.7	53.1			
August 11, 2014	Treated	Strata	14.6	7.8	6.8	46.8			
August 11, 2014	Treated	Strata	12.1	7.4	4.7	39.1			
September 29, 2015	Top Coagulation	ENV	9.9	5.6	4.3	43.6	45.0	2.0	0.42
September 29, 2015	Top Coagulation	ENV	10.0	5.4	4.7	46.4			
September 29, 2015	Top Coagulation	Strata	10.1	5.5	4.6	45.4	48.0	3.8	
September 29, 2015	Top Coagulation	Strata	10.0	4.9	5.1	50.7			
September 29, 2015	Top Sand Filter	ENV	12.0	6.7	5.3	44.1	43.8	0.5	0.0036
September 29, 2015	Top Sand Filter	ENV	11.1	6.3	4.8	43.4			
September 29, 2015	Top Sand Filter	Strata	11.0	5.4	5.6	51.0	51.2	0.4	
September 29, 2015	Top Sand Filter	Strata	11.1	5.4	5.7	51.5			
September 29, 2015	Treated	ENV	10.3	5.9	4.3	42.3	41.4	1.2	0.019
September 29, 2015	Treated	ENV	10.0	5.9	4.1	40.5			
September 29, 2015	Treated	Strata	11.0	5.8	5.2	47.6	47.5	0.1	
September 29, 2015	Treated	Strata	10.8	5.7	5.1	47.5			
January 22, 2015	Top Coagulation	ENV	14.1	8.2	5.9	42.1	42.1	0.0	0.68
January 22, 2015	Top Coagulation	ENV	13.8	8.0	5.8	42.0			
January 22, 2015	Top Coagulation	Strata	13.8	7.2	6.7	48.3	44.1	6.0	
January 22, 2015	Top Coagulation	Strata	13.6	8.2	5.4	39.8			
January 22, 2015	Top Sand Filter	ENV	13.2	8.0	5.3	39.7	40.9	1.7	0.036
January 22, 2015	Top Sand Filter	ENV	13.7	8.0	5.8	42.1			
January 22, 2015	Top Sand Filter	Strata	13.5	8.8	4.7	35.0	34.9	0.2	
January 22, 2015	Top Sand Filter	Strata	13.5	8.8	4.7	34.7			
January 22, 2015	Treated	ENV	13.9	8.1	5.8	41.8	41.9	0.1	0.0055
January 22, 2015	Treated	ENV	13.9	8.1	5.8	42.0			
January 22, 2015	Treated	Strata	14.1	7.3	6.7	47.9	48.4	0.7	
January 22, 2015	Treated	Strata	13.8	7.1	6.8	48.9			

Table 10-A: Seasonal THM formation potential measured for raw water and SPE fractions collected from the Camperville Water on January 22, 2015.

Sample	Chloroform (µg/L)	Bromodichloromethane (µg/L)	Dibromochloromethane (µg/L)	Bromoform (µg/L)	Total THMs (µg/L)	Average THM (µg/L)	Standard Deviation
Raw T1	149.5	37.7	7.5	0.0	194.6	225	33
Raw T2	201.5	49.7	9.6	0.0	260.7		
Raw T3	169.4	41.8	8.1	0.0	219.4		
ENV HPO T1	79.4	14.6	1.8	0.0	95.9	86	10
ENV HPO T2	57.6	11.6	2.3	0.0	71.5		
ENV HPO T3	71.7	13.7	2.9	0.0	88.2		
ENV HPO T4	75.9	13.8	2.8	0.0	92.5		
ENV HPO T5	64.8	12.6	2.6	0.0	80.0		
Strata HPO T1	78.4	14.5	2.7	0.0	95.6	101	15
Strata HPO T2	73.7	13.4	2.5	0.0	89.7		
Strata HPO T3	97.9	17.1	3.1	0.0	118.1		
XAD HPO T1	130.4	22.2	12.8	0.0	165.4	128	42
XAD HPO T2	115.7	12.0	9.0	0.0	136.7		
XAD HPO T3	72.9	6.7	3.7	0.0	83.4		
ENV HPI T1	8.5	7.4	3.6	0.0	19.5	27	9
ENV HPI T2	14.1	9.5	4.8	0.0	28.4		
ENV HPI T3	8.7	7.5	3.7	0.0	19.8		
ENV HPI T4	23.7	12.4	4.9	0.0	41.0		
ENV HPI T5	12.8	9.0	6.7	0.0	28.5		
Strata HPI T1	41.7	8.9	2.0	0.0	52.6	75	24
Strata HPI T2	83.1	15.1	1.5	0.0	99.7		
Strata HPI T3	58.3	11.6	2.3	0.0	72.1		
XAD HPI T1	18.3	7.5	3.7	0.0	29.5	21	8
XAD HPI T2	10.4	7.7	3.9	0.0	21.9		
XAD HPI T3	5.0	5.1	2.8	0.0	13.0		

Table 11-A: ANOVA Analysis for the STHMFP for the HPO fraction isolated from Lake Winnipegosis via the raw water intake at the Camperville Water Treatment Plant

Sample Date	Sample Location	Fractionation Type	Specific THMFP HPI-DOM (µgTHM/mgDOC)	Specific THMFP HPO-DOM (µgTHM/mgDOC)	One-way Variance for HPO STHMFP (ANOVA)	Tukey HSD Test Results for HPO STHMFP
January 22, 2015	Raw	ENV	1.92	15.40	0.080051	
January 22, 2015	Raw	ENV	2.66	12.35		
January 22, 2015	Raw	ENV	1.88	13.44		
January 22, 2015	Raw	ENV	3.79	13.78		
January 22, 2015	Raw	ENV	2.78	12.11		
January 22, 2015	Raw	Strata	4.6	12.4		
January 22, 2015	Raw	Strata	9.2	12.1		
January 22, 2015	Raw	Strata	6.6	12.7		
January 22, 2015	Raw	XAD	3.2	23.1		
January 22, 2015	Raw	XAD	2.1	20.0		
January 22, 2015	Raw	XAD	1.2	12.0		

4. Duck Bay Data

Table 12-A: Seasonal SPE fractionation data collected from Lake Winnipegosis via the Duck Bay Water Treatment Plant raw water intake.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	One-way Variance (ANOVA)	Tukey HSD Test Results
June 17, 2014	Raw	ENV	9.8	6.2	3.5	36.2	39.8	3.1	0.25353	
June 17, 2014	Raw	ENV	9.8	5.7	4.0	41.3				
June 17, 2014	Raw	ENV	10.0	5.8	4.2	41.7				
June 17, 2014	Raw	Strata	10.9	6.1	4.7	43.5	44.1	1.6		
June 17, 2014	Raw	Strata	11.3	6.4	4.8	43.0				
June 17, 2014	Raw	Strata	11.6	6.3	5.3	45.9				
June 17, 2014	Raw	XAD	10.2	5.7	4.4	43.6	39.2	5.1		
June 17, 2014	Raw	XAD	9.9	5.9	4.0	40.3				
June 17, 2014	Raw	XAD	9.9	6.6	3.3	33.6				
August 11, 2014	Raw	ENV	13.9	9.0	4.8	34.9	40.9	4.8	0.182036	
August 11, 2014	Raw	ENV	14.2	8.9	5.3	37.4				
August 11, 2014	Raw	ENV	14.3	7.6	6.7	47.1				
August 11, 2014	Raw	ENV	11.3	6.5	4.8	42.3				
August 11, 2014	Raw	ENV	14.1	8.1	6.0	42.8				
August 11, 2014	Raw	Strata	13.5	8.5	5.0	36.8	46.1	5.8		
August 11, 2014	Raw	Strata	14.2	8.6	5.6	39.5				
August 11, 2014	Raw	Strata	13.6	6.7	6.9	50.5				
August 11, 2014	Raw	Strata	13.6	6.9	6.7	49.0				
August 11, 2014	Raw	Strata	13.6	7.4	6.3	46.1				
August 11, 2014	Raw	Strata	13.6	6.9	6.7	49.1				
August 11, 2014	Raw	Strata	14.7	7.1	7.7	52.0				
August 11, 2014	Raw	XAD	14.3	8.0	6.3	44.1	40.2	5.2		
August 11, 2014	Raw	XAD	13.6	8.9	4.6	34.2				
August 11, 2014	Raw	XAD	12.2	7.1	5.2	42.2				
September 29, 2014	Raw	XAD	15.1	9.0	6.1	40.6	40.7	1.2	0.354214	
September 29, 2014	Raw	XAD	15.5	9.3	6.1	39.6				
September 29, 2014	Raw	XAD	14.9	8.6	6.3	42.0				
September 29, 2014	Raw	ENV	15.2	9.1	6.1	40.1	39.0	1.6		
September 29, 2014	Raw	ENV	14.9	9.0	5.9	39.7				
September 29, 2014	Raw	ENV	15.7	9.9	5.9	37.2				
September 29, 2014	Raw	Strata	15.1	8.7	6.4	42.3	41.3	2.6		
September 29, 2014	Raw	Strata	15.5	8.8	6.7	43.3				
September 29, 2014	Raw	Strata	15.2	9.3	5.8	38.4				
January 22, 2015	Raw	XAD	15.2	9.4	5.8	38.2	38.0	2.1	0.003675	HSD[.05]=7.55; HSD[.01] =10.98 ENV vs Strata P<.01 ENV vs XAD nonsignificant Strata vs XAD P<.05
January 22, 2015	Raw	XAD	16.2	10.4	5.8	35.8				
January 22, 2015	Raw	XAD	16.0	9.6	6.4	40.1				
January 22, 2015	Raw	ENV	15.8	10.6	5.2	32.9	33.0	2.0		
January 22, 2015	Raw	ENV	16.3	11.2	5.0	31.0				
January 22, 2015	Raw	ENV	16.2	10.5	5.7	35.1				
January 22, 2015	Raw	Strata	16.3	7.9	8.4	51.7	46.9	4.3		
January 22, 2015	Raw	Strata	16.0	9.0	7.0	43.6				
January 22, 2015	Raw	Strata	16.2	8.9	7.3	45.3				
August 19, 2015	Raw	ENV	15.6	9.7	5.9	37.7	36.2	1.3	<0.0001	HSD[.05]=5.19; HSD[.01] =7.1 ENV vs Strata P<.01 ENV vs PPL P<.01 ENV vs XAD P<.01 Strata vs PPL nonsignificant Strata vs XAD P<.01 PPL vs XAD nonsignificant
August 19, 2015	Raw	ENV	15.0	9.7	5.3	35.3				
August 19, 2015	Raw	ENV	15.9	10.2	5.6	35.6				
August 19, 2015	Raw	PPL	15.8	8.6	7.2	45.3	47.9	2.2		
August 19, 2015	Raw	PPL	15.6	7.9	7.7	49.3				
August 19, 2015	Raw	PPL	16.0	8.2	7.9	49.2				
August 19, 2015	Raw	Strata	16.0	7.7	8.3	51.7	51.6	0.3		
August 19, 2015	Raw	Strata	16.4	7.9	8.5	51.8				
August 19, 2015	Raw	Strata	15.8	7.7	8.1	51.2				
August 19, 2015	Raw	XAD	15.0	8.7	6.4	42.4	43.5	3.0		
August 19, 2015	Raw	XAD	15.5	8.2	7.3	46.9				
August 19, 2015	Raw	XAD	15.4	9.0	6.3	41.3				

