

THÈSE

Présenté à

L'UNIVERSITÉ DE LILLE

École Doctorale Sciences de la Matière, du Rayonnement et de l'Environnement

Faculté des Sciences et Technologies – Département Chimie

Pour obtenir le titre de

DOCTEUR

Spécialité : Chimie organique, minérale, industrielle

Rédigée par

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Caractérisation de la matière organique naturelle (MON) et de ses complexes formés avec des éléments traces métalliques dans des filières de potabilisation.

Date de soutenance : 28 mai 2020

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THESIS

Presented at

LILLE UNIVERSITY

Doctoral School for Materials, Radiation and Environmental Sciences

Faculty of Science and Technology – Department of Chemistry

To obtain the grade of

DOCTOR

Discipline: Organic, mineral and industrial chemistry

By

Junias ADUSEI-GYAMFI

Characterization of natural organic matter (NOM) and its complexes formed with trace metals in drinking water treatment processes.

Defended on the **28th of May 2020**

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Acknowledgment

It has been three years of adventure and I would like to thank all those contributed directly or indirectly to the success of this work. This research work was carried out at the 'LAboratoire de Spectroscopie pour les Interactions, la Réactivité et l'Environnement (LASIRe UMR 8516 CNRS)' within the 'Physico-chimie de l'Environnement (PCE)' research team. Firstly, I would like to thank the director of the laboratory Prof. Hervé Vezin and his assistant, Prof. Cyril Ruckebusch for having welcomed me into the laboratory during these three years.

I would also like to thank all the members of the jury; Pr. E.R. (Emile) Cornelissen, Dr. Emmanuelle Filloux, Dr. Koffi Marcus Agbekodo and Dr. Holly Shorney Darby for agreeing to evaluate my work. A special thank you goes to Prof. Jean-Philippe Croue and Prof. Marc Benedetti for having done me the honour of being the examiners of this manuscript and for the positive feedback given.

I am particularly grateful to Prof. Baghdad Ouddane, my thesis director and Dr. Justine Criquet my co-supervisor for having selected me for this study. Thank you for the confidence you had in me in allowing me to be as autonomous as possible. Your doors and ears were always opened to me and not only did you train me scientifically but also professionally. I was very lucky and blessed to have you as my tutors. I equally thank Prof. Luuk Rietveld of TU Delft for his great support and collaboration.

Furthermore, I would like to express my sincere thanks to Prof. Gabriel Billon, the head of the PCE research team for his ideas and great sense of humour. Within the PCE unit, I would like to express my profound gratitude to Dr. Pierre-Jean Superville for the insightful discussion and brainstorming we had. You were always patient with me and answered my questions as well as introducing me to the different modelling applications. To Dr. David Dumoulin, I say a big thank you for the technical support especially in developing a novel HPSEC-ICP-MS quantitative method. Thank you to Prof. Jean-Paul Cornard for his scientific insight in the area of fluorescence. To Véronique Brassart and Melinda George, I thank you for the technical support, I enjoyed the lunch times we spent together.

I would also like to thank Prof. Sopheak Net-David, Dr. Ludovic Lesven, Prof. Michel Wartel, Dr. Yassine kadmi, Christine Grare and Clementine Burlin for their support in diverse ways.

I also thank my office colleagues Guillaume Trommetter and Jeremy Mougin for their friendliness and fruitful discussions, I wish you good luck for the continuation of your thesis. A big thank you to Dr. Henry Mackeown for his patience and helping me to improve my French, you were more than a colleague to me. I cannot forget our one and only 'mother' in the office, Dr. Pratima Bhurtun for her encouragement and moral support, we still need to 'wash' the dirty mind of Henry.

I also thank all current and former LASIRe PhD students, Wissam, Oscar, Thomas and Silvère, Anastasia, Camille, Cecilia, Samantha, Nicole, Rayane, Marija, Diksha and Lucy as well as all the LASIRe staff such as Cécile, Catherine and Brigitte for their warm reception.

I would like to equally thank all DOC2C's partners especially to, Chris Rockey and David Metcalfe of South West water, Floris Roberto and Zheng Jumeng of PWNT, Liesbeth Verdickt and Klaas Schoutteten of Dewatergroep for their enormous collaboration and sending me samples readily to be analysed. A big thank you to the Project coordinator, Dr. Shorney-Darby Holly for her diverse support.

Last but not the least, I am utmost grateful to my sweet wife Mrs. Emmanuella Adusei-Gyamfi for her tremendous support, encouragement and prayers for me during these years. The task was lighter because you gave me a shoulder. I love you my 'Abena spirit'. I could not forget my siblings, Linda, Irene and Joel as well as their families for the continuous prayers and support.

I hope you enjoy reading this manuscript.

Glossary

BR: Burrator reservoir DAD: Diode array detector DBP: Disinfection by products DOC: Dissolved organic carbon DOM: Dissolved organic matter DWG: De Watergroep EDTA: Ethylenediaminetetraacetic acid FEEM: Fluorescence excitation emission matrix FLD: Fluorescence detector HAA: Haloacetic acid HL: Humic-like HMW: High molecular weight HPSEC: High performance size exclusion chromatography ICP-MS: Inductively coupled plasma mass spectrometry LMW: Low molecular weight NOM: Natural organic matter OCD: Organic carbon detector PARAFAC: Parallel factor analysis PL: Protein-like QY: Quantum yield **RT:** Tamar River SEC: Size exclusion chromatography SRHA: Suwannee River humic acid SRNOM: Suwannee River natural organic matter SUVA: Specific UV absorbance

SWW: South west water

THM: Trihalomethanes

TOC: Total organic carbon

UVA254: Ultraviolet absorbance at 254 nm

Résumé

Les matières organiques naturelles (MON) possèdent des ligands hétérogènes avec différents groupes fonctionnels, et pour certains d'entre eux, une densité de charge élevée les rendant capables de se lier aux cations et de contrôler leur biodisponibilité dans l'environnement. Les groupes fonctionnels les plus prédominants dans les MON sont les groupes carboxyle et phénolique, qui ont de fortes affinités pour les métaux en fonction du pH. La quantité et la nature de matière organique naturelle présente dans les ressources en eaux est une problématique majeure des filières de potabilisation. En outre les complexes MON-métal formés, ont un impact sur l'efficacité des procédés de traitement de l'eau tels que la coagulation, l'adsorption sur charbons actifs, les résines échangeuses d'ions et la filtration membranaire.

De plus, les variabilités saisonnière ou climatique observées nécessitent une adaptation des conditions opérationnelles de traitement. En effet, de nombreuses ressources en eau voient leur teneur en carbone organique augmenter au fil du temps. A titre d'exemple, au South West Water, société de traitement d'eau du sud-ouest de l'Angleterre a vu les teneurs en carbone organique total (COT) doubler en 10 ans sur une de leur ressource d'eau de surface (de 2 à 4 mg /L entre 2001 et 2011 en moyenne annuelle) et observent une plus grande variabilité des teneurs au cours de l'année.

La problématique liée à la présence et l'augmentation continue des teneurs en carbone organique, en particulier dans les eaux de surface, et son impact sur les filières de potabilisation a donné naissance au projet DOC2C's dans le but d'étudier et de proposer des moyens durables d'éliminer le carbone organique dissous (COD) des eaux. C'est un projet financé par le programme européen Interreg 2 mers couvrant la zone des mers du Nord et de la Manche. Le projet a impliqué trois partenaires industriels : PWN technologies (NL), South West Water (UK), De Watergroep (BE) et deux universités : TU Delft (NL) et l'Université de Lille (FR).

