Improving characterization of natural organic matter using high resolution techniques in peat catchments

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ABSTRACT OF THESIS submitted by: Dany AVALOS LLIGUIN for the degree of Master of Science and entitled: Improving characterization of natural organic matter using high resolution techniques in peat catchments Month and Year of submission: June, 2017.

Natural organic matter (NOM) is a complex mixture of organic compounds and plays a major role in water chemistry. The aim of this research is to improve the method for characterizing natural organic matter in peat catchments with spatial-temporal variation by combining particle separation techniques with ultraviolet-visible (UV-vis) spectroscopy and pyrolysis chromatography-mass spectrometry (Py-GC-MS). The analysis has been done in water samples collected in eroded and uneroded upland peat catchments under low and high discharge events to study the physical and chemical composition of NOM and determine which characterization technique deliver higher resolution results.

Laboratory analysis showed a positive correlation between high discharge events and increments of organic carbon (OC) concentrations. The results showed that combining Tangential flow ultrafiltration, total organic carbon analysis and UV-vis at 254nm delivered accurate particle size separation of OC which enhanced chemical characterization of NOM. Py-GC-MS was the most efficient method to determine chemical composition of NOM and recorded spatial and temporal variations in uneroded and eroded samples. The highest OC concentrations were recorded in the biggest particles sizes and the main organic compounds found in water samples were aromatics and polyaromatics and soil lipids.

Keywords: natural, organic, matter, characterization, resolution, techniques

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1. Introduction

The chemical composition of freshwater ecosystems largely relies on in-situ processes, ex-situ supply of materials, discharge and shifting factors such as climate (Dillion and Molot, 1997). Natural organic matter (NOM) is a major component of freshwaters; NOM is a complex mixture of organic compounds mainly composed by humic and fulvic materials which have the ability of interact with organic and inorganic pollutants, consequently they control water chemistry (Kim and Yu 2005); soil water chemistry; and mobility of soluble elements such as nutrients and toxic metals (Dillion and Molot 1997). The dissolved fraction of organic matter (DOM or DOC) has been defined as a complex heterogeneous mix of macromolecules that arise in surface water (McDonald et al. 2005) and has an influential role in water chemistry, because DOM's numerous weak acids, and properties of reduction and oxidation have the capability to complex toxic metals; act as a pH buffer; and control partitioning of organic materials between water and sediments (Worral et al. 2002). Traditionally, NOM has been characterized by low resolution analysis of particle size distribution (PSD), however, these techniques deliver limited information about NOM's physical properties, therefore, to understand the key role that NOM plays in water chemistry there is a requirement to improve the methods to characterize NOM. Improvement in resolution of physical and chemical properties of NOM requires suitable resources and technology. Selection of techniques used for this process is important as each technique identifies some of the main chemical compounds found in NOM making resolution a key relevant fact when for characterization of NOM. This information can be used to improve resolution of physical and chemical properties of NOM.

Peatlands have been defined as unbalanced ecosystems with high content of organic materials and low rate of decomposition, this results in carbon-rich soils which become the main source of dissolved organic carbon (DOC) from land to freshwater and marine ecosystems (Gorham 1991). Peat reservoirs play a major role in the carbon cycle, DOC in freshwater systems accounts for one of the largest source of active organic carbon (OC) on earth, predicting changes in DOC composition in peat catchments can provide important information regarding possible changes in the biogeochemical carbon cycle and subsequently on the ongoing

process of climate change (Fallowski et al. 2003). In the United Kingdom (UK), upland peatlands have the highest concentrations of carbon; disruption on its vegetation can lead higher decomposition rate and production of loosely bound NOM which is flushed into rivers or catchments and ultimately change the dynamics of DOC in water (Worral and Burt, 2004). Eroded peatlands assimilate the conditions of these types of habitats. Climate change and anthropogenic activities over the past few decades have increased the percentage of eroded peatlands. Comparing differences of NOM composition in peatlands is fundamental because it would enable to gain information about the past and the present as uneroded peatlands have been classified as a healthy environment that do not suffer disruption in their ecological processes; this information will enforce understanding of the role of NOM and predict future changes in uneroded peat catchments. Even though the larger percentages of upland peat catchments in the UK are eroded very little research has been done in these areas (Tallis et al. 1997).

Dissolved organic carbon (DOC) is ubiquitous and highly reactive to environmental alterations; these reactions can cause changes of DOC composition and structure (Hutson et al. 2007). High discharge events lead to changes in NOM and DOC concentration. Previous studies have shown that DOC increase 36 up to >50 % during short periods with high intensity rainfall, this increase can account from 31 to 66% of the annual DOC export (Inambar et al., 2006). Even tough, high flow volume increases the total mass of DOC in short periods, this process can have long term environmental implications as abrupt DOC increase could change water chemistry, pollute rivers and increase CO₂ emissions (Billet et al., 2006). Thus, it is necessary to determine the carbon concentration changes, size and composition due to discharge events in eroded and unroded peat catchments because it would enable to record the significant differences in DOC quality caused by the effects of peat and discharge on NOM. However, discharge events happen throughout the year and often times it is difficult to measure them due to inaccessibility to collection sites or other reasons that resulted from such climatic events. Because large proportions of discharge pass during these events, estimating unsampled discharge is necessary to monitor organic carbon and therefore determine with accuracy the implications of discharge events in NOM.

Characterization of NOM relies on its origins, space and time; they influence changes in NOM due to the high reactive nature of DOC. Grieve (1990a) have stated that there is a positive correlation between discharge and changes in concentration of DOC. Despite the improvements in techniques to characterize NOM, because of the complexity and heterogeneity of NOM most techniques used for characterization of NOM generate low resolution results. Therefore, the aim of this research is to improve the method to characterize NOM in eroded and uneroded peat catchments during low and high discharge events by combining particle separation techniques (low resolution, functional) with high resolution techniques to improve resolution in results, make resolution relevant by generating more accurate qualitative and quantitative data regarding the physical and chemical properties of NOM, and record the significant differences that are caused by the influence of spatial-temporal variation in NOM during discharge events in peat catchments to better understand its implications in NOM composition. The findings in this research will be of great value for future environmental applications that use NOM binding capabilities with other chemical elements for water purification treatments, and metals such as Fe and Al to control solubility of trace metals.