Table 13-A: Combined seasonal removal of DOM fractions following treatment at the Duck Bay Water Treatment Plant.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	t-Test for HPO Fraction
August 11, 2014	Top Coagulation	Strata	11.3	6.9	4.4	39.2	42.9	2.6	0.0044
August 11, 2014	Top Coagulation	Strata	11.5	6.5	5.0	43.3			
August 11, 2014	Top Coagulation	Strata	10.3	5.7	4.7	45.2			
August 11, 2014	Top Coagulation	Strata	10.4	5.9	4.6	43.8			
August 11, 2014	Top Coagulation	ENV	10.9	4.9	6.0	54.6	57.2	3.6	
August 11, 2014	Top Coagulation	ENV	10.9	4.4	6.5	59.7			
August 11, 2014	Top Sand Filter	ENV	11.0	6.7	4.3	39.2	37.3	2.7	0.063
August 11, 2014	Top Sand Filter	ENV	11.1	7.1	3.9	35.4			
August 11, 2014	Top Sand Filter	Strata	11.1	6.2	5.0	44.6	44.5	0.2	
August 11, 2014	Top Sand Filter	Strata	11.1	6.2	4.9	44.3			
August 11, 2014	Treated	ENV	8.4	5.4	3.0	35.6	34.3	1.8	0.0083
August 11, 2014	Treated	ENV	8.5	5.7	2.8	33.1			
August 11, 2014	Treated	Strata	11.1	5.8	5.3	47.9	48.1	0.3	
August 11, 2014	Treated	Strata	11.2	5.8	5.4	48.3			
September 29, 2014	Top Coagulation	ENV	11.7	7.0	4.8	40.7	41.5	1.1	0.22
September 29, 2014	Top Coagulation	ENV	11.9	6.9	5.0	42.3			
September 29, 2014	Top Coagulation	Strata	11.1	6.1	4.9	44.5	48.3	5.3	
September 29, 2014	Top Coagulation	Strata	10.9	5.2	5.6	52.0			
September 29, 2014	Top Sand Filter	ENV	10.9	5.6	5.3	48.5	46.7	2.6	0.33
September 29, 2014	Top Sand Filter	ENV	10.2	5.6	4.6	44.9			
September 29, 2014	Top Sand Filter	Strata	11.5	5.7	5.7	50.0	49.2	1.1	
September 29, 2014	Top Sand Filter	Strata	11.5	5.9	5.6	48.4			
September 29, 2014	Treated	ENV	11.4	6.6	4.8	41.9	40.1	2.5	0.038
September 29, 2014	Treated	ENV	10.6	6.5	4.1	38.4			
September 29, 2014	Treated	Strata	12.0	6.1	5.9	49.1	49.0	0.2	
September 29, 2014	Treated	Strata	12.0	6.2	5.9	48.9			
January 22, 2015	Top Coagulation	ENV	14.8	8.7	6.2	41.6	39.3	3.3	0.42
January 22, 2015	Top Coagulation	ENV	14.1	8.9	5.2	37.0			
January 22, 2015	Top Coagulation	Strata	15.0	8.5	6.5	43.6	42.1	2.1	
January 22, 2015	Top Coagulation	Strata	16.0	9.5	6.5	40.5			
January 22, 2015	Top Sand Filter	ENV	14.0	9.6	4.4	31.6	34.3	3.8	0.13
January 22, 2015	Top Sand Filter	ENV	13.7	8.7	5.1	36.9			
January 22, 2015	Top Sand Filter	Strata	13.1	7.7	5.3	40.9	43.6	3.9	
January 22, 2015	Top Sand Filter	Strata	14.4	7.7	6.7	46.4			
January 22, 2015	Treated	ENV	13.3	8.2	5.1	38.1	35.7	3.4	0.47
January 22, 2015	Treated	ENV	13.6	9.1	4.5	33.3			
January 22, 2015	Treated	Strata	12.3	8.4	3.9	31.8	33.2	2.1	
January 22, 2015	Treated	Strata	12.9	8.4	4.5	34.7			

Table 14-A: Trihalomethane formation potential from DOM fractions collected from Lake Winnipegosis via the Duck Bay Water Treatment Plant raw water intake on January 22, 2015.

Sample	Chloroform (µg/L)	Bromodichloromethane (µg/L)	Dibromochloromethane (µg/L)	Bromoform (µg/L)	Total THMs (µg/L)	Average THM (µg/L)	Standard Deviation
Raw T1	117.78	30.36	7.78	0.00	155.92	170	16
Raw T2	124.43	33.36	7.84	0.00	165.63		
Raw T3	142.80	36.67	8.18	0.00	187.65		
ENV HPO T1	101.93	9.80	0.00	0.00	111.73	119	9
ENV HPO T2	103.28	11.93	0.00	0.00	115.21		
ENV HPO T3	115.47	13.10	0.00	0.00	128.57		
Strata HPO T1	124.45	12.83	0.00	0.00	137.28	147	13
Strata HPO T2	128.71	13.31	0.00	0.00	142.02		
Strata HPO T3	144.04	14.90	2.05	0.00	160.99		
XAD HPO T1	137.51	6.52	4.16	0.00	148.19	146	9
XAD HPO T2	124.45	8.23	2.96	0.00	135.64		
XAD HPO T3	130.68	19.02	3.03	0.00	152.73		
ENV HPI T1	3.00	3.94	2.34	0.00	9.28	14	4
ENV HPI T2	7.59	6.62	3.52	0.00	17.73		
ENV HPI T3	5.68	5.91	3.17	0.00	14.76		
Strata HPI T1	21.71	11.08	4.89	0.00	37.68	42	12
Strata HPI T2	18.22	10.00	4.45	0.00	32.67		
Strata HPI T3	39.43	12.05	3.82	0.00	55.30		
XAD HPI T1	27.42	11.55	5.39	0.00	44.36	44	5
XAD HPI T2	23.57	10.47	4.90	0.00	38.94		
XAD HPI T3	30.42	12.50	6.04	0.00	48.96		

Table 15-A: ANOVA Analysis and Tukey HSD Test results for STHMFP for the HPO fraction collected from Lake Winnipegosis via the raw water intake at the Duck Bay Water Treatment Plant.

Sample Date	Sample Location	Fractionation Type	Specific THMFP HPI-DOM (µgTHM/mgDOC)	Specific THMFP HPO-DOM (µgTHM/mgDOC)	One-way Variance for HPO STHMFP (ANOVA)	Tukey HSD Test Results for HPO STHMFP
January 22, 2015	Raw	ENV	1.39	21.48	0.001066	HSD[.05]=2.08 HSD[.01]=3.03 ENV vs Strata P<.05 ENV vs XAD nonsignificant Strata vs XAD P<.01
January 22, 2015	Raw	ENV	1.58	22.87		
January 22, 2015	Raw	ENV	0.88	22.69		
January 22, 2015	Raw	Strata	4.16	19.12		
January 22, 2015	Raw	Strata	6.14	19.72		
January 22, 2015	Raw	Strata	4.26	19.37		
January 22, 2015	Raw	XAD	4.74	25.61		
January 22, 2015	Raw	XAD	4.70	23.34		
January 22, 2015	Raw	XAD	4.07	23.90		
August 19, 2015	Raw	ENV	2.15	18.87	0.034863	HSD[.05]=4.3 HSD[.01]=5.87 ENV vs Strata nonsignificant ENV vs PPL nonsignificant ENV vs XAD nonsignificant Strata vs PPL nonsignificant Strata vs XAD nonsignificant PPL vs Strata nonsignificant
August 19, 2015	Raw	ENV	2.61	23.42		
August 19, 2015	Raw	ENV	2.32	22.39		
August 19, 2015	Raw	Strata	4.61	19.63		
August 19, 2015	Raw	Strata	3.43	16.17		
August 19, 2015	Raw	Strata	4.53	18.25		
August 19, 2015	Raw	PPL	3.14	17.26		
August 19, 2015	Raw	PPL	3.43	17.16		
August 19, 2015	Raw	PPL	3.48	18.36		
August 19, 2015	Raw	XAD	5.26	22.36		
August 19, 2015	Raw	XAD	4.28	19.85		
August 19, 2015	Raw	XAD	4.16	21.20		

Table 16-A: Trihalomethane formation potential from DOM fractions collected from Lake Winnipegosis via the Duck Bay Water Treatment Plant raw water intake on August 19, 2015.