Le sujet présenté ici s'intéresse principalement à la caractérisation de la MON et de ses complexes avec les métaux. Pour caractériser la MON et ses complexes avec les éléments traces, une chromatographie d'exclusion stérique (HPSEC) a été couplée à des détecteurs spectraux (UV et Fluorescence) et à un détecteur élémentaire (ICP-MS). Ainsi, les propriétés spectrales et complexantes des différentes fractions de la MON peuvent être étudiées après avoir été séparées par taille. La séparation par exclusion stérique (HP-SEC) est réalisée sur une colonne Agilent Bio SEC-5 (Diamètre interne 7.8 mm, longueur 300 mm, taille de particule 5 μ m - 100 ~ 100,000 Da).

L'éluant était constitué d'un tampon phosphate à pH 6.8 limité à 1g/L pour éviter un encrassement prononcé de l'ICP-MS. Les temps de rétention de la colonne ont été étalonné avec des standards de l'IHSS et par la comparaison d'eaux naturelles analysées sur notre instrument et par SEC-OCD par le HET Laboratorium (Allemagne). Une nouvelle méthode innovante de quantification a été développée pour évaluer la complexation des métaux sur les différentes fractions de la MON. La méthode analytique a été développée sur un spectromètre de masse couplé à une torche à plasma de dernière génération (ICP-MS, Agilent 7900) installé dans une salle blanche. Cet ICP-MS est couplé à un système de chromatographie liquide (HPSEC Agilent 1260 Bio-Inerte). Cette méthode a été étalonnée et validée à l'aide d'EDTA et d'étalons de matière organique (SRHA, SRFA) de IHSS pour les ligands et les solutions étalons d'Al, Cr, Cu, Pb et Zn.

Notre colonne permet une séparation relativement fine des différentes fractions de matière organique naturelle en fonction de leur taille (biopolymers, substances humiques, building blocks, acide et composés neutres de faible poids moléculaires). Cette méthode a été appliquée aux eaux des unités pilotes de l'usine de production d'eau potable d'Andijk aux Pays-Bas (PWNTechnologies) et de De Watergroep en Belgique. D'après les chromatogrammes obtenus, l'étape de coagulation élimine presque tous les biopolymères (confirmé par SEC-OCD), les masses moléculaires les plus hautes des substances humiques (38-80% d'après les données d'absorbance) et une très faible partie des composés de plus faible poids moléculaire (neutres, 'building blocks' et acides). A noter toutefois que cette étape élimine usuellement une plus grande partie des substances humiques. L'ultrafiltration n'a pas permis d'éliminer significativement plus de matière organique hormis la fraction résiduelle des biopolymères.

Les SEC-UV/FLD ont démontré leur capacité à être utilisés comme substituts aux détecteur COD, en particulier pour la caractérisation de la substance humique et des building blocks. Ces détecteurs de substitution ont été étalonnés avec des échantillons de référence pour des mesures semiquantitatives précises. Le PARAFAC a été utilisé pour identifier les différents fluorophores présents dans les échantillons. La méthode indice de rapport d'absorbance UV (URI), qui n'est possible qu'après fractionnement, a permis de mieux comprendre la composition chimique de cette fraction sans interférence d'autres fractions de la MON. Les signaux de chromatographie ont été utilisés pour relier directement les spectres de fluorescence des MON à la polarité et à la taille moléculaire, ce qui a permis de surveiller et de prévoir le potentiel de formation des sous-produits de désinfection (SPD). En comparant deux voies de traitement de l'usine de De Watergroep, on a observé que l'eau brute contenait un mélange de composants hydrophobes et hydrophiles qui contribuaient au potentiel de formation de SPD à des degrés divers. La fraction de substances humiques de la MON, dont on sait qu'elle est plus hydrophobe, a contribué le plus à la formation de SPD, tandis que les fractions des composés de plus faible poids moléculaire et des hydrophiles y ont contribué le moins. La résine anionique a été efficace même pour éliminer (35%) les composés de plus faible poids moléculaire neutres qui ont une faible densité de charges. Il a été observé que la réduction du THMFP (potentiel de formation des trihalométhanes) pendant les processus de traitement était due à l'élimination préférentielle des précurseurs organiques THM hautement réactifs et à la transformation progressive de la MON vers des fractions moins réactives.

Nous avons aussi suivi l'évolution du chromatogramme (eau naturelle) avec des quantités croissantes d'aluminium et de zinc (de $0 a 150 \mu$ M). Une décroissance du signal UV était observée pour la plupart des fractions. La décroissance était plus forte pour les hauts poids moléculaire et plus intense avec l'aluminium que le zinc. Les décroissances observées sont liées à la diminution de la concentration du ligand libre en solution suite à la complexation de la matière organique avec ces métaux. Un comportement différent est mis en évidence pour les composés acides de faible poids moléculaire. En effet, pour ces composés, un décalage du temps de rétention est observé, signe d'une modification de taille de l'espèce.

Grâce au couplage de HPSEC-ICP-MS, nous avons pu analyser en continu les métaux de la matrice réelle (sans dopage) après séparation de la matière organique éventuellement complexée sur la colonne de chromatographie. D'après le chromatogramme, le cuivre est complexé à toutes les fractions de matière organique, contrairement au zinc et au plomb qui se complexent préférentiellement aux building blocks (produits de dégradation des substances humiques). Cette méthode permet donc la spéciation des métaux complexés à la matière organique à des concentrations très faibles (de l'ordre du µg/L). Des tests de compétition ont été réalisés par dopage des eaux. A titre d'exemple, dans un échantillon d'eau naturelle avec des concentrations initiales de Cu (4,3 μ g/L) et de Zn (50 μ g/L), le pic de complexation en Zn disparaît complètement après addition de 20 µg/L de Cu, démontrant une compétition entre les complexes forts et faibles. Pour pouvoir quantifier la concentration de métaux complexé, l'appareillage a été étalonné avec des complexes d'EDTA et des éléments traces métalliques : Cu, Mn et Zn. Vue la nécessité d'avoir un métal complexé pour calibrer la méthode, l'EDTA a été choisi pour son fort pouvoir complexant. Une procédure de lavage de la colonne a également été mise en place entre les analyses pour éviter les interférences résiduelles entre échantillons. Une réponse linéaire sur les domaines de concentrations choisis a été obtenue (1-10 µg/L pour Cu et 10-50 µg/L pour Mn et Zn). L'analyse de complexes des éléments traces métalliques avec des extraits de matière organique a permis de caractériser plus finement la méthode et d'évaluer les fractions complexées des métaux.