1.1 Characterization of natural organic matter

Characterization of NOM requires the isolation of DOC from freshwater systems. The dissolved fraction of organic matter has been referred with terms DOM and DOC as previously mentioned, while there is a distinction between the two terms, often times they are used interchangeably (Allan 1995). DOM is measured by chemical oxidation methods whereas DOC commonly, uses combustion in samples through automated carbon analysers (Kalbitz et al. 2000). DOC has been defined operationally by the organic matter that filters through a 0.45 µm filter, nevertheless, this method is considered as inadequate due to the removal of colloidal species and the compromise of flow rate and rejection of clay minerals (McDonald et al. 2004).

DOC is constituted of two parts. First, the non humic fraction, a group of biomolecular compounds that include amino acids, proteins, waxes, resins, lipids, polysaccharides and carbohydrates (Piccolo, 2001). Second, the humic fraction, a mix composition of refractory organic substances with high molecular weight with a

range of yellow to black colors (MacCarthy et al. 1990). These macromolecules are important for water chemistry because its abundance and reactivity (Thurman et al. 1982).

The most influential component of DOC is the humic fraction, also known as humic substances (HS), they account from 50 to 75% of DOC in water and is ubiquitous in sediments, soil and water; according to its solubility is categorized into three components: humic acids (HA), fulvic acids (FA), and humin (HU). Humic acids can made up 90% of coloured surfaced water, is the main component of NOM in peat catchments and is a complex mix of phenolate and carboxyl groups (Hessen and Tranvik 2013). Humic acids are not soluble at pH below 2, but soluble at higher pH; its MW ranges from 1500 to 5000 Da and high oxygen contents. Fulvic acids can comprise 50% of groundwater, FA are soluble at all pH levels and its MW ranges from 600 to 1000 Da. Humin is not soluble at any pH level (Malcom et al. 1990). This complex heterogeneous mixture of non-humic and humic materials makes accurate characterization of NOM challenging. Thus, separating humic acids, fulvic acids and humic by size from peat catchments samples that vary in space and time is the first step to understand the chemistry of HS and its influence in the degradation process of NOM.

1.1.1 Techniques for characterization of natural organic matter

There plenty of methods for characterization of NOM, however, most methods present limitations. XAD resin fractions DOC to classify organic solutes per their hydrophilic and hydrophobic fractions (Swietlik et al, 2004). However, the strong acid conditions of this method could result in uncontrolled fractioning (Leenheer, 1981). Tangential-flow filtration (TFF) uses membranes of from 0.2 um to 0.65 µm to separate molecules by particles. Still, there are some limitations for this method as fluids which could contain more molecules tent to remain in the membranes after filtration. High performance size exclusion chromatography (SEC) traditionally uses UV detection to isolate molecular weight distribution of particles by size. Nevertheless, the main issue of SEC is the undefined standards to calibrate the column which can over estimate MW of HS by a factor of 5 or more (Peuravuori, 1999). Hence, this method produce limited information when it comes to analysing

small MW that includes organic carboxylic acids (Kim and Yu, 2005). Although the techniques mentioned above apply well developed technology, they only generate partial or incomplete information and inaccurate data of NOM characterization.

The combination of particle separation methods (low resolution analysis) with high resolution techniques has been accepted as appropriate methods to assess the physical and chemical properties of NOM (Maurice et al., 2002). In order to determine the physical properties of NOM is necessary to separate its organic carbon by fractions. Then the total organic carbon from each fraction need to be measured, this is done through the use of a total organic carbon (TOC) analyser (Danielsson 1982). Selecting the most appropriate high resolution techniques is one of the keys to improve resolution because they could deliver a detailed list of the NOM's chemical compounds. Therefore, for this research to effectively assess the spatial and temporal variations and the influence of high discharge in characterization of NOM, first of all, fractions of NOM will be isolated by size pressure filtration and tangential flow ultrafiltration (TFU), followed by TOC measurement of all different fraction sizes. Secondly, pyrolysis chromatographymass spectrometry (Py-GC-MS) is considered to be state of the art technology that generates higher selectivity, sensitivity and resolution of chemical properties of NOM and therefore will be used as the main high resolution technique. Additionally, ultraviolet-visible spectroscopy by E4/E6, E2/E3 ratios and SUVA₂₅₄, which is considered as a surrogate high resolution technique will also be used to determine the chemical properties of NOM; this will allow comparison of resolution between both techniques and determine the reliability in resolution of Py-GC-MS for characterization of NOM.

1.1.2 Tangential Flow Ultrafiltration

Ultrafiltration is a method that refines particle size separation to a finer scale (Quynh 2009). TFU is also known as cross-flow and uses membranes in which solvents and solutes that are smaller the membrane filters tangentially through and isolate DOM according to specific pore size fractions when hydrostatic pressure is applied leaving a retentate and a filtrate than can be reused to refine to a lower particle size by repeating this process with a smaller membrane pore size (Everett and Chin 1999).

Moreover, TFU aids mitigation of distortion of shape and coagulation of particular matters (Buffle and Leppard 1995).

TFU is considered a fast and efficient method to isolate organic and inorganic materials from freshwater because it uses various membranes of different ranges (i.e. 0.2 μ m, 10 KD) rather than the standard 0.45 μ m filter. Benner et al. (1997) stated that molecules that are retained are assumed to have higher molecular weight than the membrane pore size; this makes possible to recover DOM within a specific pore size rich in organic carbon content and then make DOM available for chemical characterization. HA has molecular size of about 100 KD and FA of under 10 KD, consequently organic carbon that filters through the 0.2 μ m may be HU instead of HA, using ultrafiltration in a range of >0.2 μ m to <10 KD complies with all the requirements to isolate HA by PSD.

TFU could have similar limitations than the TFF even though TFU uses membrane pore sizes that refine fraction size to a much smaller scale, DOM molecules could potentially remain in the membrane and subsequently this can lead to inaccurate measurement of TOC of the fractions size. An innovative method that is applied in this research to prevent miss measurement of TOC is to measure retentate through ultraviolet-visible spectroscopy at 254nm wavelength (UV₂₅₄) after short intervals, this will allow calculation of HA to the point where HA constant mass is achieved to ensure complete fraction size isolation and accurate TOC measurement and therefore improvement in resolution PSD separation techniques.