Sample	Chloroform (µg/L)	Bromodichloromethane (µg/L)	Dibromochloromethane (µg/L)	Bromoform (µg/L)	Total THMs (µg/L)	Average THM (µg/L)	Standard Deviation
Raw T1	173.9	35.7	12.0	0.0	221.6	238	16
Raw T2	198.8	29.0	9.9	0.0	237.6		
Raw T3	210.3	30.7	13.0	0.0	254.0		
ENV HPO T1	86.6	8.5	0.0	0.0	95.0	104	8
ENV HPO T2	101.5	7.6	0.0	0.0	109.0		
ENV HPO T3	99.8	9.3	0.0	0.0	109.1		
PPL HPO T1	118.3	9.6	0.0	0.0	107.5	116	11
PPL HPO T2	103.6	8.9	0.0	0.0	112.5		
PPL HPO T3	98.8	8.8	0.0	0.0	128.0		
Strata HPO T1	127.6	7.9	0.0	0.0	135.4	124	10
Strata HPO T2	107.9	8.1	0.0	0.0	115.9		
Strata HPO T3	112.4	8.8	0.0	0.0	121.2		
XAD HPO T1	122.5	9.9	0.0	0.0	132.4	126	7
XAD HPO T2	116.6	10.7	0.0	0.0	127.3		
XAD HPO T3	108.3	9.7	0.0	0.0	118.0		
ENV HPI T1	12.4	8.4	0.0	0.0	20.9	23	3
ENV HPI T2	17.7	9.0	0.0	0.0	26.7		
ENV HPI T3	14.8	7.8	0.0	0.0	22.6		
PPL HPI T1	13.3	11.5	0.0	0.0	24.9	28	3
PPL HPI T2	18.2	9.8	0.0	0.0	27.9		
PPL HPI T3	16.6	13.4	0.0	0.0	30.0		
Strata HPI T1	21.1	14.7	0.0	0.0	35.7	33	5
Strata HPI T2	16.3	10.2	0.0	0.0	26.5		
Strata HPI T3	24.4	11.4	0.0	0.0	35.9		
XAD HPI T1	32.1	13.6	0.0	0.0	45.6	39	5
XAD HPI T2	26.2	9.0	0.0	0.0	35.2		
XAD HPI T3	25.9	11.7	0.0	0.0	37.6		

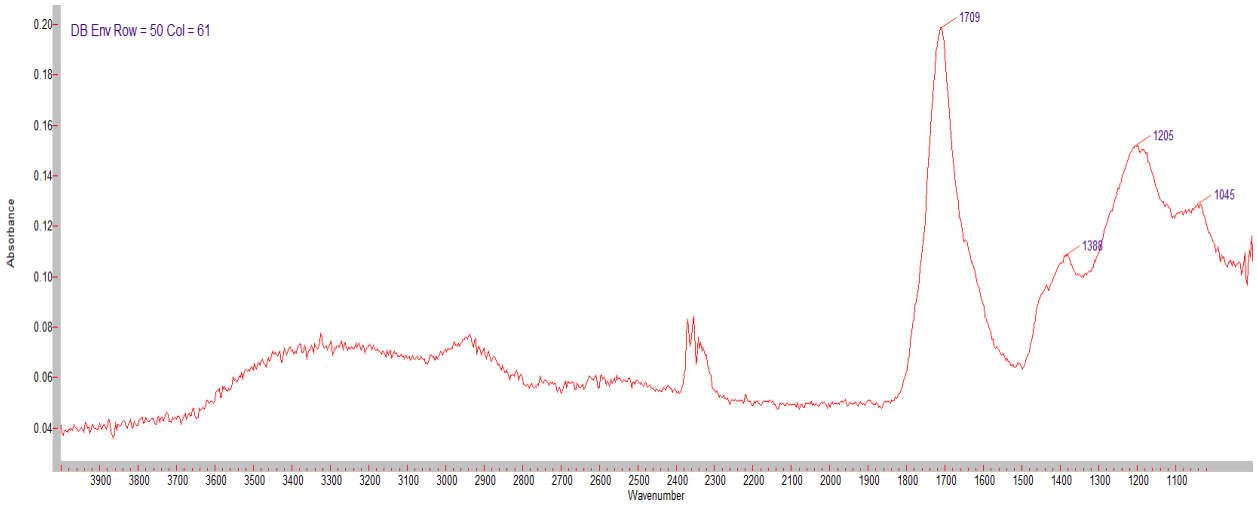

Figure 15-A: FITR spectrum of the hydrophobic fraction isolated from Lake Winnipegosis (Duck Bay) using Bond Elute ENV. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

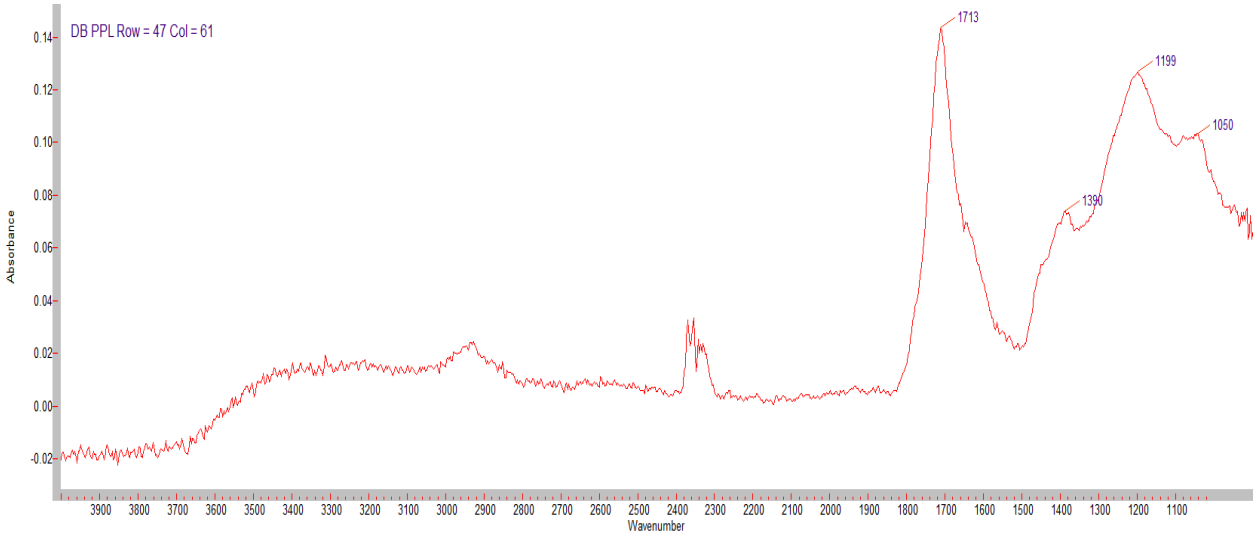

Figure 16-A: FITR spectrum of the hydrophobic fraction isolated from Lake Winnipegosis (Duck Bay) using Bond Elute PPL. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

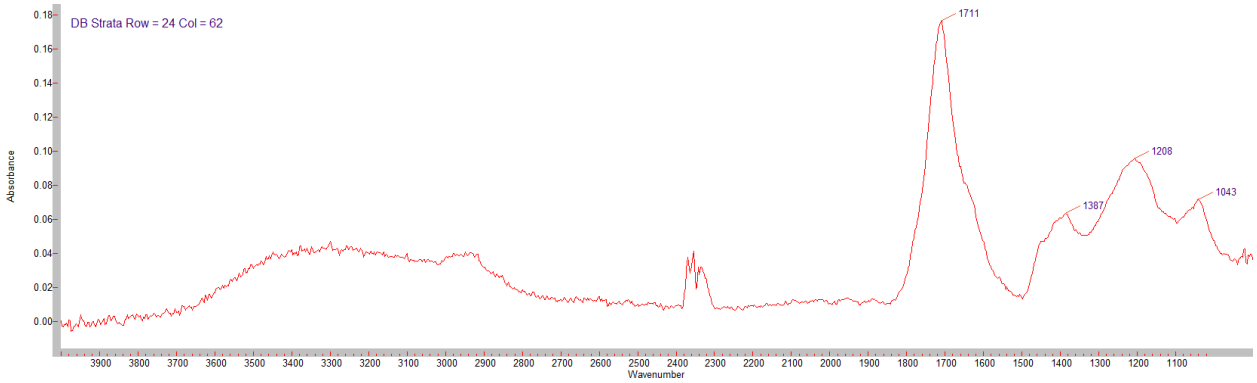

Figure 17-A: FITR spectrum of the hydrophobic fraction isolated from Lake Winnipegosis (Duck Bay) using Strata X. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

5. Pine Creek First Nation Raw Data

Table 17-A: Seasonal SPE fractionation data collected from Lake Winnipegosis via the Pine Creek First Nation Water Treatment Plant raw water intake.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	One-way Variance (ANOVA)	Tukey HSD Test Results
September 29, 2014	Raw	ENV	11.2	7.0	4.2	37.6	37.6	3.8	0.051664	
September 29, 2014	Raw	ENV	10.8	6.8	4.0	37.3				
September 29, 2014	Raw	ENV	12.0	6.9	5.1	42.3				
September 29, 2014	Raw	ENV	11.7	7.8	3.9	33.0				
September 29, 2014	Raw	Strata	10.9	5.9	5.1	46.3	43.9	3.8		
September 29, 2014	Raw	Strata	10.8	6.0	4.8	44.3				
September 29, 2014	Raw	Strata	11.8	6.3	5.5	46.6				
September 29, 2014	Raw	Strata	11.3	7.0	4.3	38.4				
September 29, 2014	Raw	XAD	12.2	6.6	5.6	46.0	44.3	2.4		
September 29, 2014	Raw	XAD	13.2	7.2	6.0	45.3				
September 29, 2014	Raw	XAD	13.0	7.6	5.4	41.5				
January 22, 2015	Raw	XAD	16.0	9.7	6.3	39.2	38.8	2.7	0.008212	HSD[.05]=5.49 HSD[.01]=8 ENV vs Strata P<.01 ENV vs XAD nonsignificant Strata vs XAD P<.05
January 22, 2015	Raw	XAD	15.8	9.2	6.5	41.3				
January 22, 2015	Raw	XAD	15.6	10.0	5.6	35.9				
January 22, 2015	Raw	ENV	15.5	10.2	5.4	34.6	36.6	2.6		
January 22, 2015	Raw	ENV	15.6	9.5	6.2	39.5				
January 22, 2015	Raw	ENV	15.4	9.9	5.5	35.7				
January 22, 2015	Raw	Strata	15.4	8.5	6.9	44.6	45.0	0.6		
January 22, 2015	Raw	Strata	15.6	8.5	7.1	45.7				
January 22, 2015	Raw	Strata	15.3	8.5	6.9	44.7				