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Introduction

General Introduction

For the past three decades, the concentration of natural organic matter (NOM) in water bodies has been observed to increase as well as vary significantly. This seasonal variability and elevated concentration of NOM poses a challenge to water treatment facilities in terms of operational optimization and proper process control (Sillanpää 2014, Sillanpää et al., 2018). The presence of NOM can result in unpleasant colour, odour, regulation of the bioavailability of trace elements, promotion of bacterial reproduction and biofilm formation and interaction with disinfectants forming toxic disinfection by products (DBPs) (Metsämuuronen et al., 2014; Golea et al., 2017). The concern over the continuous increase in dissolved organic carbon (DOC) levels particularly in surface waters and its impact on drinking water treatment and processes gave birth to the DOC2C's project in 2016 with the aim of investigating and proposing innovative, research based and sustainable ways of removing DOC from source waters and promoting green economy especially in the 2 seas area (North sea and the English Channel regions). For instance, a drinking water treatment company in the Netherlands (PWNT) which depends on the Ijsselmeer Lake as its primary water source has reported that, while the average DOC concentration in the raw water appears not to vary much over the last decade, the composition of NOM varies significantly especially between seasons. Another drinking water treatment facility in the United Kingdom (SWW) has reported over 100% increase in the yearly average DOC over a decade. The project had a 4-year (Jan 2016- Dec 2019) duration and is through the European Interreg 2 seas programme covering the 2 seas area, thus England, France, Netherlands and Belgium (Flanders) as shown in Figure 1.

To realize this objective, an inclusive consortium which consisted of two universities (Lille University (FR) and Delft University of Technology (NL)) and three drinking water treatment companies (PWNT (NL), South West Water (UK) and, De Watergroep (BE)) was set up to enhance a comprehensive exchange of knowledge and experience. Our (Lille University) main responsibility as a project partner was to find an innovative implied approach for the characterization of NOM which would help in future design and optimization of treatment processes for enhanced NOM removal.

General Introduction



Figure 1: A map of the project partners in the DOC 2 seas region; Univ Lille-France, DWG-Belgium, Tu Delf & PWNT-Netherlands and SWW-United Kingdom.

To fully understand the effect of NOM on water treatment, water treatment facilities have in the past employed simple techniques to characterize NOM based on colour, total organic carbon (TOC), dissolved organic carbon (DOC), chemical oxygen demand (COD), pH, turbidity and ultraviolet absorbance at 254 nm (UVA₂₅₄) to monitor water quality and treatment (Chow et al., 2008). These bulk analytical techniques are preferentially used to characterize bulk NOM because they do not require any pre-concentration, fractionation or enormous sample preparation. Spectroscopic techniques for instance, which can be used for both qualitative and semi-quantitative analysis of NOM require very small sample volume and minimal sample preparation (Li et al., 2017). UVA₂₅₄ is commonly used as a surrogate for measuring NOM in waters and has been reported to correlate well with DOC especially in high hydrophobic waters. Likewise, the specific UV absorbance (SUVA₂₅₄), which describes the UV absorbance normalized with the DOC has been proposed as a surrogate to measure the hydrophobicity of NOM as well as its aromatic character (Chow et al., 2008; Matilainen et al., 2010). Fluorescence excitation-emission matrix (FEEM) coupled with parallel factor analysis (PARAFAC) has also been used to categorize NOM samples into a combination of several groups of fluorophores with similar optical properties (Henderson et al., 2009).

However, the challenge is that, some vital information needed in designing and optimizing treatment processes may be lost when some of these bulk techniques are used exclusively. For example, a water sample with high biopolymer fraction (>> 20 KDa) needs some pre-treatment to remove this fraction which is known to be the main fraction responsible for membrane fouling. Using only bulk techniques like TOC, UV and fluorescence would not be able to identify the presence of this fraction and may reduce the efficiency and durability of the

membrane. Non-destructive techniques like high performance size exclusion chromatography (HPSEC) that can fragment NOM before its characterization are therefore needed to fill in this information gap. An HPSEC separates the bulk NOM into different fractions based on their molecular size or weight. In this study, an HPSEC was coupled with three different detectors (UV spectrometer, fluorescence spectrometer and inductively coupled plasma mass spectrometer). Combining both fragment and bulk techniques, gives enough information to understand and predict the fate of NOM in water in order to make informed decisions on how to effectively optimize treatment processes.

Objectives of this study

The specific objectives of this PhD within the context of the global objectives of the DOC2C's project can be summarized as follows:

- To synergize the physical and spectroscopic properties of NOM fractions to enhance their characterization in drinking water treatment.
- To use the spectral properties of NOM to predict and monitor precursors responsible for the formation of disinfection by products (in collaboration with another PhD student on the project).
- To develop a novel quantitative and qualitative method for the distribution of metals in NOM fractions.

Outline of the thesis

This thesis has been divided into five chapters. The content of each chapter has been organized in a manner that the results of each has been (or will be) published in an international peer reviewed scientific journal. This method makes it easier to approach each chapter as nearly independent of the other chapters. A brief description of the content of the various parts is as follows:

Each chapter begins with a precise bibliographical information related to the specific chapter which is complimentary to chapter I.

In Chapter I, a comprehensive review of NOM-cations complexation and its impact on water treatment is presented. Different cations have been presented and their effect on several treatment techniques reviewed. This chapter provides a good justification and platform to respond to our third objective.

General Introduction

Chapter II highlights the different analytical methods and experimental conditions that were used in this study. A detailed description of project partners and the origin of both natural water samples as well as NOM extracts are also provided.

Chapter III presents the results of combining both the physical and spectroscopic properties of NOM fractions to enhance their characterization in drinking water treatment. Bulk detectors were combined with fractionation techniques to monitor the evolution of DOC and NOM fractions within a full-scale treatment plant.

Chapter IV is issued from a collaborative work with Henry MacKeown, a PhD student recruited on the project who focused on the formation potential of disinfection by-products. The spectral properties of NOM are further probed for the prediction and monitoring of precursors that may be responsible for the formation of DBPs.

In Chapter V, an innovative method for quantitative analysis of NOM-metal complex using HPSEC-ICP-MS is presented. The distribution of metals in NOM fractions during treatment processes has also been discussed. Unfortunately, this part was not fully developed due to logistical challenges as access to the HPSEC-ICP-MS was limited than expected. The initial plan was to verify the findings in Chapter I by quantitatively assessing the impact of NOM-metal complexation on drinking water treatment techniques at natural metal concentrations. Membrane filtration was chosen to be tested at this phase of the project in collaboration with TU Delft. Unfortunately, TU Delft was unable to host the proposed experiment. This phase of the experiment would be revisited with the next available opportunity.

The thesis report ends with a conclusion which presents a summary of the main findings and conclusions of this study as well as some recommendations and perspectives for future research.

This thesis work was carried out within the "Physical chemistry of the Environment" team of the 'LAboratoire de Spectroscopie pour les Interactions, la Réactivité et l'Environnement' (LASIRE UMR CNRS 8516), at Lille University in collaboration with Delft University of Technology – Faculty of Civil Engineering and Geosciences – Department of Water Management.

This project has received funding from the Interreg 2 Seas programme 2014-2020 co-funded by the European Regional Development Fund under subsidy contract No 2S01-013"

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Chapter I

Natural organic matter-cations complexation and its impact on water treatment: a critical review

This chapter is based on the following published paper:

Adusei-Gyamfi, J., Ouddane, B., Rietveld, L., Cornard, J.-P., Criquet, J., 2019. Natural organic matter-cations complexation and its impact on water treatment: A critical review. Water Res. 160, 130–147.