1.1.3 Ultraviolet-Visible (UV-vis) Spectroscopy

UV-vis spectroscopy is a cheaper and simpler method to describe the molecular properties of HA (Shrishova et al. 2006). UV-vis spectra of HA is difficult to measure because HA are featureless, broad and decrease monotonously as wavelength increases, nonetheless there is a range in which UV-vis regions and absorbance of spectra could be utilized to detect some absorbing groups and therefore to analyse HA (Uyguner and Bekbolet 2005). Mot research and studies have tested absorbance at 254 nm showing this wavelength as the most suitable for examining HS spectra, consequently absorbance at 254 nm has been considered as a surrogate for DOC

due to the dominance of aromatic HS in freshwater and to a lesser extend visible in these absorb light in the UV (Edzwald et al. 1985). For this research, UV-vis at different absorption wavelengths will be determinant in improving physical and chemical characterization of NOM because of its close link to DOC just as it was explained in the above paragraph.

1.1.4 Total Organic Carbon

Total organic carbon supplies quantitative data of NOM, the data is a key component to determine qualitative information regarding characterization of NOM and further combination with other techniques. A total organic carbon (TOC) analyser measures continuous flow and calculate the amount of total organic carbon from freshwater which can be used as an indicator of water quality (Leenheer and Croue, 2003). A traditional measurement of TOC requires recording the total amount of carbon present in the sample and the inorganic carbon (IC) and then subtracted it from the total carbon (Wang and Anderson 2008).

1.1.5 E4/E6, E2/E3 and SUVA₂₅₄

A humification index is an important tool use in this research to characterize the humificaiton process that closely connects the rate of transformation of NOM, development of HS with parameters that describe chemical properties of formed materials (Lu et al. 2001).

The relation between absorbance at 465 nm and 665 nm is defined as the E4/E6 ratio and can be used as humifcaiton index that correlates to the percentage of aromaticity and extend of condensation (Kononova 1966). A low E4/E6 indicates a high degree of aromaticity, particle size and molecular weight, but the presence of low quantities of aliphatic structures (Uygyner et al. 2004). However, Chin et al. (1994) stated that E4/E6 ratios could be correlated to percentage of carbon, oxygen and acidity and the quantities of the COOH group. E4/E6 ratios have established to be smaller than 5 for HA and within the range of 6 – 8.5 for FA with higher aromaticity in HA compared to FA (Chen and Schnitzer 1977). Kukkonen (1992)

determined that E4/E6 ratios should be with the ranges of 3.8 - 5.8 for HA and 7.6 - 11.5 for FA.

The E2/E3 ratio uses absorbance at 254 nm and 365 nm and has been used in studies showing correlation of molecular size and aromaticity (Peuravuori et al. 1997). Other studies show that E2/E3 ratio have correlation of humic solutes and molecular weight consequently if molecular size increment, the E2/E3 ratio will decrease because particle molecules with high molecular weight requires stronger long absorption at longer wavelength (Doubbs et al. 1972; Peuravuori and Pihlaja 1997).

Specific UV absorbance (SUVA) is a measurement of absorbance taken from a 254 nm wavenumber and then divided by DOC of the solutus (Edzwald et al. 1985). SUVA 254 measures aromaticity and has a positive correlation with hydrophobicity; freshwater with high SUVA is an indicator of high hydrophobic NOM, for instance, HS (Edzwald and Tobiason 1999). SUVA of >4 values are rich in hydrophobic and aromatic material (Ilina et al. 2014)

1.1.6 Pyrolysis Chromatography-Mass Spectrometry (Py-GC-MS)

Pyrolysis gas chromatography-mass spectrometry degrades DOC into small components which could be related back to the structural information of the original source-sample and to the degradation process and in some ways it could be considered a structural fingerprint (Mc Donald et al., 2005). Py-GC-MS is a very powerful technique because of its capability to separate, identify, relative quantify individual chemical compounds and complex chemical mixtures of NOM (Kozyatnyk et al. 2016). Consequently, Py-GC-MS can be used to determine very detailed information due to its high sensitivity which allows detecting minor compositional changes at the molecular level and therefore highly improving resolution in characterization of chemical properties of NOM (Parsi et al. 2007)

Py-GC-MS uses controlled thermal degradation from NOM samples to breakdown large complex DOC molecules into small fragments which then are paired with gas chromatograph column for separation before final identification by the mass spectrometer (Vancampenhout et al. 2009). Schulten and Gleixner (1999) used Py-GC-MS to analyse lake water and HS and determined that chemical compounds such as peptides, lingins, aromatics, lipids, phenols, sterols, nitrogen and suberins were found.

Even though Py-GC-MS is one of the most efficient techniques for characterization of NOM sample preparation requires accuracy to isolate sediments form the bulk of water. A widely accepted method for isolation and concentration of NOM in Py-GC-MS analysis is physical scraping and collecting NOM from freeze dried samples (Croue 2004). However, this method involves a long process and does not ensure accuracy of sample collection because a considerable amount of organic materials from the freeze dry sample could be left behind during scraping and collecting process. A novel method studied by Faster et al. (1998) showed the efficiency of the application of methanol as an extraction solvent for organic residues in dry freeze samples. Therefore, comparing the results in freeze dried samples using scraping and dissolution of methanol as Py-GC-MS analysis methods would enhance higher quality results for characterization of chemical properties of NOM and consequent improvement in resolution.

Py-GC-MS application and analysis is long process that starts with the sample collection, preparation of samples and finishes with the application of the Data Analysis software for GC-MS analysis, processing data and final statistical reports. Science relies in quantitative methods that depend in repeated experiments or observation. Additionally, environmental reports are required to provide evidence that a result can be reproducible in the long term (Vaux *et al.* 2012). Standard error (SE) determines the accuracy in which the samples can deviate from the population mean. However, because of the difficulty in sample processing, analysing and reporting a variability analysis from the same sample with a second inter-operator will be conducted to assess the differences in data interpretation and evaluate how significant these differences could influence the results.

1.2 Objectives

The following objectives have been stablished to comply with the aim of the research and have been set to follow the research step by a step in a chronological order:

- 1. Collecting samples from eroded and uneroded peat catchments during low and high discharge events.
- Determine the significant differences using low resolution analysis, including testing the consistency of general classification based particle size distribution.
- Determine whether differences are apparent when using E4/E6, E2/E3, SUVA₂₅₄ and pyrolysis chromatography-mass spectrometry.
- 4. Conduct a variability analysis between inter operators to validate chemical characterization data.
- 5. Determine which method for sampling Py-GC-MS is more efficient by showing differences in resolution of organic coumpounds.
- 6. Conduct a Py-GC-MS analysis of chemical composition of OC
- Determine if the differences are more apparent when using a combination of particle distribution analysis and subsequent high resolution techniques analysis.
- 8. Relate the observed changes to environmental processes.