Table 18-A: Combined seasonal removal of DOM fractions following treatment at the Pine Creek First Nation Water Treatment Plant.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	t-Test for HPO Fraction
September 29, 2014	Coagulation	Strata	10.7	5.7	5.0	46.5	47.2	1.0	0.070
September 29, 2014	Coagulation	Strata	11.0	5.7	5.3	47.9			
September 29, 2014	Coagulation	ENV	12.8	7.6	5.2	40.3	37.8	3.6	
September 29, 2014	Coagulation	ENV	11.0	7.1	3.9	35.3			
September 29, 2014	RO	Strata	6.9	3.6	3.4	48.4	47.7	1.0	0.243
September 29, 2014	RO	Strata	7.0	3.7	3.3	47.1			
September 29, 2014	RO	ENV	9.1	4.9	4.1	45.5	42.2	4.7	
September 29, 2014	RO	ENV	8.4	5.2	3.3	38.8			
September 29, 2014	Treated	ENV	12.4	8.0	4.5	36.0	38.1	2.9	0.077
September 29, 2014	Treated	ENV	13.5	8.1	5.4	40.1			
September 29, 2014	Treated	Strata	12.2	5.9	6.3	51.5	49.0	3.5	
September 29, 2014	Treated	Strata	11.2	6.0	5.2	46.5			
January 22, 2015	Coagulation	ENV	14.9	9.4	5.5	37.1	37.8	1.1	0.422
January 22, 2015	Coagulation	ENV	14.9	9.1	5.7	38.6			
January 22, 2015	Coagulation	Strata	14.2	8.8	5.4	37.9	44.9	9.9	
January 22, 2015	Coagulation	Strata	14.9	7.2	7.7	51.9			
January 22, 2015	Dissolved Air Flotation	ENV	15.0	11.0	4.1	27.2	30.4	4.6	0.055
January 22, 2015	Dissolved Air Flotation	ENV	16.0	10.6	5.4	33.7			
January 22, 2015	Dissolved Air Flotation	Strata	16.3	9.2	7.1	43.4	44.3	1.2	
January 22, 2015	Dissolved Air Flotation	Strata	16.3	8.9	7.4	45.2			
January 22, 2015	Reverse Osmosis Membrane	ENV	11.8	8.0	3.7	31.8	34.2	3.4	0.100
January 22, 2015	Reverse Osmosis Membrane	ENV	10.4	6.6	3.8	36.6			
January 22, 2015	Reverse Osmosis Membrane	Strata	11.6	6.8	4.8	41.4	43.4	2.8	
January 22, 2015	Reverse Osmosis Membrane	Strata	11.7	6.4	5.3	45.3			
January 22, 2015	Treated	ENV	15.8	8.8	7.0	44.1	42.8	1.9	0.102
January 22, 2015	Treated	ENV	16.0	9.4	6.6	41.5			
January 22, 2015	Treated	Strata	16.9	8.6	8.3	49.2	48.0	1.7	
January 22, 2015	Treated	Strata	15.2	8.1	7.1	46.8			

Table 19-A: Trihalomethane formation potential from DOM fractions collected from Lake Winnipegosis via the Pine Creek First Nation Treatment Plant raw water intake on January 22, 2015.

Sample	Chloroform (µg/L)	Bromodichloromethane (µg/L)	Dibromochloromethane (µg/L)	Bromoform (µg/L)	Total THMs (µg/L)	Average THM (µg/L)	Standard Deviation
Raw T1	156.6	40.0	8.5	0.0	205.1	206	17
Raw T2	144.5	37.7	7.7	0.0	189.8		
Raw T3	173.5	41.1	9.1	0.0	223.7		
ENV HPO T1	107.9	11.5	0.0	0.0	119.4	109	12
ENV HPO T2	85.6	9.4	0.0	0.0	95.1		
ENV HPO T3	100.8	10.4	0.0	0.0	111.3		
Strata HPO T1	67.8	7.8	0.5	0.0	76.1	90	14
Strata HPO T2	81.0	9.3	0.0	0.0	90.3		
Strata HPO T3	94.6	9.6	0.0	0.0	104.2		
XAD HPO T1	129.0	20.1	4.0	0.0	144.7	161	21
XAD HPO T2	129.0	20.1	4.0	0.0	153.1		
XAD HPO T3	113.0	48.0	23.2	0.0	184.2		
ENV HPI T1	32.6	9.8	3.7	0.0	46.0	38	7
ENV HPI T2	19.3	10.3	5.1	0.0	34.7		
ENV HPI T3	18.7	10.1	4.8	0.0	33.6		
Strata HPI T1	5.3	3.7	2.3	0.0	11.3	9	2
Strata HPI T2	1.1	4.2	2.6	0.0	7.9		
Strata HPI T3	1.3	4.7	2.7	0.0	8.6		
XAD HPI T1	23.1	10.5	4.5	0.0	38.1	41	3
XAD HPI T2	27.3	11.2	4.5	0.0	43.0		
XAD HPI T3	30.5	6.5	4.3	0.0	41.3		

Table 20-A: ANOVA Analysis and Tukey RSD Test Results for STHMFP of the HPO fraction collected from Lake Winnipegosis at Pine Creek First Nation.

Sample Date	Sample Location	Fractionation Type	Specific THMFP HPI-DOM (µgTHM/mgDOC)	Specific THMFP HPO-DOM (µgTHM/mgDOC)	One-way Variance for HPO STHMFP (ANOVA)	Tukey HSD Test Results for HPO STHMFP
January 22, 2015	Raw	ENV	4.53	17.69	0.000227	HSD[.05]=4.23 HSD[.01]=6.16 ENV vs Strata P<.05 ENV vs XAD P<.01 Strata vs XAD P<.01
January 22, 2015	Raw	ENV	3.67	19.36		
January 22, 2015	Raw	ENV	3.39	20.27		
January 22, 2015	Raw	Strata	4.6	13.2		
January 22, 2015	Raw	Strata	9.2	14.6		
January 22, 2015	Raw	Strata	6.6	11.1		
January 22, 2015	Raw	XAD	4.3	24.5		
January 22, 2015	Raw	XAD	4.1	28.3		
January 22, 2015	Raw	XAD	4.3	25.8		

6. Sanford Raw Data

Table 21-A: Seasonal SPE fractionation data collected from the La Salle River via the Macdonald Water Treatment Plant raw water intake.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	One-way Variance (ANOVA)	Tukey HSD Test Results
May 9 2014	Raw	ENV	12.5	6.6	5.9	47.2				
May 9 2014	Raw	ENV	12.0	6.7	5.4	44.7				
May 9 2014	Raw	ENV	12.1	6.9	5.3	43.4	44.4	1.7		
May 9 2014	Raw	ENV	13.9	7.6	6.3	45.3				
May 9 2014	Raw	ENV	13.6	7.8	5.9	43.0				
May 9 2014	Raw	ENV	13.7	7.8	5.8	42.6			<0.0001	HSD[.05]=4.01 HSD[.01]=5.58 ENV vs Strata P<.01 ENV vs XAD P<.01 Strata vs XAD P<.05
May 9 2014	Raw	Strata X	13.0	5.5	7.5	57.4				
May 9 2014	Raw	Strata X	12.6	5.3	7.3	57.8	57.0	0.8		
May 9 2014	Raw	Strata X	13.3	5.7	7.5	56.8				
May 9 2014	Raw	Strata X	13.1	5.7	7.3	56.1				
May 9 2014	Raw	XAD	12.7	6.1	6.6	52.1	52.1	0.0		
June 11, 2014	Raw	ENV	12.2	5.7	6.5	47.1	47.4	0.5		
June 11, 2014	Raw	ENV	11.7	6.1	5.6	47.7				
June 11, 2014	Raw	Strata	12.2	5.9	6.3	51.7	48.2	4.9	0.890	
June 11, 2014	Raw	Strata	12.3	6.8	5.5	44.7				
June 11, 2014	Raw	XAD	13.0	6.6	6.4	49.5	48.8	1.0		
June 11, 2014	Raw	XAD	12.7	6.6	6.1	48.1				
July 10, 2014	Raw	ENV	15.1	9.4	5.7	37.9				
July 10, 2014	Raw	ENV	15.2	9.4	5.8	38.1	38.0	0.1		
July 10, 2014	Raw	Strata	14.8	8.0	6.7	45.5			0.0109	HSD[.05]=4.1 HSD[.01]=9.15 ENV vs Strata P<.05 ENV vs XAD P<.05 Strata vs XAD nonsignificant
July 10, 2014	Raw	Strata	14.7	7.8	6.9	46.7	46.1	0.8		
July 10, 2014	Raw	XAD	14.1	8.0	6.1	43.3	43.3	0.0		
July 24, 2014	Raw	ENV	14.1	7.6	6.5	45.8	45.4	0.6		
July 24, 2014	Raw	ENV	14.1	7.8	6.4	45.0				
July 24, 2014	Raw	Strata	13.9	6.2	7.7	55.2	55.4	0.3		
July 24, 2014	Raw	Strata	13.9	6.2	7.8	55.7			0.0497	HSD[.05]=9.77 HSD[.01]=15.63 ENV vs Strata P<.05 ENV vs XAD nonsignificant Strata vs XAD nonsignificant
July 24, 2014	Raw	XAD	11.1	5.3	5.8	51.9				
July 24, 2014	Raw	XAD	13.1	7.3	5.8	44.1	47.3	4.1		
July 24, 2014	Raw	XAD	13.4	7.2	6.1	45.9				
September 24, 2014	Raw	ENV	16.8	9.1	7.7	46.0				
September 24, 2014	Raw	ENV	17.1	8.8	8.3	48.5	47.0	1.3		
September 24, 2014	Raw	ENV	16.6	8.9	7.8	46.7				
September 24, 2014	Raw	Strata	16.5	6.6	9.9	59.9				
September 24, 2014	Raw	Strata	16.8	7.9	8.9	53.2	55.2	4.1	0.012966	HSD[.05]=6.71 HSD[.01]=9.76 ENV vs Strata P<.05 ENV vs XAD nonsignificant Strata vs XAD P<.05
September 24, 2014	Raw	Strata	14.8	7.0	7.8	52.6				
September 24, 2014	Raw	XAD	16.2	8.6	7.6	47.1				
September 24, 2014	Raw	XAD	16.1	8.9	7.2	44.7	46.7	1.8		
September 24, 2014	Raw	XAD	16.1	8.3	7.8	48.3				
March 4, 2015	Raw	ENV	26.2	15.3	10.9	41.6				
March 4, 2015	Raw	ENV	26.5	15.2	11.3	42.6	42.5	0.9		
March 4, 2015	Raw	ENV	25.8	14.6	11.2	43.4				
March 4, 2015	Raw	Strata	24.6	14.6	10.0	40.7				
March 4, 2015	Raw	Strata	25.2	14.9	10.3	40.8	41.1	0.7	0.40048	
March 4, 2015	Raw	Strata	26.0	15.1	10.9	41.9				
March 4, 2015	Raw	XAD	26.2	16.3	10.0	38.0				
March 4, 2015	Raw	XAD	26.3	15.2	11.1	42.2	40.8	2.4		
March 4, 2015	Raw	XAD	26.0	15.0	11.0	42.2				
June 30, 2015	Raw	ENV	14.9	8.7	6.2	41.7				
June 30, 2015	Raw	ENV	15.2	8.9	6.3	41.5	41.5	0.2		
June 30, 2015	Raw	ENV	15.5	9.1	6.4	41.3				
June 30, 2015	Raw	Strata	15.7	7.5	8.2	52.5				
June 30, 2015	Raw	Strata	15.2	7.4	7.8	51.5	50.9	1.9		
June 30, 2015	Raw	Strata	15.0	7.7	7.3	48.8				
June 30, 2015	Raw	PPL	14.7	7.1	7.7	52.2			0.004223	HSD[.05]=7.33 HSD[.01]=10.02 ENV vs Strata P<.05 ENV vs PPL P<.05 ENV vs XAD nonsignificant Strata vs PPL nonsignificant Strata vs XAD P<.05 PPL vs XAD P<.05
June 30, 2015	Raw	PPL	14.8	7.2	7.7	51.7	51.2	1.3		
June 30, 2015	Raw	PPL	15.1	7.6	7.5	49.7				
June 30, 2015	Raw	XAD	15.8	9.9	5.9	37.2				
June 30, 2015	Raw	XAD	16.4	9.1	7.3	44.7	43.0	5.1		
June 30, 2015	Raw	XAD	16.4	8.7	7.7	46.9				
July 23, 2015	Raw	ENV	18.7	9.7	9.0	48.2				
July 23, 2015	Raw	ENV	17.7	9.6	8.1	45.6	46.6	1.5		
July 23, 2015	Raw	ENV	17.8	9.6	8.1	45.8				
July 23, 2015	Raw	Strata	18.1	9.8	8.3	45.6				
July 23, 2015	Raw	Strata	17.7	9.3	8.4	47.5	46.4	1.0		
July 23, 2015	Raw	Strata	18.0	9.7	8.3	46.1			0.319884	
July 23, 2015	Raw	PPL	19.9	9.2	10.6	53.5				
July 23, 2015	Raw	PPL	18.1	9.9	8.2	45.3	50.2	4.4		
July 23, 2015	Raw	PPL	19.0	9.2	9.8	51.8				
July 23, 2015	Raw	XAD	18.2	9.4	8.8	48.3				
July 23, 2015	Raw	XAD	17.9	9.3	8.6	48.0	46.4	3.0		
July 23, 2015	Raw	XAD	17.8	10.2	7.7	43.0				