I. Natural organic matter-cations complexation and its impact on water treatment: a critical review

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Abstract

The quality and quantity of Natural organic matter (NOM) has been observed to evolve which poses a challenge to water treatment facilities. Even though NOM may not be toxic, its presence in water has aesthetic effects, enhances biological growth in distribution networks, binds with pollutants and controls the bioavailability of trace metals. NOM has heterogeneous functional groups, with the predominant ones being the carboxyl and the phenolic groups which have high affinities for metals depending on the pH. The properties of both the NOM and the trace elements influence the binding kinetics and preferences. Ca^{2+} prefers to bind with the carboxylic groups especially at a low pH while Zn²⁺ prefers the amine groups though practically, most cations bind to several functional groups. The nature of the chemical environment (neighbouring ligands) the ligand finds itself equally influences its preference for a cation. The presence of NOM, cations or a complex of NOM-cations may have significant impact on the efficiency of water processes such as coagulation, adsorption, ion exchange resin and membrane filtration. In coagulation, the complexation between the coagulant salt and NOM helps to remove NOM from solution. This positive influence can further be enhanced by the addition of Ca²⁺. A negative influence is however, observed in lime-softening method as NOM complexes with Ca²⁺. A negative influence is also seen in membrane filtration where divalent cations partially neutralize the carboxyl functional groups of NOM thereby reducing the repulsion effect on NOM and increasing membrane fouling. The formation of disinfection byproducts could either be increased or reduced during chlorination. The speciation of products formed is modified with generally the enhancement of haloacetic acid formation observed in the presence of metal cations. This current work, presents in detail the interaction of cations and NOM in the environment, the preference of cations for each functional group and the possible competition between cations for binding sites, as well as the possible impacts of the presence of cations, NOM, or their complex on water treatment processes.

Keywords: Natural organic matter, Cations, Ligands, water treatment, complexation

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1. Introduction

The presence of natural organic matter (NOM) in water bodies has recently been observed to increase as well as vary significantly (Eikebrokk et al. 2004). High seasonal variability and elevated concentrations of NOM would pose challenges to water treatment plants in terms of operational optimization and proper process control (Sillanpää 2014, Sillanpää et al., 2018). Even though NOM may not be toxic, it has the tendency of changing both the chemical and physical properties of water. It may result in colour and odour of water, influences the concentrations of dissolved O, N, P, and S and serve as a carrier of toxic pollutants like pesticides and radionuclides (Knauer et al., 2017; Santschi et al., 2017). In addition, NOM serves as both a source and sink for carbon, which may have an indirect climatic effect (Delpla et al., 2009; Dore, 2005; Hruška et al., 2009).

NOM refers to a complex mixture of different organic compounds which are principally formed by the weathering or decay of living material and are present in fresh water (Stevenson 1994; Iskrenova-Tchoukova et al. 2010). The different chemical constituents of NOM do not make it practically feasible to characterize it based on individual compounds. There even exists some ambiguity on whether NOM is chemically a true macromolecular entity (Kononova, 1961; LeBoeuf and Weber, 2000) or just an assembly of relatively small molecules held together by relatively weak non-covalent interactions (primary electrostatic and hydrogen bonding) to form a supramolecule (Kalinichev and Kirkpatrick 2007). Notwithstanding NOM being a complex mixture of organic compounds, there are fractions with distinct chemical formulae such as carbohydrates, fats, waxes, alkanes, peptides, amino acids, proteins, lipids and organic acids. The other fractions, with no distinct chemical formulae and unknown unique identity, are referred to as humic substances. The non humic fractions are easily degraded while the humic fraction, together with soil minerals, can persist for centuries (Pettit, 2004). The elemental analysis of the different components of humic substances reveals that they are primarily composed of carbon (55-57%), oxygen (34-36%), hydrogen (4-6%), nitrogen (0.9-3%), and sulphur (0.4-1.8%) in a complex carbon chains (Dixon et al. 1989; Rice and MacCarthy 1991; Pettit 2004; Bravo et al., 2017). Very little is known about the nitrogen and sulphur containing functional groups (Filella, 2008). Humic substances can be further divided into three classes based on their solubility and molecular weight (MW). Humin (MW range of approximately 100,000 to 10,000,000) is insoluble in aqueous systems at all pH. Humic acid (mixture of weak aliphatic and aromatic organic acids with MW range of approximately 10,000 to 100,000) is

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soluble at pH >2 and fulvic acid (mixture of weak aliphatic and aromatic organic acids with MW range of approximately 1,000 to 10,000) is soluble at all pH. Fulvic and humic acids are ubiquitous in water systems, and constitute about 10–30% of dissolved NOM in seawater, 70–90% of dissolved NOM in wetland water, 40–90% of dissolved NOM in streams and about 50% of dissolved NOM in lake waters, depending on hardness (Xue and Sigg 1999; Thurman 2012; Lipczynska-Kochany 2018a). This is because the presence of hardness cations apparently suppresses the solubility of high molecular weight fulvic acids (Aiken and Malcolm 1987; Breault et al. 1996).

NOM could also be defined by its origin, *i.e.* allochthonous or autochthonous. Allochthonous NOM, on the one hand, refers to NOM that originates from a distant place. It may have been transported to its present matrix by agents of erosion and are susceptible to degradation and removal processes during its transport. Allochthonous NOM is typically derived from lignin-containing plants, the degradation of which yields fulvic acids which are relatively rich in aromatic carbon content and phenolic compounds but low in nitrogen content. Autochthonous NOM, on the other hand, refers to NOM that is indigenous to the matrix and its often algal derived (Luider et al., 2004). Aquatic algal derived NOM has a relatively higher nitrogen content and lower concentration of aromatic and phenolic compounds (Fabris et al., 2008).

NOM can further be classified based on its polarity and hydrophobicity (Sillanpää et al., 2015). The hydrophobic (less polar) fraction, which, generally constitutes a higher percentage of the NOM fraction, consists of aromatic rings with conjugated double bonds and phenolic structures, while the hydrophilic (more polar) fraction is mainly made up of aliphatic carbons and nitrogenous compounds like proteins, sugars and amino acids (Baghoth, 2012). Some functional groups observed in NOM are the substituted alkyl carbons, unsaturated carbons, amides, carboxylic groups, aldehydes and ketones, amino groups, alcohols and phosphate esters, sulphur (Torbjörn et al. 2006; Mopper et al. 2007). Figure I-1, gives a summarized scheme of the broad characteristics of NOM.

Because of its high reactivity, NOM also controls the bioavailability and biogeochemical cycling of trace elements in the environment (Xia et al. 1997). Since it is rare to find a metal un-complexed, 'naked', in the environment, metal-NOM complexation occurs by the metal binding to an already existing ionized site or by displacing a proton from its position. This can be due to an increase in the electrostatic interaction between the deprotonated functional groups and the cations as they are deprotonated at higher pH.

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NOM and climate change affect each other; whereas NOM contributes to climate change through the release of greenhouse gases, climate change influences the quantity, structure and reactivity (biotic and abiotic interactions with aquatic pollutants such as trace elements) of NOM thereby affecting surface and groundwater quality. Climatic conditions that are known to have a positive correlation with DOC concentration in waters include high precipitation, high temperature, storm, floods and runoff (Ågren et al., 2010; Bhurtun et al., 2019). An increase in temperature for instance can increase biological activities and the decomposition of organic matter, which can subsequently be eroded into water bodies during floods or rainfalls. Hurricane Irene which occurred in 2011 is estimated to have contributed to 19% of the 2011 annual dissolved organic matter (DOM) exports from a forested catchment in Cecil County, Maryland (Dhillon and Inamdar, 2013). Areas of mean temperatures of about 5°C and 30°C would have 10% and 3% carbon loss respectively with a temperature increase of 1°C. The change in temperature would also influence the bioavailability and toxicity of pollutants since adsorption processes are temperature dependent (Ripszam et al., 2015). The activity of microbial organism is further enhanced as humic substances act as electron donors acceptors during anaerobic respiration (Lau et al., 2015; Lipczynska-Kochany, 2018b). Available database shows that the increase in DOC concentration started in the early 1980s (Evans et al., 2005). Data on 315 records from across Britain show that about 69% of the sites studied indicated a significant increase in DOC concentration (up to 16% annual percentage change) between 1975 and 2002. A lower proportion (38%) has been found across Europe and northern American countries (189 sites – 11 years) (Skjelkvåle, 2003).