1.3 Research Background

The Crowden Great Brook is a peat upland study area suitable for this research due to its soil and hydrological physiochemical conditions. Located in the Peak District National Park, the study area comprises 7 Km², 70% of which are peatlands. Previous studies have measured a 0.5 Km² eroded surface and 3 Km² of uneroded surface that have direct connection to sub-catchments scattered across the study area (Phai 2012). Elevation of the selected study sites range from 220 m to 550 m.a.s.l. (Todman 2005).



Figure 1 Crowden Great Brook location

The Crowden Great Brook hydrology is influenced by two contrasting subcatchments. The first one is surrounded by extensive bare peat patches result of an ongoing erosion process (Evans et al. 2006). The second a uneroded catchment enclosed with vegetated peat that includes bog-forming *Sphagnum sp*. (Gaffney et al. 2008). The sub-catchments flow into Black Hill where they feed a stream which outflows into the Torside reservoir, a reservoir of the Longdendale chain reservoir that supplies East-Manchester with 102 mega litres of drinking water a day. Because of similarity in topography sample collection from the uneroded peat catchment (site 30) and eroded peat catchment (site 50) shown in Figure 2 comply with the environmental conditions needed to compare characterization physical and chemical properties of NOM with spatial variations. Moreover, sample collection under low (base flow) and high discharge event conditions in site 30 ND 50 would allow comparison of temporal variation of NOM.

Source: Google Maps (©2017) data Tele Atlas, GeoBasis-DE/BKG (2009); Google Earth (©2017), GeoBasis-DE/BKG (2009)



Figure 2 Map of Crodwen Great Brook, Location site 30 and site 50 Source: Edina Digimap, Ordnance Survey (©2017), adapted from Todman (2005)

2. Materials and Methods

2.1 Collection of water samples

Samples were collected from site 30 and 50 on November 2nd 2016 at the Crowden Great Brook and transported for storage at the University of Manchester labs. For base flow samples a 5 L polypropylene bottles were used for manual collection In each catchment. For discharge events, a float switch with a data logger for measurements of electrical conductivity (EC) and a Sigma SD900 auto sampler was placed on site 30 with grid coordinates SE 06172 02965 and site 50 with grid coordinates SE 05650 00943 for collection samples and recording of data. The float switch was placed at a specific height and was activated once the water levels increase during discharge events collecting samples in hourly basis with maximum capacity storage of 24 bottles. Nonetheless, the water level needs to remain above the specific height otherwise water sample collection would stop automatically.

Samples for discharge events were collected in 500 ml polypropylene bottles. All bottles were pre-washed with HON₃ at 10% analytical grade from Fisher Chemicals, UK).



Figure 3 Sigma SD900 auto sampler for high discharge events and illustration of float switch and data logger

Samples for site 30 were auto collected by the Sigma SD900 on September 30th, 2016 starting at 15:22 hours until 14:22 on October 18st. In site 50 auto sample collection was divided into two events: the first one, started on October 18th from 9:34 hours until October 19th at 2:38 hours; the second one, the process initiated on October 28th at 03:48 hours and ended at 17:42 hours. A total of 12 samples were collected in each discharge event.

2.2 Determination of the discharge

Discharge (Q) was determined by the application of the salt dilution gauging (Wood and Dykes, 2002). This technique uses pre weighted sodium chlorine (NaCl) as conservative water tracer to determinate its EC. In order to calculate the value of the discharge measurements between results from EC, mass of tracer and time need to be combined. For measurement of the parameters a four pole EC probe, set with a range of measurement of 0 to 2500 μ S/cm, pre-calibrated with at a known concentration of NaCl standard needs to be connected to a Sentry II data logger. For

recording data the Sentra II logger was set to log at two seconds intervals and then placed into the peat catchments to record the initial EC. Next, 400 g of NaCl was realised into the catchments approximately 15 m upstream form the EC reading point in an area where water flow is constant to prevent longer flow. This process would continue until EC returns to the initial reading. The following equation uses EC value, mass of NaCl and time from the logger to calculate the discharge:

$$Q = \frac{m}{A}; A = (t * m) - (t * b) * n$$

Gauging equation where Q = discharge (L/s); m = mass of NaCl (g), A = area under the curve; t = time interval (s); b = baseline (g/L); n = number of data points

2.3 Determination of the unsampled discharge

It is important to determine the unsampled discharge because of the long lasting influence of increments of DOC due to high discharge events in freshwater. Studies in the Great Crown Brook over a number of years were carried out by Todman (2005), Gafney (2008), Do (2012) and Rae (2014). These studies used data from high discharge events to create a stage discharge curve from which a flow duration chart was created. Through calculation of the area under the curve above the maximum discharge from prior research it was possible to estimate the amount unsampled annual discharge. Figure 5 shows for site 30 (120 l/s) and 12 l/s for site 50 (Rojas 2016).



Figure 4 Flow duration chart with the area under the curve used for calculation of unsampled discharge for site 30 and site 50

2.4 Tangential Flow Ultrafiltration

Pressure filtration was used to filter all original collected water samples through 1.0 μ m cellulose nitrate membrane (Whatman, UK). The filtrate from these samples was size separated into different size fractions using TFU membrane plates of 0.2 μ m, 100 KD and 10 KD pore size cut-off. TFU was performed on a Vivaflow 50 from Viva Science, UK and Sartorius masterflex pump head.

An initial 1000 ml of base flow sample was used for TFU for sites 30 and 50. For discharge samples 1280 ml was and 1200 ml was use for site 30 and 50 respectively. Discharge samples for both sites were prepared mixing a set of 3 subsamples collected from the Sigma SD900. The criterion to select the sub samples was based on samples that registered highest TOC concentration and availability after pressure filtration at 1.0 μ m. For site 30 subsamples 8, 9 and 10 and for site 50 subsamples 4, 5 and 6 were selected.