Table 22-A: Combined seasonal removal of DOM fractions by the Macdonald Water Treatment Plant.

Sample Date	Sample Location	Fractionation Type	Raw DOM (mg/L)	HPI-DOM (mg/L)	HPO-DOM (mg/L)	HPO-DOM (%)	Average HPO-DOM (%)	Standard Deviation	t-Test for HPO Fraction	Tukey HSD Test Results
July 10, 2014	UF Permeate	Strata	11.8	4.8	7.1	59.7	58.7	1.4	0.021	N/A
July 10, 2014	UF Permeate	Strata	11.8	5.0	6.8	57.7				
July 10, 2014	UF Permeate	ENV	12.1	6.7	5.4	44.8	46.3	2.1		
July 10, 2014	UF Permeate	ENV	12.1	6.3	5.8	47.8				
July 10, 2014	RO	ENV	0.9	2.2	-1.3	-141.0	N/A	N/A	N/A	
July 10, 2014	RO	Strata	0.9	0.9	0.0	-3.8				
July 10, 2014	Blend	ENV	3.4	2.1	1.3	38.9	36.3	3.7	0.118	
July 10, 2014	Blend	ENV	3.3	2.2	1.1	33.6				
July 10, 2014	Blend	Strata	3.4	1.9	1.5	42.8	43.4	0.9		
July 10, 2014	Blend	Strata	3.4	1.9	1.5	44.1				
July 24, 2014	UF Permeate	ENV	13.0	7.3	5.8	44.3	44.4	0.0	0.00049	N/A
July 24, 2014	UF Permeate	ENV	13.0	7.2	5.8	44.4				
July 24, 2014	UF Permeate	Strata	12.6	5.4	7.2	57.2	56.9	0.4		
July 24, 2014	UF Permeate	Strata	12.6	5.5	7.1	56.6				
July 24, 2014	RO	N/A	1.0	N/A	N/A	N/A	N/A	N/A	N/A	
July 24, 2014	Blend	Strata	3.7	1.9	1.8	49.3	46.9	3.4	0.060	
July 24, 2014	Blend	Strata	3.7	2.0	1.6	44.5				
July 24, 2014	Blend	ENV	3.5	2.2	1.3	37.5	37.4	0.2		
July 24, 2014	Blend	ENV	3.5	2.2	1.3	37.3				
September 24, 2014	UF Permeate	ENV	15.6	7.6	8.0	51.0	49.1	2.7	0.143	N/A
September 24, 2014	UF Permeate	ENV	15.4	8.2	7.3	47.2				
September 24, 2014	UF Permeate	Strata	16.0	7.4	8.6	53.6	55.4	2.6		
September 24, 2014	UF Permeate	Strata	16.7	7.1	9.6	57.3				
September 24, 2014	RO	BDL							N/A	
September 24, 2014	Blend	Strata	4.3332	2.2717	2.0615	47.57	47.5	0.1	0.796	
September 24, 2014	Blend	Strata	4.4301	2.3286	2.1015	47.44				
September 24, 2014	Blend	ENV	3.0712	1.8345	1.2367	40.27	45.9	7.9		
September 24, 2014	Blend	ENV	4.4477	2.1595	2.2882	51.45				
March 4, 2015	UF Permeate	ENV	25.7	14.6	11.2	43.4	42.2	1.7	0.064	N/A
March 4, 2015	UF Permeate	ENV	25.6	15.1	10.5	41.0				
March 4, 2015	UF Permeate	Strata	24.2	12.7	11.4	47.3	48.4	1.6		
March 4, 2015	UF Permeate	Strata	24.2	12.2	12.0	49.5				
March 4, 2015	RO	BDL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
March 4, 2015	Blend	Strata	4.7	3.1	1.6	33.4	32.5	1.3	0.073	
March 4, 2015	Blend	Strata	4.7	3.2	1.5	31.6				
March 4, 2015	Blend	ENV	5.3	4.0	1.3	25.2	22.5	3.8		
March 4, 2015	Blend	ENV	4.8	3.9	1.0	19.8				
June 30, 2015	UF Permeate	ENV	13.9	7.7	6.2	44.6	40.9	5.2	0.03*	HSD[.05]=14 HSD[.01]=24.9 ENV vs Strata P<.05 ENV vs PPL nonsignificant Strata vs PPL nonsignificant
June 30, 2015	UF Permeate	ENV	13.2	8.3	4.9	37.2				
June 30, 2015	UF Permeate	Strata	13.8	5.9	7.9	57.2	57.1	0.1		
June 30, 2015	UF Permeate	Strata	13.8	5.9	7.9	57.0				
June 30, 2015	UF Permeate	PPL	13.8	6.2	7.6	55.1	53.3	2.5		
June 30, 2015	UF Permeate	PPL	13.5	6.5	6.9	51.5				
June 30, 2015	RO	N/A	0.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A
June 30, 2015	Blend	ENV	4.3	3.2	1.1	26.0	29.6	5.1	0.0097*	HSD[+A1:K51.05]=12.42 HSD[.01]=22.09 ENV vs Strata P<.05 ENV vs PPL nonsignificant Strata vs PPL P<.05
June 30, 2015	Blend	ENV	4.2	2.8	1.4	33.2				
June 30, 2015	Blend	Strata	4.0	2.1	2.0	49.1	49.4	0.4		
June 30, 2015	Blend	Strata	3.8	1.9	1.9	49.6				
June 30, 2015	Blend	PPL	4.2	3.0	1.2	28.8	28.3	0.8		
June 30, 2015	Blend	PPL	4.2	3.1	1.2	27.7				

* Represents significant results using ANOVA analysis

Table 23-A: Trihalomethane formation potential from DOM fractions collected from the La Salle River via the Macdonald Water Treatment Plant raw water intake on March 4, 2015.

Sample	Chloroform (µg/L)	Bromodichloromethane (µg/L)	Dibromochloromethane (µg/L)	Bromoform (µg/L)	Total THMs (µg/L)	Average THM (µg/L)	Standard Deviation
Raw T1	133.2	25.3	9.3	0.0	168	186	17
Raw T2	156.4	29.2	3.8	0.0	189		
Raw T3	166.3	30.6	4.0	0.0	201		
ENV HPO T1	163.0	7.2	0.0	0.0	170	190	20
ENV HPO T2	202.2	8.4	0.0	0.0	211		
ENV HPO T3	180.5	9.0	0.0	0.0	189		
Strata HPO T1	270.3	17.6	0.0	0.0	288	266	27
Strata HPO T2	219.7	16.4	0.0	0.0	236		
Strata HPO T3	252.9	19.7	0.0	0.0	273		
XAD HPO T1	225.6	20.8	3.7	0.0	250	257	23
XAD HPO T2	213.9	20.3	3.1	0.0	237		
XAD HPO T3	262.4	18.4	2.0	0.0	283		
ENV HPI T1	66.3	16.5	4.9	0.0	88	83	15
ENV HPI T2	72.9	17.8	5.2	0.0	96		
ENV HPI T3	48.1	13.9	4.4	0.0	66		
Strata HPI T1	76.7	17.7	3.1	0.0	97	90	23
Strata HPI T2	87.0	20.0	2.7	0.0	110		
Strata HPI T3	52.3	9.7	2.3	0.0	64		
XAD HPI T1	57.6	8.7	5.6	0.0	72	60	11
XAD HPI T2	38.7	6.1	4.6	0.0	49		
XAD HPI T3	46.1	10.7	1.7	0.0	58		

Table 24-A: Trihalomethane formation potential from DOM fractions collected from the La Salle River via the Macdonald Water Treatment Plant raw water intake on July 23, 2015.