Even though underground waters are less sensitive to climate change than surface waters, its quality is also expected to deteriorate due to its exchanges (recharge and discharge) with surface waters (Kjøller et al., 2004) which would have grave consequences on drinking water production and may increase treatment cost. The browning of surface waters by NOM reduces UV disinfection capacity as it shields pathogens by absorbing the UV rays (Williamson et al., 2017; Lipczynska-Kochany, 2018). The formation of disinfection by products (DBPs) in drinking waters is expected to rise as well as the release of trace metals into the environment (Lipczynska-Kochany, 2018). Acidification of groundwater due to acid rains would results in less than 10% NOM-metal complexes making about 90% of trace metals bioavailable (Kjøller et al., 2004).

Due to its highly complex structure and heterogeneity, numerous papers and reviews are focused on NOM classification and/or characterization. (Edzwald et al. 1985; Aiken et al. 1992;

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Bryan et al. 2000; Luider et al. 2004; Sutton et al. 2005; Karlsson et al. 2006; Kalinichev and Kirkpatrick 2007; Mopper et al. 2007; Fabris et al. 2008; Sillanpää et al., 2015). The aim of this review is to (1) extensively examine the interactions of cations and NOM in the environment. Much attention is given to the major functional groups and cations as well as the different types of interactions that can exist between them, (2) examine the preference of cations for each functional group and the possible competition between cations for binding sites, (3) assess the impact of the presence of cation, NOM, or their complex on drinking water treatment processes.



Figure I-1: A summary of the major characteristic classes of NOM

2. Theoretical aspects of the complexation of metals to NOM

2.1 Central atom and ligands

Bonding that occurs through the donation of electron pairs from an orbital of an atom (electron pair donor or ligand) to a partner atom (electron pair acceptor) is referred to as coordinate bonding or coordinate covalent bonding. The ligand can be an ion (possibly an ionic form of the central metal atom) or a molecule. Broadly speaking, the electron pair acceptors are generally referred to as Lewis acids while the electron pair donors, Lewis bases (Lawrance 2010). Once coordination happens, the structural properties such as the number of bonds, bond angles and bond distances of the new substance formed causes it to have an altered physical property compared to their individual components. Thus, the new physical properties are dependent on the nature of the central atom (either same or different atom type) and the ligands involved. The electron pair acceptor, which serves as the central atom, is usually a metal or a metalloid. When there is only one central atom involved, the newly formed structure is called a monomer. When the number of linked units are low, the structure is considered to be an oligomer (Naka, 2014). If several central atoms are involved, they would either be held together by an atom-atom bond or be bridged by a ligand making them a polymer. This linkage can be done through a common donor atom, simultaneously binding to the central metal atoms, or through different donor atoms. The metal atoms can also be encapsulated in a polydentate ligand with each metal coordinating with several donors (Lawrance 2010).

Ligands can be grouped into two main classes; simple and heterogeneous ligands according to their structural properties and the ability to chemically define their structures and metal complexation properties. In the case of a simple ligand like EDTA, its molecular structure (composition and geometry), concentration (activity) of itself and its complexes, stoichiometry of its complexation with metals, metal-ligand formation free energy or thermodynamic equilibrium constant are known or can be known. However, heterogeneous ligands like humic substances may exhibit characteristics that are opposite to that of simple ligands; polyfunctionality which results in high electrical charge density, structural modification as a function of e.g. pH, ionic strength, metal binding, and variation in molecular weights (Buffle et al., 1990; Filella, 2008). Thus, the ligand can present either a single or multiple donor atom; if
only one donor atom is coordinated, it is referred to as a monodentate ligand. If multiple donor atoms are involved, it is referred to as polydentate ligands. The 'denticity' therefore defines the number of donor atoms or groups that are available or used in bonding. For polydentate ligands, the subsequent lone pair after the first coordination, is oriented in a way that prevents them from coordinating with the same central atom. In an instance where the existing covalent bond is deformed, two lone pairs from a ligand can be attached to the same metal thus forming a cyclic compound referred to as a chelate. Even though the shape of the ligand influences the strength of the complex formed and the coordination, there are no rules to really predict an expected shape to be formed by a ligand in complex structure. In general, chelate rings exhibit an enhanced stability compared to monodentate ligand since the size of the stability constant is almost invariably proportional to the number of coordinated donor atoms in a ligand (Lawrance 2010). Figure I-2, shows various possible coordination of a central metal atom to a ligand.



Figure I-2: Possible coordination of metals (M) to ligands (L).

Carbon can act as a ligand (*e.g* carbanion (H₃C⁻)) and effectively donate electrons to coordinate to a metal. This type of coordination can occur either by σ covalent bonds or π covalent bonds when the sides of the carbons equidistance from the central metal are involved. The d_z² and d_x². y² orbitals of the transition metals are also often involved in σ bonding while the other three more stable orbitals d_{xy}, d_{xz}, d_{yz} are mostly involved in π bonding. They have incomplete dorbitals and an electronic configuration of ns, (n-1)d, np with varied oxidation numbers (Lawrance 2010). The oxidation number indicates the number of available spaces that have been created as a result of the loss or gain of electrons, which influences the tendency to coordinate with ligands to make up for this loss. For metals with different oxidations states, the chemistry of each state distinctively differs from the others. The effect of ionic interactions on the valence electrons (p orbitals for main group, d orbitals for transition group and f orbitals for lanthanides) influences its bonding and coordination. The valence shell of a d-block elements

can be considered as having a total of 9 orbitals (18 electrons) which comprises, 5 nd orbitals, (n+1)s and (n+1)p orbitals respectively. To achieve a stable oxidation state, elements in the d-block (transition metals) of the periodic table ought to have a partially filled nd subshell. The five d $(d_{xy}, d_{xz}, d_{yz}, d_z^2 \text{ and } d_x^2 g^2)$ orbitals occupy different spatial orientations and present two different shapes. Two of the five orbitals lie along the axes while the other three orbitals lie between the axes of the coordinate system. This is however different from elements in the main block which require either a fully occupied or empty subshell to attain oxidation stability.

When there is a direct linkage between the metal atom and the ligand, it is referred to as a direct or inner sphere complexation. If, however the metal atom is bridged to the ligand by a water molecule, it is referred to as indirect or outer sphere complexation. For carboxylic groups, the metal atom can be directly linked to one of the oxygen atoms of the carboxylate (monodentate) or to both oxygen atoms (bidentate) as shown in Figure I-3. However, this behaviour depends on the metal ion involved as *e.g.* in the case of Ca^{2+} as the central atom, there can be a proton transfer from the water to the carboxylic group at a low water content (Aquino et al., 2011). Small ions preferentially form monodentate outer sphere complexes to avoid steric hindrance (Allen, 1993; Dudev and Lim, 2004; Falck, 1989; Kunhi Mouvenchery et al., 2012). An increase in the number of water molecules in the coordination shell of the cations favours more monodentate coordination than bidentate coordination.