Figure 5 Viva system 50 and master-flex pump

TFU started at 0.2 µm membrane plate, the remaining filtrate went through the 100 KD plate and the same process was applied to the 10 KD membrane plate. As explained in section 1 UV₂₅₄ was measured from the retentate after filtrating volumes that ranged from 500 ml – 800 ml to determine the end point were HA constant mass was achieved (Figure 7). However, this is a long process that requires large volume of sample, consequently 15M Ω deionized water (DIW) at the same know volumes that went through filtration was added until fraction size separation was completed. The great addition of DIW resulted in dilution of the original sample and therefore results measurements need conversion in order to obtain premier concentration. Figure 5 shows a diagram of the TFU process.

After TFU each size plates, samples were taken from the retentate and the last filtrate of the 10 KD plate for measurement of TOC, and UV-vis. Thus, four NOM size fractions were obtained: <1 μ m, >0.2 μ m; <2 μ m, >100 KD; <100 KD, > 10 KD; and <10 KD.



Figure 6 HA mass and TFU running time process to achieve complete fraction size separation, measured at UV254 from retentate

2.5 Total Organic Carbon

After fraction sizes separation, 20 ml of retentate samples from 1 μ m; <1 μ m, >0.2 μ m; <2 μ m, >100 KD; <100 KD, > 10 KD; and <10 KD fractions sizes were stored in universal tubes with name tags for TOC analysis.



Figure 7 samples from site 30 (4, 5, 6) and site 50 (8, 9, 10). 20 ml stored in universal tubes for TOC analysis at 1 μ m; <1 μ m, >0.2 μ m; <2 μ m, >100 KD; <100 KD, > 10 KD; and <10 KD fractions sizes

The total carbon analyser (Shimmazu, Japan) was used to obtain the TOC concentrations. Error in the Shimmazu total carbon analyser is set to ± 0.1 mg/l, consequently when the concentration of TOC shows very small difference between the retentate and the filtrate this could result in apparent negative concentration of TOC in the size fraction. Errors that fall within this range have been set to cero. Figures 12, 13 and 14 show cero values due to error in TOC analysis.

2.6 Ultraviolet-Visible Spectroscopy

UV-vis measurements were taken with UVvis analyser BioMateTM 3 in all base flow and discharge samples for all 5 size fractions: 1 µm; <1 µm, >0.2 µm; <2 µm, >100 KD; <100 KD, > 10 KD; and <10 KD. Wavelengths at 254 nm (Abs²⁵⁴) divided by 365 nm ((Abs³⁶⁵) and 465 nm (Abs⁴⁶⁵) divided by 665 nm (Abs⁶⁶⁵) were used to determined E2/E3 and E4/E6 ratios respectively; SUVA₂₅₄ was calculated by multiplying the last UV₂₅₄ measurement taken from retentate of the 5 size fractions and multiplied by 100 and divided by the TOC of each size fraction (UV_{254} *100/TOC). Humification indexs (E4/E6, E2/E3, SUVA₂₅₄) were used to assess characterization of chemical properties of NOM.

2.7 Pyrolysis Gas Chromatography-Mass Spectrometry

Samples from size fractions <1 μ m, >0.2 μ m; <2 μ m, >100 KD; <100 KD, > 10 KD; and <10 KD were placed in 1L freeze resistant glass vilas in a measurement of 150 ml for freeze drying in the Edwards, K4 Modulyo Freeze-Dryer. 4L of liquid nitrogen was measured in a 5L glass dewar, samples were then dipped into the dewar. To reduce freeze drying time samples the vials needed to keep turning to increase ice surface area. Freezing samples took 90 minutes and 2.5 days to complete freeze drying. Upon completion samples were taken out with a clean metal spoon for every sample and stored in 20 ml vials at 5 °C.

Two methods were applied for sample packaging:

- Freeze dried samples 11 and 12 from site 50 from high discharge events were scraped with a metal rod and collected in a metal spoon. 10 mg of freeze dried samples were weighted for further Py-GC-MS analysis.
- Methanol at a known measure was used as dissolvent for the remaining freeze dried samples. After further evaporation of methanol had taken place, 10 mg of NOM was weighted for additional Py-GC-MS analysis.

Samples 1 and 2 were used to mark the beginning of the discharge events. Samples 11 and 12 determined the end of discharge event for site 50, while samples 23 and 24 marked the end of discharge in site 30. Base flow samples are constant.

Scraped and diluted samples were placed into a pre-combustion quartz and pyrolysed by heating 720 °C for 20 seconds using the Chemical Data Systems 5200 Pyroprobe. The fragments that were liberated went through further analysis using the Aligent 7890A Gas Chromatograph fitted with a Zebron ZB – 5 MS column (30 m length, inner diameter 0.25 mm, film thickness 0.25 μ m) and paired to a Aliegant 5975A MSD which was operated with electron ionization mode with a scanning m/z of 104 50 – 600 at 2.7 scans s-1, with ionization energy 70 eV and a solvent delay of

1minute (Rojas 2016). Lines for pyrolysis transfer and injection were set a temperature of 350 °C, heated interface at 230 °C and the quadrupole MS at 150 °C. Samples were introduced in split mode at a ratio of 5:1 using helium as a carrier gas. Oven was set from 40 °C for 5 minutes to 320 °C at 4 °C min⁻¹ and held for 10 minutes at this temperature (Rae 2014).

Identification of chemical compounds was based on reports and literature from Vancampenhout et al. (2009). This study divides chemical compounds into 6 groups: aromatics and polyaromatics, soil lipids, lignin compounds, polysaccharides, phenols and nitrogen compounds (N-compounds). The variability analysis to determine the differences in interpretation of data between the inter operators and consequent validation will be carried out with data from Ashwell (2017). Finally, it is important to acknowledge that preparation and analysis of samples for Py-GC-MS was carried out by PhD candidate Rojas. Consequently, for this part of the research TOC data and Py-GC-MS files from Rojas were used for identification of chemical compounds and further analysis. The Agilent MSSD Productivity ChemStation for GC and GC/MS Systems Data Analysis Application' software was used for identification of chemical compounds.

3. Results



3.1 Discharge – TOC and PSD analysis

Figure 8 TOC concentration and water stage in eroded 30 and eroded (50) sites

Sample 24 in the eroded site was not recorder due to a possible due to malfunction of the auto sampler. Discharge for the eroded site was divided in two stages and samples were collected by the auto sampler 10 days apart from each other due to the low levels of discharge (Figure 8) and not in hourly basis. On the contrary, samples for the uneroded site were collected 18 days prior collection of eroded samples.