Sample	Chloroform (µg/L)	Bromodichloromethane (µg/L)	Dibromochloromethane (µg/L)	Bromoform (µg/L)	Total THMs (µg/L)	Average THM (µg/L)	Standard Deviation
Raw T1	193.3	16.8	8.7	0.0	218.8	222	13
Raw T2	211.5	17.1	7.7	0.0	236.3		
Raw T3	188.3	13.0	9.1	0.0	210.4		
ENV HPO T1	102.0	3.7	1.6	0.0	107.2	113	18
ENV HPO T2	93.5	4.2	1.7	0.0	99.3		
ENV HPO T3	126.5	4.0	3.1	0.0	133.6		
Strata HPO T1	141.3	3.9	1.5	0.0	146.7	125	19
Strata HPO T2	105.4	3.1	2.4	0.0	110.9		
Strata HPO T3	112.3	3.0	2.2	0.0	117.4		
XAD HPO T1	157.3	3.6	2.5	0.0	163.4	152	10
XAD HPO T2	138.4	3.9	1.6	0.0	143.9		
XAD HPO T3	142.0	4.7	1.9	0.0	148.6		
PPL HPO T1	166.5	4.3	0.0	0.0	170.8	152	17
PPL HPO T2	141.0	3.9	1.5	0.0	146.4		
PPL HPO T3	132.5	3.0	2.2	0.0	137.7		
ENV HPI T1	47.4	2.6	1.4	0.0	51.3	42	10
ENV HPI T2	25.2	4.2	2.2	0.0	31.6		
ENV HPI T3	36.2	5.0	1.6	0.0	42.8		
Strata HPI T1	61.6	2.1	1.7	0.0	65.3	39	23
Strata HPI T2	21.7	3.7	0.0	0.0	25.4		
Strata HPI T3	18.9	4.1	1.9	0.0	24.9		
XAD HPI T1	29.7	4.9	23.6	0.0	58.1	40	17
XAD HPI T2	17.5	4.4	1.8	0.0	23.7		
XAD HPI T3	29.1	6.0	2.6	0.0	37.7		
PPL HPI T1	35.0	6.1	2.6	0.0	43.7	42	9
PPL HPI T2	41.1	5.8	3.1	0.0	50.0		
PPL HPI T3	24.5	5.4	2.3	0.0	32.2		

Table 25-A: ANOVA Analysis and Tukey RSD test results for SPTHMP of the HPO fraction collected from the La Salle River via the Macdonald Water Treatment Plant

Sample Date	Sample Location	Fractionation Type	Specific THMFP HPI-DOM (µgTHM/mgDOC)	Specific THMFP HPO-DOM (µgTHM/mgDOC)	One-way Variance for HPO STHMFP (ANOVA)	Tukey HSD Test Results for HPO STHMFP
March 4, 2015	Raw	ENV	4.4	22.9	0.005047	HSD[.05]=4.99 HSD[.01]=7.26 ENV vs Strata P<.01 ENV vs XAD P<.05 Strata vs XAD nonsignificant
March 4, 2015	Raw	ENV	3.2	21.0		
March 4, 2015	Raw	ENV	3.9	25.3		
March 4, 2015	Raw	Strata	5.7	15.6		
March 4, 2015	Raw	Strata	6.3	18.6		
March 4, 2015	Raw	Strata	4.5	16.9		
March 4, 2015	Raw	XAD	6.7	27.2		
March 4, 2015	Raw	XAD	4.3	23.0		
March 4, 2015	Raw	XAD	7.3	26.4		
July 23, 2015	Raw	ENV	6.2	18.6	0.016725	HSD[.05]=3.63 HSD[.01]=4.96 ENV vs Strata nonsignificant ENV vs PPL nonsignificant ENV vs XAD P<.05 Strata vs PPL nonsignificant Strata vs XAD nonsignificant PPL vs XAD nonsignificant
July 23, 2015	Raw	ENV	2.5	17.3		
July 23, 2015	Raw	ENV	3.7	18.8		
July 23, 2015	Raw	Strata	5.3	14.8		
July 23, 2015	Raw	Strata	3.3	12.3		
July 23, 2015	Raw	Strata	4.4	13.2		
July 23, 2015	Raw	PPL	6.6	13.4		
July 23, 2015	Raw	PPL	2.7	17.4		
July 23, 2015	Raw	PPL	2.6	14.2		
July 23, 2015	Raw	XAD	4.7	16.0		
July 23, 2015	Raw	XAD	5.0	14.9		
July 23, 2015	Raw	XAD	3.5	16.8		

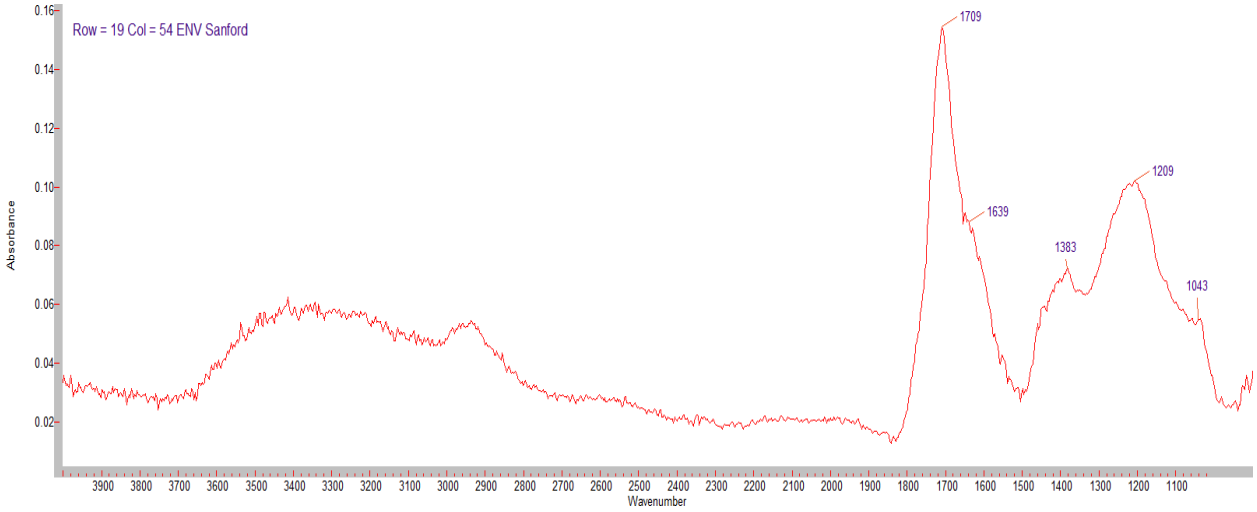

Figure 18-A: FITR spectrum of the hydrophobic fraction isolated from the La Salle River using Bond Elute ENV. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

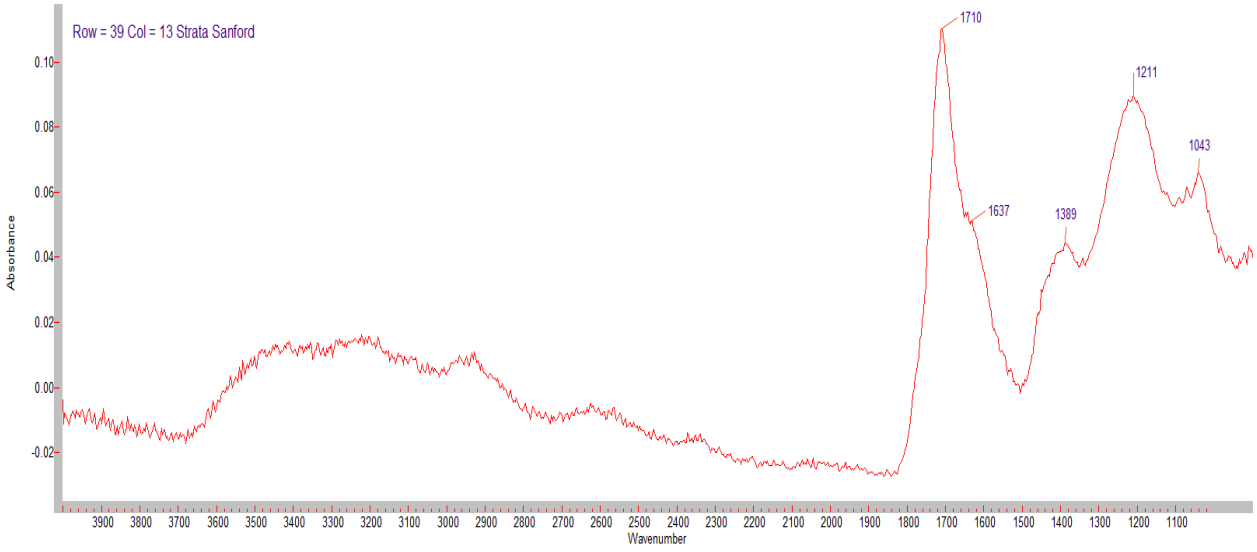

Figure 19-A: FITR spectrum of the hydrophobic fraction isolated from the La Salle River using Strata X. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector.