Figure I-3: An image of bidentate inner sphere (M1), outer sphere coordinated (M2) and monodentate inner sphere complexation (M3) contact ion pairs for two carboxylic groups respectively.

2.2 Preferential binding sites

Two major types of functional groups, usually indicated as being of greatest importance with respect to NOM-cation binding, are the carboxyl and phenolic groups (Kalinichev and Kirkpatrick 2007; Chappaz and Curtis 2013). Phenolic groups are much less abundant than carboxylic groups. In assessing the proportion of these two main functional groups in 14 IHSS standards, Ritchie and Perdue, (2003), concluded that carboxyl groups constitute 78% to 90% of the total acidity for fulvic acids and 69% to 82% for humic acids. Thus, the average ratio of phenolic-to-carboxyl groups for the IHSS samples studied was about 21:79. The high affinity of these two groups to cations compared to other minor groups (ester, amine, alcohols, aldehydes and ketones) is highly dependent on pH (Mota et al., 1996; Nederlof et al., 1993). At high pH, there is an increase in deprotonation, which increases the available binding sites. Figure I-4, gives a summary of the preference of the two main functional groups of NOM and

their characteristic paths in complexation.



Figure I-4: A scheme of the Characteristic of the carboxyl and phenolic (PhOH) groups in complexation with metals (M) and hydro-metal (MOH).

2.2.1 Carboxylic group

The carboxylic group which is more acidic than the phenolic group starts to dissociate at pH > 4.4; e.g the dissociation of the carboxylic group of Suwanee river humic substance occurs at pH of 4.42 (Baalousha et al. 2006) making it responsible for the formation of negative sites on DOM (Aquino et al. 2011). During dissociation, a net negative charge is created which causes the production of large potentials at the surface of humic substance, and the formation of a double layer in their surrounding (Bryan et al., 2000). For humic substance to achieve steric stability there should be a repulsion of like charges. This repulsion of the negative charges causes an electrical double layer to be formed at each interface, which in effect increases the surface area for smaller sized particles as well as their binding affinities with cations. Figure I-5, shows some of the different carboxylic structures likely to be found in humic substances. As counter ions approach the locality of macro ions such as humic substances, they electrostatically interact with any available site (non-specific) or a targeted site (specific). The choice of which site can be quite difficult to determine. Some surface complexation models that have sought to improve the prediction of metal behaviour and their speciation in the environment are the NICA and Donnan Models. While the NICA model describes specific association, the Donnan model rather describes non-specific association (Kinniburgh et al., 1996). However for all models, one of the basic requirements is that both the functional groups and the humic substance should be simplified (Zhao, 2008).



Figure I-5: Carboxylic group moieties likely to be found in humic substances (Deshmukh et al., 2007).

Aside the affinities of the individual ligands, the nature of the environment in which they find themselves cannot be ignored. Thus carboxylate binding, depends on both the availability and type of interactions between the central metal atom and the carboxylate O atom, as well as between the carboxylate O atoms and its neighbouring ligands (Dudev and Lim, 2004). The

presence of functional groups containing heteroatoms in the close vicinity of COOH groups affects their ionization efficiency due to inductive effects. Electron withdrawing substituents on C atoms that are α positioned to the COOH groups, decreases the electron density leading to an increase in the asymmetric vibrational modes, enhances their acidity, and may also participate in chelation reactions (Hay and Myneni, 2007). Additionally, in macro ions such as humic molecules, where multiple ionizable groups are in proximity, electrostatic effects result in transmission of polarization through the solvent medium. This may affect the carboxylic groups' pKa values, which is a measure of their reactivity (Deshmukh et al., 2007).

2.2.2 Phenolic group

Phenols are considered as one of the most important groups for complexation with metal atoms (Chappaz and Curtis, 2013). Phenolic compounds are naturally leached from plant materials into the soil and water matrix and constitute about 60% of the plant dry mass (Cates and Rhoades 1977). Figure I-6, shows some phenolic structures that can be found in humic substances. If two hydroxyl groups are positioned ortho to each other, they can bidentatively complex with a metal atom (Guan et al. 2006). At higher pH (> 9.5), the phenolic group is more involved in complexation as it is easily deprotonated at this pH. The dissociation of the phenolic groups of Suwanee river humic substance occurs at pH of 9.68 (Benedetti et al. 1995; Baalousha et al. 2006). When molecules get larger in size, they tend to become less soluble and so its tendency to be coordinated to a metal ion is limited even though the rules of coordination do not change (Lawrance 2010). The presence of phenolic groups enhances the complexation capacity of carboxylic groups especially when it is located at the ortho position and at lower pH. This is because the ortho-phenolic-oxygen increases the electron density of the carboxylic group, which subsequently favours inner sphere complexation between the carboxylic group and the metal hydroxide even though aromatic carboxylic acids usually form outer sphere complexes with metal hydroxides (Nordin et al. 1998; Guan et al. 2006).



Figure I-6: Phenolic moieties that can be found in humic substances (Rappoport, 2003).

2.2.3 Other functional groups

Amines, a derivative of ammonia that has one or more of its hydrogen atoms substituted by an alkyl or aryl group constitutes a nitrogen atom with a lone pair of electrons. While Ammine, is a complex compound that contains ammonia molecule. This difference is important because the substitution of the hydrogen atom alters the electronic effect and polarity as well as the size of the molecule, which subsequently redefines the preferences of the ligands. At high pH, a monodentate ammonia ligand already coordinated to a metal atom can deprotonate (NH_2^-) to increase its lone pair of electrons to two and forms a bridge with a second metal atom. Thus, ligands can coordinate to two metals either by using a lone pair from each of the two donor atoms or using two lone pairs from the same donor atom. Amines are less 'sterically efficient' than carboxylate because the size of amine causes it to bump into other ligands when occupying coordination sites around a metal which often leads to a lower stability (Lawrance 2010).

Aldehydes and ketones although not abundant, are known to serve as ligands in transition metal complexes (Huang and Gladysz 1988). They consist of a carbonyl functional group (C=O) where the tetravalent carbon atom has two available sites for bonding. Aldehydes and ketones species are produced as by-products during ozonation in drinking water treatment (Shilov and Shul'pin 1997; Choi et al. 1998; Świetlik et al. 2004). Figure I-7, gives some of the ketones and aldehydes found in humic substances or in drinking waters as by-products. Ketonyl metal complexes can be formed either through trans-metallation (transfer of ligands from one metal to another), oxidative addition or by the interaction of electrophilic metal centres with ketones in their enol or enolate form. When the central metal atom is softly electrophilic during keto-enol equilibrium, a complex with a π -bonded enol can be formed which can evolve into a

ketonyl complex (Barone et al., 2012). The absorbance and emission properties (especially at visible wavelengths) of humic substances, can be greatly influenced by the presence of ketones and aldehydes, because they act as the primary acceptors in charge-transfer transitions within these samples (Vecchio et al. 2017).



Figure I-7: Ketone and aldehyde moieties that can be found in humic substances (Shilov and Shul'pin, 1997).