There is a positive correlation between TOC and discharge in stage 1 and in stage 2 of the eroded site. Figure 8 shows almost a perfect correlation which is more noticeable in the middle period of stage 1, when discharge was almost at its lowest 0.16 l/s at the beginning of the event and OC had a concentration of 34.35 mg/l, and then both sharply increase before reaching their pick 4.23 l/s and 35.75 ± 0.1 mg/l within a period of 2 and 4 hours respectively. From that point on OC decreased to its lowest concentration 32.51 ± 0.1 mg/l at a minimum discharge of 0.018 l/s. Stage 2 registered a partial correlation starting in the early period of the event when TOC 24.04 ± 0.1 mg/l and reached its pick at 35.1 ± 0.1 mg/l in four hour. Discharge in the early stage increased from a minimum 0.125 l/s to its maximum 79.15 l/s in 2.5 hours. While TOC maintained similar concentrations in the middle and late stages of the event ending at 30.58 ± 0.1 mg/l, discharge steadily decreased in four hours to 4.41 l/s and finishing at 0.55 l/s. High TOC concentrations and slow decrease could have been caused by the long lasting effects on DOC concentrations due discharge events, for this case, stage 1 event. The uneroded site presented negative correlation particularly in the early stages with TOC starting at its highest at 20.64 ± 0.1 mg/l decreasing abruptly to 18.31 ± 0.1 mg/l in the first five hours followed by intervals of small of increase and decrease periods reaching its minimum in the late stages at $17.42 \pm 0.1 \text{ mg/l}$ and . Discharge registered 80.33 l/s at the beginning of the event and reach its pick at 156.48 l/s within the three hours and slowly decreasing until the last stage where there is abrupt drop to the lowest point 36.99 I/s. It is important to highlight that discharge was much higher in minimum and maximum values in the uneroded site, however OC was much lower which goes against the reviewed literature cited in the introduction.

Discharge measurements from the above paragraph in eroded and uneroded sites confirm the trend from previous studies and from the reviewed literature which stated how much a single high discharge event could influence the overall yearly discharge. Moreover, figure 8 shows the effects of discharge events in water stage. Unsampled discharge was calculated in figure 4, in uneroded site amounts for 971 mega litres



(MI), this equals to 70.89% of the annual discharge; and the eroded site amounts to163 MI and 71.77%.



Figure 9 TOC and discharge in uneroded (30) and eroded (50) sites. Samples 8, 9, 10 for site 30 were collected in September 30th 2017. Samples 4, 5, 6 for site 50 stage 1 and 2, samples were collected on October 18th and 28th 2017 respectively. Error of ± 0.1 mg



Figure 10 TOC in eroded and uneroded sites for base flow and discharge

Filtering samples at 1 µm shows that TOC under base flow in the uneroded samples is 2.6 times higher than the eroded samples; this represent a large spatial variation. Contrastingly, during discharge events the eroded site contains 1.92 times more organic carbon than the uneroded site. On the other hand, TOC in the uneroded sites increases 1.54 times from the influence of discharge from 11.7 mg/l to 18.1 mg/l while whereas in the eroded site TOC increases 7.75 times due to the influence of discharge increasing OC from 4.5 mg/l at base flow to 34.9 mg/l.



Figure 11 PSD and TOC in uneroded (30) and eroded (50) sites under base flow and high discharge events

In terms of PSD, <1 μ m >0.2 μ m makes up the larger proportion of TOC in all samples, the eroded sample from high discharge event recorded the largest amount-percent 36.5 mg/l - 59.53% of OC. On the contrary, <0.2 μ m >100KD fraction recorded the lowest OC of all samples reaching the lowest amount-percentage of 0.95 mg/l - 1.53% in the in eroded site during discharge conditions. The second largest proportion of TOC was found at <100 KD > 10 KD in the uneroded samples, this fraction size at base flow accoutns for 44.63% of the TOC. There are no clear spatial-temporal variations besides two in the <0.2 μ m >100KD fraction size. The spatial variation changed TOC from 20.03 the eroded site to 6.10% in the uneroded one; the temporal variation, occurred in the eroded site when OC shifted from 20.03% in base flow to 1.53% during discharge in the eroded site.

3.2 Chemical characterization of OC

3.2.1 E4/E6, E2/E3, SUVA₂₅₄ analysis

Once calculated E2/E3, E4/E6 and SUVA ratios, the 1 μ m fraction size show overall the heaviest humification and the highest absorptivity of all samples besides the eroded sample during discharge conditions. At 1 μ m E2/E3 ratios in base flow and discharge samples of the uneroded site, and discharge sample from the eroded site meet humification index that indicates heavy coloured water (about 4), the base flow sample from the eroded site does not meet this criterion. SUVA values are higher than 4, implying high aromaticity and positive correlation with hydrophobicity. E4/E6 ratios are low and do not meet Kukkoner (1992) criterion of 3.8 – 5.8 for HA and 7.6 – 11.5 for FA which indicates high aromaticity, nonetheless Chin et Schnitzer (1977) stated that ratios below 5 for HA are indicators of aromaticity, consequently is most likely that the low values could indicate high degree of condensation and aromaticity.

After TFU <100 KD >10 KD fraction size shows the highest E2/E3 ratio with the lowest molecular weight, particularly in the eroded site under discharge conditions which represents heavy coloured water and high molecular weight. At this fraction size 2.24 is the highest E4/E6 ratio, making this closest ratio that nearly meets Kukkoner (1992) criterion and should represent high degree of aromaticity, nonetheless Chin et al. (1994) stated that E4/E6 ratios that are lower than 5

represent high aromaticity independently of other humification indexes, consequently at this fraction size aromaticity is the lowest. This can be confirmed with low SUVA ratio 6.71 which even though surpass the 4 SUVA humification index that indicates high aromaticity, this value is 3 to 4 times smaller in relation with SUVA values found at other fraction sizes (<0.2 μ m > 100KD, <10 KD).

E4/E6 ratios at <10 KD are the lowest in all samples ranging from 1.04 to 1.15 showing the highest aromaticity. Although these values can be influenced by the large addition of DIW diluted which can lower sensitivity. However, the SUVA index showed the highest ratios which ranged from 17.81 - 20.50 indicating high percentage of hydrophobic compounds and therefore high degree of aromaticity at a low molecular weight. A similar tendency was found at <0.2 μ m > 100KD fraction size. HS at the high molecular weight level were found in the <1 μ m >100 KD fraction size which shows high E2/E3 ratios. These rations are contrasting with low E4/E6 and SUVA ratios. This relation implies that samples that show E2/E3 with the biggest molecular weights contain the lowest aromatic structures. Table 1 and figure 12 shows PSD and humification indexes values.