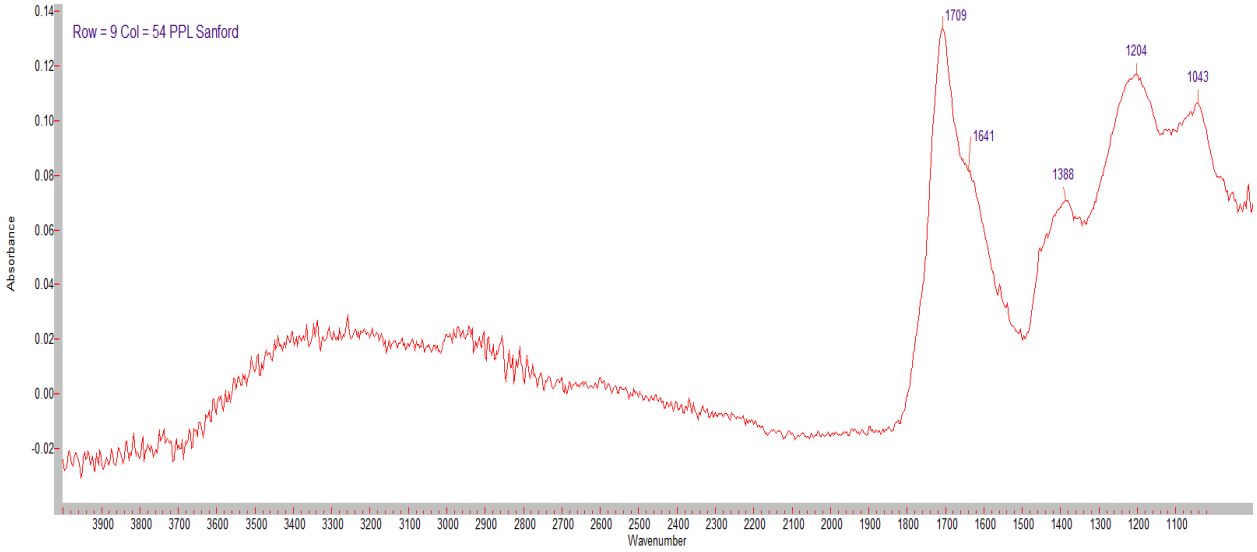

Figure 20-A: FITR spectrum of the hydrophobic fraction isolated from the La Salle River using Bond Elute PPL. FTIR spectrum obtained using the Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 infrared microscope equipped with a Focal Plane Array mercury cadmium telluride (MCT) detector

7. Images of Before and After ATR Touchdown

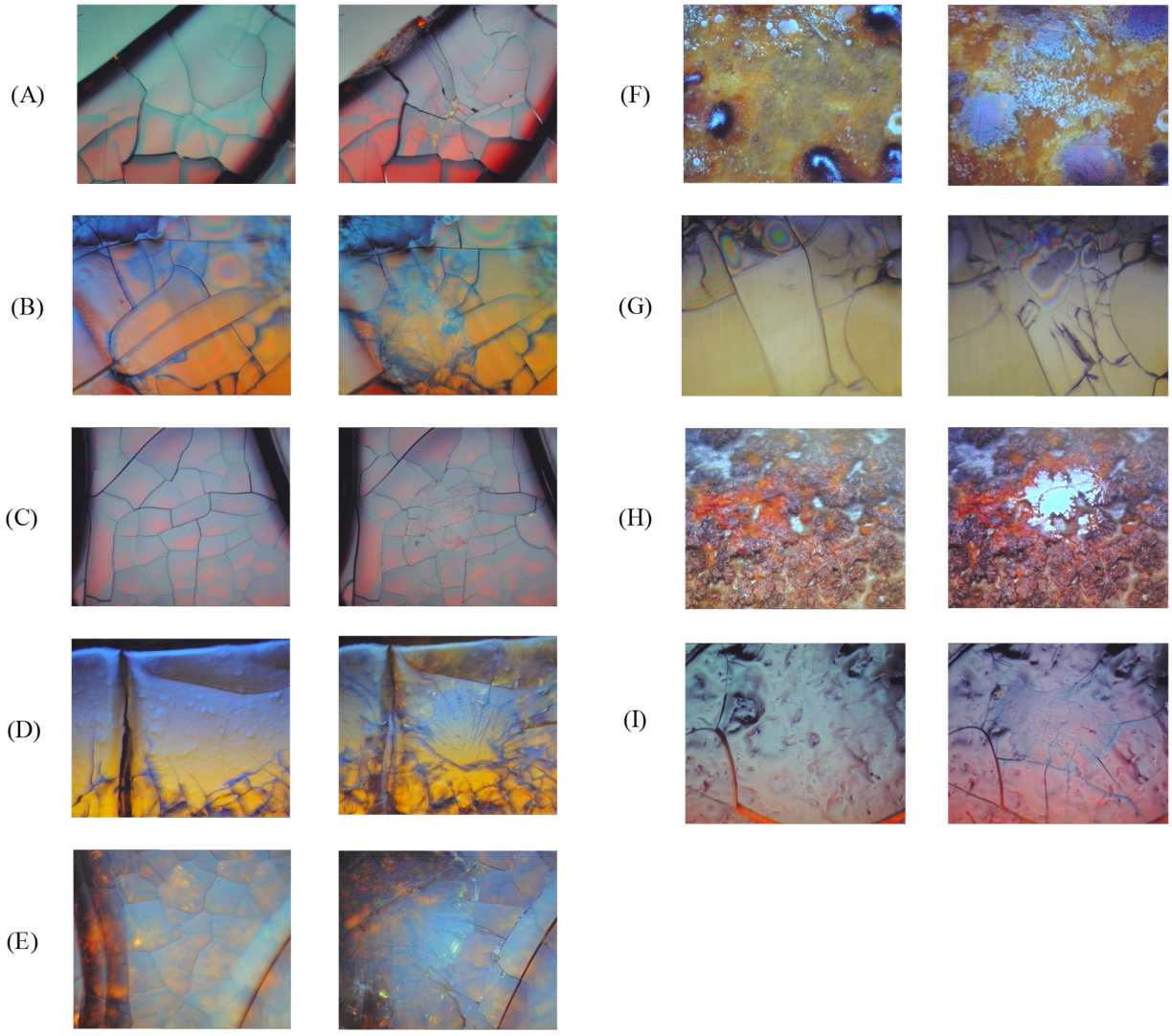

Figure 21-A: Images showing before (left) and after (right) ATR touchdown on microscope slide. Image was collected using the FTIR microscope at 15x magnification. Images are: (A) Bond Elute PPL – IHSS DOM standard (B) Bond Elute ENV – Sanford (C) Bond Elute PPL – Sanford (D) Bond Elute PPL (E) Bond Elute PPL – Waterhen (F) Bond Elute ENV – Waterhen (G) Strata X – Waterhen (H) Bond Elute ENV – IHSS DOM standard (I) IHSS DOM standard dissolved in DI (unfractionated).

Appendix B – SPE Method for Operators

Procedure for Fractionation using Solid Phase Extraction

Basic Outline

The 9 steps below outline the process of fractionation using solid phase extraction (SPE) cartridges. Full descriptions of each step are attached.

1. Collect 1 L of water to be fractionated
2. Rinse a 0.45 micron filter with clean water (either deionized or reverse osmosis)
3. Filter sample through the clean 0.45 micron filter
4. Run 10 mL of methanol through the SPE cartridge
5. Run 1 L of clean water through the SPE cartridge
6. Lower the pH of water to be tested to 2.0 using sulphuric acid
7. Fill a vial with a sample of water
8. Run 1 L of water at pH of 2 through the SPE cartridge (this is the process of fractionating the sample)
9. Fill a vial with fractionated water

Figure 1: SPE cartridge used for fractionation

Water Sample Preparation

Water Collection

Collect at least 1L of water that is to be fractionated, if there is too much water, excess can be poured out later as exactly 1L is needed for testing.

Water Filtration

Prior to fractionation, all water should be filtered through a 0.45 micron filter. Filter 2L of clean water (either deionized [DI] or reverse osmosis [RO]) through the filter before running any of the test water through, this ensures there is not residual organic matter that may contaminate the sample.

Once the filter is clean, pour the 1L of sample through the filtration system. If flow rate becomes slow, gently wiping the filter can help to remove any fouling. Once the water is filtered it is ready to be prepped.

Figure 2: Filtration set-up

pH Reduction and 1st Sample Collection

Collect 1 litre of water that is to be fractionated and insert a pH probe. In order to run the sample the pH must be brought down to 2.0 using sulphuric acid (H_2SO_4). Slowly add drops of concentrated acid until the desired pH is reached. To aid with mixing a stir plate can be used.

At this point fill a vial with the sample and label it with the date and something to indicate that it has not been fractionated like “Non-Fractionated”.

Cartridge Preparation

Methanol Rinse

Each cartridge should be rinsed with 10mL of methanol prior to use, to do this insert the cartridge into a rubber cork and place on top of a glass flask that can be connected to a pump hose. Connect the set up to a pump to pull the methanol through the cartridge. Continue to run the pump for a couple minutes after filtration to allow the cartridge to dry.

Note: the Agilent Bond Elut ENV cartridges only hold 5mL of liquid, so they will need to be filled with methanol, allowed to drain, and then re-filled in order to run 10mL of methanol.

Figure 3: Set up for methanol rinse of the SPE cartridge

DI or RO Rinse

The cartridge must now be rinsed with clean water. Collect 2 liters of clean water (either DI or RO) in a beaker. Place the cartridge in a rubber cork on top of a large Erlenmeyer flask. Insert a cap into the top of the cartridge and a tube into the cap. The tube should run from the cap to the collected water, take care not to bend or kink the tube. Finally attach the pump to the set up and begin running water. The pressure on the pump should be between 10 and 15 psi. Pressure is adjusted by twisting the knob on the right directly above the pressure gauge. Once all water has been transferred the cartridge is ready to run a sample, this process should take about an hour, but does not need to be monitored.

Figure 5: Image of an SPE cartridge, cap, and tube prior to assembly

Ensure that all tubes and corks are well fitted, creating an air tight seal, if there are air leaks, the process will be much slower, or possibly not run at all.