Esters, which are mostly formed by the replacement of a hydrogen of an acid with an alkyl or any organic group are known to form partial bonds with transition metals (Verkade et al., 1965). The presence of metal ions can increase the hydrolysis of amino esters. Hence, in the absence of a suitable metal ion, the hydrolysis of amino esters is very slow, but upon the addition of a metal ion, a rapid reaction is observed due to the cleavage of the metallic complex and pH. Upon further increase in the metal concentration, a 1:1 ester-metal complex formation is observed (Kroll, 1952). Phosphate di-esters, on the one hand, do not exhibit a chelating mode of coordination but rather prefers bridging to two adjacent metal ions. Phosphate monoesters, on the other hand, are likely to attract more metal ions around them and form larger aggregates due to the presence of two acidic protons and one phosphoryl oxygen (Murugavel et al., 2008). Sulphur functional groups, although present in humic substances in various oxidation states as thiol, thiophene or disulfide, sulfoxide, sulfone, sulfonate and sulphate esters are considered as minor functional groups (Filella, 2008). Figure I-8, shows some Sulphur moieties that could be present in NOM.



Figure I-8: Sulphur in organic compounds (Filella, 2008).

According to Bloom et al. (2001), a humic acid (HA) containing 0.2% of reduced sulphur has $63 \ \mu molg^{-1}$ of thiol sites. In peatland, sulphate reduction leads to the formation of sulphide which then reacts with metal(oid)s like antimony (Sb) or arsenic to form complexed precipitate at low or neutral pH values (Rittle et al., 1995; Dijkstra et al., 2004). The high affinity of Sb(III) for thiol can result in up to 44% of total Sb forming a 3-fold coordination to sulphur at a distance of 2.46 Å (Xia et al., 1999). Besold et al., (2019), however recently found that sulphide-reacted peat increases sorption of antimonite to as high as 98%. Carboxylic and phenolic groups are likely to make available additional sorption sites for antimonite in this particular matrix only when the amount of reduced sulphur is low or no metal (oxyhydr)oxides are available. In the case of low Hg concentration, thiols groups are seen to be the most preferred binding sites and likewise responsible for the binding of methylmercury (CH₃Hg⁺) (Skyllberg et al. 2003).

2.3 **Preferences of central atoms (cations) for ligands**

Protons are present in aqueous systems and originally occupy most of the binding sites of NOM. Thus, metal ions must compete with the protons for binding sites. When a metal (M) reacts with equimolar amounts of ligands L_A and L_B , the amounts of ML_A and ML_B, will depend on the cation preference and are rarely equivalent. This section looks at some S, D and P block cations that are present in the environment at significant concentrations and their preference for ligands.

2.3.1 s-block (calcium, magnesium, sodium)

Even though calcium (Ca^{2+}) exhibits a relatively weak bonding with humic acids, compared to trivalent cations, it cannot be ignored considering its high concentration in most fresh waters. Ca^{2+} predominantly binds with carboxylic sites at low pH but would equally bind with phenolic sites at high pH (Kinniburgh et al., 1999). Amine moieties show the same binding preference

as carboxylic acid towards calcium. However calcium has a preference for binding to larger and more negatively charged molecules because they generally have large ligand groups (Cabaniss, 2011). In calculating the potential mean force for the interaction of cations and carboxylic groups of NOM in aqueous solutions, Iskrenova-Tchoukova et al. (2010), found that, Ca^{2+} binding depended on the distance between the Ca^{2+} and the carbon atom of the carboxylic moieties. The presence of Ca^{2+} also results in competition with other cations for available binding sites (Cao et al. 2006) and the modification of the redox transformation of some competing cations like Fe (Jiang et al. 2017). Iglesias et al. (2003), concludes that at concentration above 2.5 mM, calcium can significantly compete with copper of concentration 1 μ M for adsorption sites on NOM. The presence of Ca^{2+} further causes relatively small NOM to aggregate and form larger colloidal particles of supramolecular structure (Kalinichev and Kirkpatrick 2007). This role (probably specific to Ca^{2+} , as the same observations were not made for other cations studied, Na⁺ and Mg²⁺), can be explained in two main geometrical paths (Iskrenova-Tchoukova et al. 2010);

- Directly, by cationic bridging between carboxylic groups from different NOM molecules. Generally metal bridging can occur at a monodentate or a bidentate configuration through inner and outer sphere coordination depending on the ion charge and the local ligand environment (Tipping and Hurley 1992; Kalinichev and Kirkpatrick 2007). Bidentate complexes can occur only if the two functional groups involved are close enough to undergo interaction with the same cation otherwise monodentate complex may be formed (Mouvenchery et al. 2012).
- 2. Indirectly, by Ca²⁺ simultaneously complexing with two or more carboxylic groups of the same NOM molecules. This coordination reduces the net charge and repulsion of the complex, thus allowing the complexes to approach each other more readily.

Magnesium (Mg²⁺) is with calcium, the primary cations responsible for the hardness of water. When dissolved in water, magnesium interacts with NOM present and results in the formation of soluble and sorbed complexes, and a variety of solid phases. Mg²⁺ equally competes with other cations for binding sites. Kalinichev and Kirkpatrick, (2007), suggest that, the strongly held hydration shell makes Mg²⁺ interact little with NOM. This is however, contradicted by Yan et al. (2015), who in studying Mg²⁺-NOM interaction using differential absorbance spectroscopy confirmed the existence of strong interactions between Mg²⁺ and NOM. This is however dependent on pH and the ability of Mg²⁺ to deprotonate phenolic and carboxylic groups of NOM. While there was an increase in differential absorbance with increase in Mg²⁺

concentration, an increase in pH (5 to 11), increases the concentration of Mg bound onto SRHA and SRFA although the effect of pH was more pronounce for SRHA. This noticeable effect in humic acid is probably because it has about 2 third less of carboxyl group content compared to fulvic acids. The phenolic content in fulvic and humic acids are statistically similar (Ritchie and Perdue, 2003). An explanation to the supposed minimal interaction could be due to the bond between Mg²⁺ and NOM which is mainly by nonspecific Donnan electrostatic interaction and so the quantity of Mg²⁺ that is bonded to NOM is minimal (Li and Elimelech 2004). The carboxylic group, forms strong association with Mg²⁺ compared to the phenolic group (Ahn et al. 2008). Even at higher pH, Mg²⁺ shares similar properties with Ca²⁺ and can still bond to the carboxylic groups and to a lesser extent to the phenolic groups of the NOM (Iglesias et al., 2003; Lu and Allen, 2002; Yan et al., 2015). This binding was accompanied by the replacement of protons in the protonation-active phenolic and carboxylic groups (Korshin et al., 2009).

Regarding sodium (Na⁺), on the one hand, it is considered that this cation forms only very weak outer-sphere complexes with NOM and the ions remain almost entirely in the solution to be hydrated by the water molecules. Therefore, Na⁺ does not bind to NOM and even its outer-sphere coordination to the carboxylate groups is statistically weak (Kalinichev and Kirkpatrick 2007). On the other hand, it is considered that Na⁺ could act as a relatively effective cross-linker for NOM (Aquino et al., 2011). An increase in the cation charge density increases equally the tendency to form a contact ion pair (Kalinichev and Kirkpatrick 2007). Iskrenova-Tchoukova et al. (2010), however, has disagreed with the conclusion of Kalinichev and Kirkpatrick, (2007) that Na⁺ does not bind to NOM, and states that Na⁺, like Ca²⁺, rather shows a strong preference for bidentate carboxylate groups even though the bond for the latter may be weak.