Table 1 UV-vis analysis at 254 nm wavelength

Site	Size	HA (ppm)	TOC (mg/l)	UV254	E2/E3	E4/E6	SUVA	Site	Size	HA (ppm)	TOC (mg/l)	UV254	E2/E3	E4/E6	SUVA
	1 um	34.35	11.70	0.73	3.40	1.56	29.02	30 DS	1 um	38.93	18.10	0.93	3.44	1.98	5.14
30 BF	< 1um >0.2um	14.97	3.50	0.31	2.56	1.40	23.19		< 1um >0.2um	20.38	6.64	0.44	2.88	1.48	6.64
	<0.2um >100KD	5.95	0.72	0.10	1.81	1.07	33.45		<0.2um >100KD	7.50	0.74	0.87	1.75	1.05	27.85
	<100KD >10KD	17.59	5.25	0.37	3.24	1.17	21.59		<100KD >10KD	35.74	11.64	0.81	3.47	1.63	6.93
	<10KD	14.68	2.29	0.31	1.50	1.16	20.50		<10KD	38.38	1.50	0.68	1.93	1.04	19.45
	1 um	11.32	4.50	0.86	1.60	1.17	19.11	50 DS	1 um	78.74	34.90	1.68	3.30	2.56	4.81
	< 1um >0.2um	13.00	4.53	0.25	1.62	1.17	5.56		< 1um >0.2um	91.49	36.50	2.14	3.11	3.12	5.85
50 BF	<0.2um >100KD	8.51	2.50	0.16	2.56	1.11	6.32		<0.2um >100KD	5.52	0.94	0.08	1.76	1.03	8.26
	<100KD >10KD	11.74	4.13	0.24	1.63	1.16	5.70		<100KD >10KD	62.59	21.57	1.45	3.53	2.24	6.71
	<10KD	12.28	1.32	0.25	1.45	1.15	18.75		<10KD	52.90	2.30	1.21	2.27	1.08	17.87

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Figure 12 HA, TOC and PSD with E2/E3, E4/E6 and SUVA ratios

Overall, there is one spatial-temporal variation in the base flow samples form the uneroded site which shows the highest SUVA values at all fraction sizes and then decrease the ratio at discharge but still remain higher than the eroded site under base flow and discharge conditions. After PSD analysis, lower aromaticity is found at <1 μ m >100 KD and <100 KD >10KD fractions but high molecular weight. High aromaticity was registered at the remaining fractions sizes. Hydrophobicity is the very high at <10KD, because hydrophobicity and aromaticity have a positive correlation this should implicate that at this fraction size aromaticity should be very high as well. Although through this technique changes of chemical properties in NOM according to PSD can be assessed; coloured water, grade of aromaticity and hydrophobicity only provide general and limited information regarding the chemical properties of NOM. Consequently, Py-GC-MS analysis was performed in order to determine if UV-vis shows the necessary high resolution results be considered a surrogate technique that can improve the method to characterize NOM.

3.2.2 Py-GC-MS analysis

3.2.2.1 The variability report

Previously, it was stated that Py-GC-MS analysis requires large amounts of sample. Additionally, sample preparation requires long time and a very accurate preparation method. Because it is difficult to run triplicate samples to calculate the error; a variability analysis was conducted, figure 13 shows a combination of charts from two sets of data from the same sample analysed in this research and a second inter operator. The results were used to develop an analysis that would replace the error and validate the data.



Figure 13 Variability analysis of organic compounds from different inter-operators at end of discharge event on site 30. Fraction size <0.2 um, >100 KD falls into the +-0.1 mg/I TOC error, consequently a 0 value has been given

The variability report analysed samples from base blow, start of discharge event and end of discharge event for all fractions size. For the most parts, both analysis reported the dominance of aromatics and polyaromatics, and soil lipids with little variance in percentage (5 – 10%). Other chemical compounds such as phenols and polysaccharides were found at the beginning of the event but in much smaller amounts with much smaller variance (\leq 4%) with fall into 95% confidence level at 5% error. The biggest variability was recorded in figure 12; variability between inter operators is a qualitative concept and is mainly caused form interpretation of results because some compounds are very similar chemical composition, even though they are belong to a different compound group.

3.2.2.2 The method analysis

After validating these data, sampling techniques using the scraping and methanol methods was conducted. Figures 13 and 14 show results from the scraping and methanol method.

It is important to acknowledge the only sample provided from the scraping method is the one shown in Figure 14 and to compare the efficiency of the novel methanol method the same site under normal base flow condition was selected for the analysis. The base flow sampled presented the lowest TOC (5.53 mg/l) of al Py-GC-MS samples, according to the reviewed literature this sample should contain less number of organic compounds. On the other hand, the end of event sample has the highest TOC (32.78 mg/l) of all samples and should contain a large quantity of organic compounds. Both samples contain two compound groups, aromatics and polyaromatics, and soil lipids but both experience a major shift from and overall 26.85% to aromatics and polyaromatics in baseflow to 85.79% at the end of the event; and 73.25% to 14.21% in soil lipids. Table 2 also show the number of organic compounds in each fraction size



Figure 14 TOC and percentage of organic compounds using the scraping method in freze dried samples in the eroded site at the end of high discharge event

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Figure 15 TOC and percentage of organic compounds using the scraping method in freeze dried samples in the eroded site during normal base flow conditions. TOC values in <100 KD >10 KD fall into the +- 0.1 mg/l TOC error, consequently a 0 value has been



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	PSD									
Stage	Group	< 1um >0.2um	<0.2um >100KD	<100KD >10KD	<10KD					
Base flow	Aromatics and polyaromatics	5	6	7	9					
	Soil lipids	13	14	13	13					
	Total	18	20	20	22					
End of event	Aromatics and polyaromatics	12	11	8	8					
	Soil lipids	7	4	4	10					
	Total	19	15	12	18					

Table 2 number or organic compounds in base flow and at the end of high discharge in the eroded site

The analysis from PSD shows more organic compounds in most fraction sizes in the base flow samples, expect at the <1 μ m >0.2 μ m fraction which contains one more organic compound (19) at the end of discharge event. Based on percentages of aromatic groups and soil lipids, the base flow sample contains a higher ratio or organic compounds per dominant compound group at a specific fraction size i.e. at a PSD <1 μ m >0.2 μ m, 12 compounds were found in 90.46% of aromatics and polyaromatics at the end of the event; compared to 13 compounds in 69.70% of soil lipids in base flow. Images from the Data Analysis software in figures 14 and 15 also showed more consistency in picks of base flow samples where organic compounds are found. The method analysis demonstrates higher resolution for identification and quantification of organic compounds using methanol for sampling which consequently improves the resolution of NOM.