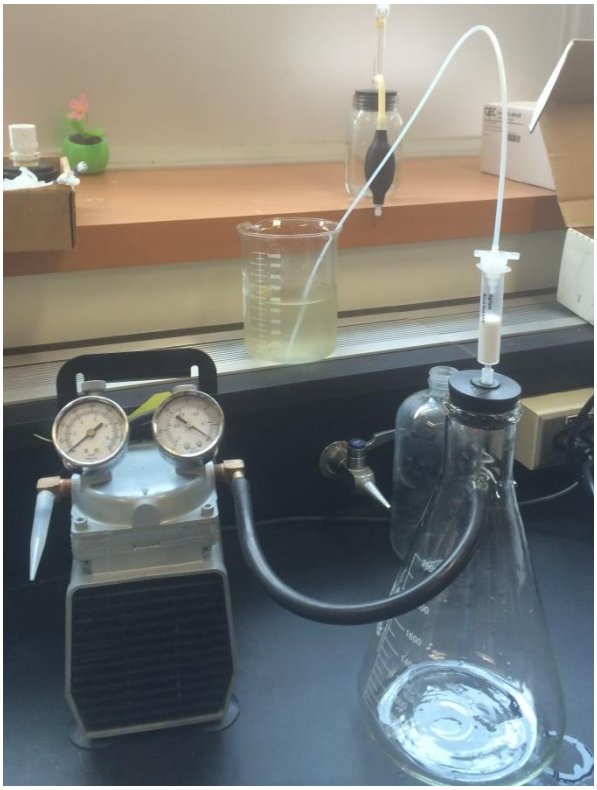

Figure 4: Final set up for DI/RO rinse of SPE cartridge and fractionation process

Fractionating the Sample

The set up for this stage is the same as rinsing the cartridge. Place the tube coming from the cartridge into the sample of water which should have a pH of 2 at this point. Make sure there is no water in the Erlenmeyer flask and turn the pump on to begin fractionation. Maintain a pressure of no higher than 15 psi. This process takes slightly longer than running the set up with clean water but can also be left without observation once set up.

When the process is complete, fill a vial with fractionated water, and label it the same as the previous vial, but this time indicate that the sample has been fractionated.

Place both vials together in a fridge to store until they can be picked up and tested.

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Title: Comparison of XAD macroporous resins for the concentration of fulvic acid from aqueous solution

Author: G. R. Aiken, E. M. Thurman, R. L. Malcolm, et al

Publication: Analytical Chemistry

Publisher: American Chemical Society

Date: Sep 1, 1979

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Charles Goss, M.Sc.
University of Manitoba
Department of Civil Engineering

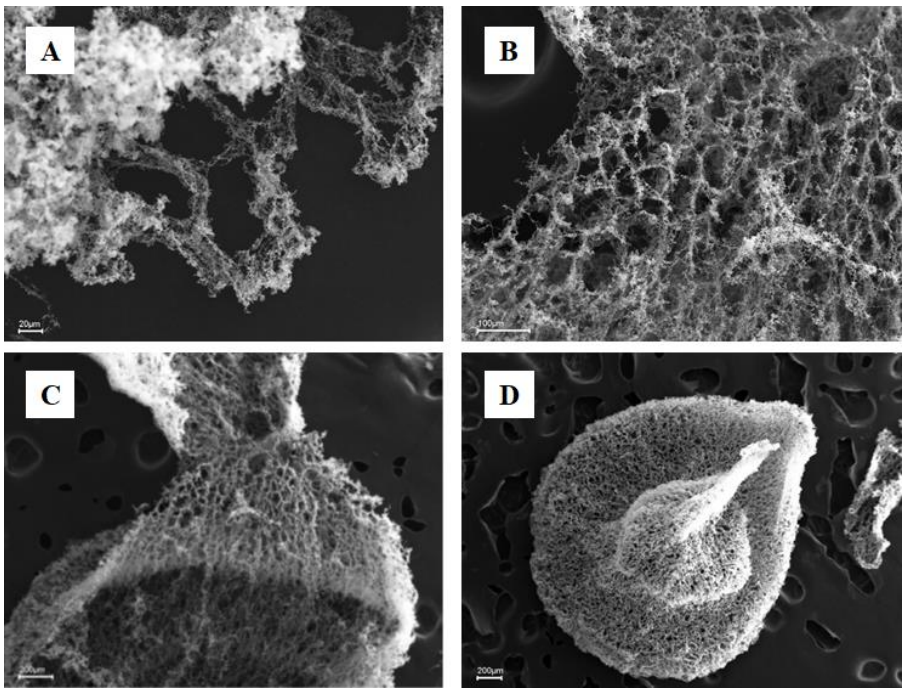

Figure 1: Scanning electron microscope images from of humic acid isolated from lignite showing honeycomb configuration (Images A-C). Humic acid in a cylindrical nanotube membrane configuration, or nanobud, showing a characteristic fishnet structure. Image created from (Tan, 2011(a); Tan, 2011(b))

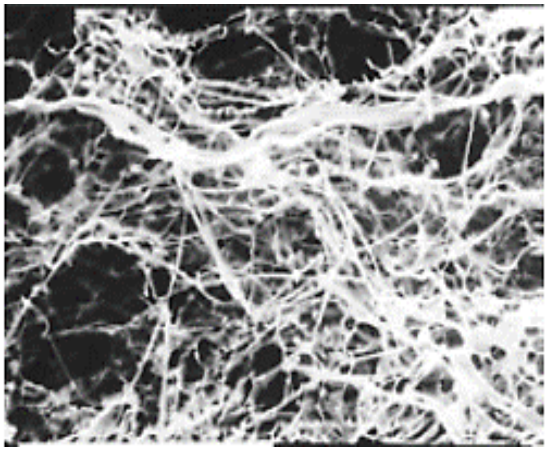

Figure 2: Scanning electron microscope image of a fulvic acid sample collected from Satilla River, Georgia, USA showing carbon nanotube bundles. (Tan, 2011(b))

Shaw Webmail **charlesgoss1@shaw.ca**

Re: New Form Entry: Contact Form

From : [redacted] Tue, Feb 05, 2019 11:48 AM
Subject : Re: New Form Entry: Contact Form 1 attachment
To : Budi Tan <buditan@hotmail.com>

Hello Mr. Tan,

Yes I did some follow-up trying to find current contact information for your father and found out of his unfortunate passing. I was in contact with your father a year and a half ago regarding his work and I feel very privileged to have had discussions with him regarding his research. The figures I would like to use are from documents he had on his website (https://drkhtan.weebly.com/). I attached a letter requesting permission to use the figures and provided the figures themselves to show him what figures I was intending to use.

I would like to send my deepest condolences to you and your family regarding the passing of your father.

Thank you kindly for taking the time to respond to me regarding this matter.

Charles D. Goss, M.Sc.

[redacted]

From: "Budi Tan" <buditan@hotmail.com>
To: "charlesgoss1[redacted]
Sent: Tuesday, February 5, 2019 11:32:57 AM
Subject: Re: New Form Entry: Contact Form

Hi Charles - Dr. Tan (my father) passed away last April.

I am administrator of his estate - so I give you permission to use the figures you mentioned below one time.

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Budi Tan

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Thank-you,

Charles Goss

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If you do not control the copyright on the above-mentioned work, I would appreciate any contact information you can provide regarding the proper rights holder.

Thank you for your consideration. If you require further information, please don't hesitate to contact me.

Sincerely,

Charles Goss, M.Sc.
University of Manitoba
Department of Civil Engineering

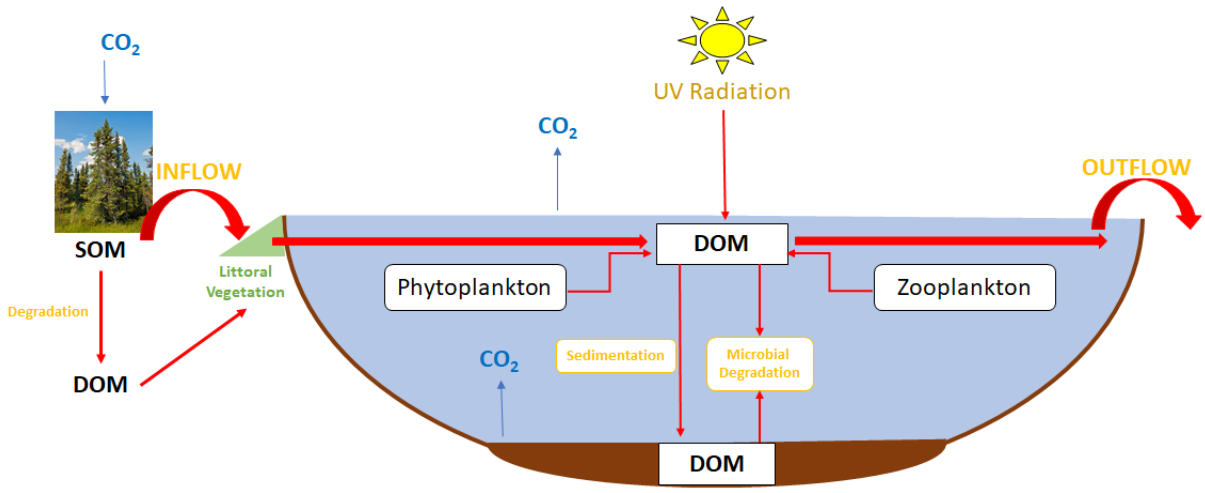

Figure 1: Flux of DOM in a fresh water lake. Diagram recreated from (Tulonen, 2004).

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Sent: Tuesday, February 5, 2019 1:04 AM
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Subject: RE: request for permission to use a figure from your PhD thesis

Dear Charles Goss,

I'm pleased that you have considered to use my figure in your thesis and certainly I give the permission to use it.

Best wishes

Tiina Tulonen

From: [redacted]
Sent: 4. helmikuuta 2019 22:04
To: Tulonen, Tiina V <tiina.tulonen@helsinki.fi>
Subject: request for permission to use a figure from your PhD thesis

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8.5 The licensing transaction described in the Order Confirmation document shall be governed by and construed under the law of the State of New York, USA, without regard to the principles thereof of conflicts of law. Any case, controversy, suit, action, or proceeding arising out of, in connection with, or related to such licensing transaction shall be brought, at CCC's sole discretion, in any federal or state court located in the County of New York, State of New York, USA, or in any federal or state court whose geographical jurisdiction covers the location of the Rightsholder set forth in the Order Confirmation. The parties expressly submit to the personal jurisdiction and venue of each such federal or state court.If you have any comments or questions about the Service or Copyright Clearance Center, please contact us at 978-750-8400 or send an e-mail to info@copyright.com.

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