3.2.2.3 Chemical characterization of OC by Py-GC-MS application

Chemical composition of OC was determined by the Vancampenhout et al. (2009) compound classes. Eroded and uneroded sites show the dominance of aromatics and pyarolamtics, and soil lipids which make up from 3.90% to 75.66% and 20.79% to 96.10% respectively in the uneroded samples and 5.40% to 82.90% in the eroded samples. In fact, aromatics and polyaromatics were the only organic compounds

found in base flow samples and discharge samples in the eroded and uneroded sites.

There is a spatial and temporal variation in uneroded samples which show the presence of polysaccharides and phenols at the start of the discharge event and added Nitrogen (N) compounds at end of the event. These compounds were found in small percentages; at the start of the event polysaccharides and phenols were only present in the <0.2 μ m >100KD fraction size and made up 1.57% 1.99% respectively. By the end of the event phenols (0.59%) were found in the <1 μ m >0.2 μ m sample; N-compounds in the <0.2 μ m >100 KD (6.17%) and the <100 KD > 10KD (2.23%) samples; and polysaccharides (7.64%) were found in the <10 KD sample. None of these compounds were found in the eroded samples.

TOC in PSD has the highest concentration of OC at the <1 μ m >0.2 μ m fraction in all samples, the lowest concentration is found in the eroded base flow sample 54.30% and the highest in the uneroded site at the start of the discharge event 84.65%. It is important to acknowledge TOC concentrations because there is a strong positive correlation between TOC which is directly link to discharge events and the aromatic and polyaromatics compounds. This can be found in five out the six samples at the <1 μ m >0.2 μ m particularly in the eroded site, the highest correlation was registered in the eroded samples; where aromatics and polyaromatics increased from 30.30% at base flow to 90.46% by the end of the event. The lowest correlation was registered at the <0.2 μ m >100 KD fraction size in the uneroded samples.



Figure 16 Percentage of organic compounds and TOC (mg/l) concentrations in eroded and uneroded base flow, start and end of discharge event samples. Site 30 end of discharge event, fraction size <0.2 um, >100 KD; and site 50 base flow, fraction size <100 KD, >10KD

Eroded samples showed a temporal variation in all fractions size which started with strong presence of soil lipids over aromatics and polyaromatics in base flow samples and shifted completely by the end of the discharge event. Figure 9 shows changes in compound percentage content and composition for all samples. The biggest temporal change occurred in the <100 KD >10KD in base flow samples, soil lipids accounted for 82.90% and aromatics and polyaromatics for the remaining 17.10%; at the end of discharge aromatics accounts for 94.60% and soil lipids for 5.40%. The lowest temporal variation was registered in the <10 KD fraction size with an initial composition of 30.77% for aromatics and polyaromatics and 69.33% for soil lipids shifting to 67.64% and 32.36% respectively.

4. Conclusion and discussion

Discharge events have positive correlation with increments of TOC in eroded samples. OC registered increments of 1.54 times in uneroded samples and 7.75 times in eroded samples. Spatial temporal variations in PSD were found in eroded site at <100KD >10KD. TFU combined with UV-vis at 254nm wavelength allowed to achieved HA constant mass and improved low resolution analysis for further analysis of physical and chemical properties of NOM. The notable increase of 7.75 TOC concentrations in eroded samples at a lower discharge compared to uneroded samples can be environmentally unbeneficial because high OC from eroded peat lands increase decomposition rates and produce loosely bounds of NOM which are flushed into catchments changing the dynamics of DOC in water and therefore disrupting the carbon cycle. The low OC increment in uneroded peat catchments shows that natural ecological processes in uneroded peat catchments are much more stable which is an indicator of healthy ecosystems (Tallys et al. 1997).

The highest TOC were recorded at <1 μ m >0.2 μ m and <100KD >10KD and E2/E3 ratios determined that at these fraction sizes there is greater molecular weight and heavy coloured water. E4/E6 and SUVA ratios showed that the remaining fraction sizes have higher aromaticity and hydrophobicity at the lowest molecular weight. Accurate PSD and TOC measurements determined which are dominant fraction sizes of OC samples and therefore also determine the dominant chemical properties of NOM in eroded and uneroded peatlands using UV-vis as a surrogate technique.

Humification indexes provided only limited information about chemical composition of NOM (degree of aromaticity, hydrophobicity properties and heavy coloured water). Consequently, it cannot be used as a surrogate high resolution technique because it does not provided very detailed information and quantitative data. Py-GC-MS showed very detailed quantitative and qualitative information of chemical composition of OC. Moreover, UV-vis does not provided consistency as data particularly in E4/E6 ratio which do not meet in of the Kukkonen (1992) and Chen and Schnitzer (1977) criterions and do not follow the same trend of SUVA values. Additionally, Py-GC-MS showed that the higher aromaticity is found at <1 μ m >0.2 μ m fraction size contrasting the results of humification indexes which showed the lowest aromaticity at the same fraction size.

Py-GC-MS shows much more consistency in all samples. The dominant compound groups aromatic and polyaromatics and soil lipids follow and specific trend in all samples. Accuracy is PSD and OC concentrations allowed to stablish a very strong positive correlation between TOC and aromatics and polyaromatics compounds. The combination of low resolution analysis with a Py-GC-MS have proofed to improve the method of characterizing NOM and made resolution relevant by using quantitative and qualitative data to determine stabilising spatial and temporal in eroded and uneroded samples.

Finally, there was very low presence of polysaccharides, phenols, and Ncompounds, and no presence of lignin compounds. This is contrasting from previous studies from Nguyen (2009), Do (2012) and Rae (2014) which used Py-GC-MS to determine chemical composition of NOM in the same sites and recorded good presence of the these compound groups. This can lead to the assumption that water chemistry could be changing and therefore further research using Py-GC-MS is need it to update data or show the possible changes in chemical composition of NOM.

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