2.3.2 d-block (cadmium, copper, iron, zinc)

Cadmium (Cd²⁺) complexes with NOM under natural water conditions (Hertkorn et al. 2004). Cd²⁺ shows, a relatively weak binding, even though they prefer amine-containing sites to pure carboxylates and bind to smaller, less aromatic molecules (Cabaniss, 2011, 2009). Li et al. (1998), however, have stated that Cd²⁺ is rather primarily coordinated to O donor atoms (carboxyl groups) and would be coordinated to N donor atoms only at high pH with no observed coordination with S. In disagreement, Hertkorn et al. (2004), have stated that the coordination with S is possible. This has been evidenced in the case of soil organic matter where Cd²⁺ at a concentration of below 1000 μ g/g is exclusively bonded to the reduced organic sulphur ligands (thiols). Cd²⁺ forms inner-sphere complexes with two S groups in an octahedral geometry (Xia et al. 1997; Karlsson et al. 2005). At higher concentration, however, there is a contribution from

the organic O and N containing ligands (Karlsson et al. 2005; 2007). According to the bond distances and coordination numbers, in soil organic matter, Cd^{2+} is complexed to a mixture of a 4-coordination with S (thiols) and 4- and 6-coordinations with O and N containing ligands respectively (Karlsson et al. 2005).

In the environment, Copper (Cu²⁺) can exist in four different oxidation states (0, +1, +2 and +3), with the most abundant speciation found in pure water being Cu²⁺. The speciation of copper is influenced by pH and available oxygen (WHO 2003). The type of coordination it undergoes is determined by several factors, the most important being the ligand-to-metal ratio (Carrera et al., 2004). Cu²⁺ is very strongly bounded to humic acids even though this interaction is pH dependent, especially at trace concentrations. At a pH between 4 and 8, most of the binding can be attributed to the phenolic sites (Kinniburgh et al., 1999). However, Cabaniss (2011) has suggested that at pH below 7, the largest reactive sites in NOM are the carboxylate fractions and can form a vast number of coordination complexes with Cu²⁺. In terms of strength, the strongest bonds are expected to be formed with the carboxyl ligands. Cu²⁺ also shows high affinity for amine containing sites which are often located on small (MW < 1000 Da) lower aromaticity molecules (Cabaniss 2011). Cu²⁺ is complexed by O and N functional groups in NOM, forming a structure involving one or two five-membered chelate rings (Karlsson et al. 2006).

The remarkable affinity of Cu^{2+} towards NOM, compared to the other cations, may probably be due to the excellent match in size between the cupric ion and one or several ligands (*e.g* oxygen and sulphur) (Kinniburgh et al., 1999; Manceau and Matynia, 2010). Thus NOM has a high sorption capacity and selectivity for Cu^{2+} over a large concentration range (Gao et al. 1997). The size-match fit (strain energy), is a function of the bond-length and bond-angle deformation, the torsional strain of the chelate, and van der Waals interactions among non-bonded atoms (Manceau and Matynia, 2010). Considering the poly-functional nature of NOM, Cu^{2+} is likely bonded to more than one functional group in proportions that may vary with the metal concentration, the pH and the chemical composition of the organic matter. In the presence of other cations however, Cu^{2+} would have to compete for binding sites. Chappaz and Curtis (2013), in studying the simultaneous interaction of Cu-Al-DOM, observed that Cu is strongly complexed with the high molecular weight fractions of NOM than the low molecular weight fractions. Due to the availability of relatively sensitive and inexpensive electrochemical methods for the measurement of Cu^{2+} in Cu-NOM complexation, copper complexation has been the model choice of many studies (Filella, 2008).

Iron (Fe) mainly forms complexes with NOM as Fe^{2+} or Fe^{3+} with the speciation being highly influenced by pH. The presence of humic substances, however, slow down the oxidation of Fe^{2+} as well as the aggregation of the Fe particles (Pédrot et al. 2011). Even though the hydroxide and oxide forms of Fe have low solubility in water, it is still possible to find high concentrations of such Fe speciation associated with organic colloids in waters rich in organic matter (Pokrovsky et al. 2005; Pédrot et al. 2011). Humic substances equally inhibit the crystallization of iron hydroxide and iron oxides, by forming Fe nano-(hydr)oxides coated with DOM and Fe(III)-DOM complexes (Pédrot et al. 2011). At pH lower than 3 and higher than 6, Fe species bind to humic acids thereby indicating that it has the same relative affinity for carboxylic and phenolic humic acid sites (Dudev and Lim, 2004; Marsac et al., 2013).

In the presence of EDTA in wastewater, transition metals especially Fe^{3+} because of its increased valence electrons interacts strongly with the nitrogen groups of EDTA (Muñoz and von Sonntag, 2000).

Zinc (Zn) in the aquatic environment can either be linked to the organic or inorganic phases. At low concentrations, the sorption of Zn^{2+} occurs mostly on pH-dependent binding sites of oxyhydroxides competing with its binding to organic matter. Zn could sorb to clay minerals by ion exchange processes (Mertens and Smolders, 2013). Zn²⁺ generally shows a weaker binding strength compared to other transition metals like Cu²⁺ and Ni²⁺, and prefers to bind rather to amine groups and smaller less aromatic molecules instead of carboxylates (Cabaniss, 2011, 2009). Zinc is most likely to be involved in inner sphere complexation and can be coordinated to four or six O atoms and also form 4 coordination with N and S ligands (Karlsson et al. 2007).

2.3.3 p-block (aluminium, lead)

In the aquatic environment, aluminium (Al³⁺) complexes with NOM, which subsequently modifies its speciation and influences its toxicity. The exchange reaction between Al³⁺ and H⁺ and the hydrolysis of Al³⁺ on organic matter exchange sites are important factors to buffer pH and control the activity of Al³⁺. In the presence of other competing cations like Cd²⁺ and Pb²⁺, Al³⁺ can reduce the amount of Pb²⁺ ions bound to the carboxylic functional group by a factor of 2 to 3 especially at lower pH of 3 to 5.5 (Pinheiro et al. 2000). This competition effect is solely dependent on its affinity for the carboxylic, phenolic or chelate ligands, which is pH dependent. Al³⁺ like Fe³⁺ strongly influences the speciation of rare earth element in organic rich waters. At pH \geq 3, Al³⁺ competes with rare earth elements/lanthanides suggesting that Al³⁺ is bound to the carboxylic sites rather than the phenolic sites. At neutral pH such as in natural waters, Al³⁺ is expected to bond to weak carboxylic groups (Dudev and Lim, 2004; Marsac et

al., 2012). Other studies observed that Al³⁺ has a unique preference for phenol-containing sites and larger, more aromatic molecules (Cabaniss, 2011).

In the natural aquatic environment, Lead (Pb) may exist in two oxidation forms (+2 and +4) with the +2 being dominate. Pb^{2+} exhibits a binding strength which is similar to that of Cu^{2+} and form strong binding with amines dominated NOM. It equally binds with other groups such as phenols, alcohols, ether groups and larger molecules but forms slightly weaker bonds with carboxylate (Cabaniss, 2011, 2009). The large ionic radius of Pb^{2+} (1.19 Å) makes it possible for it to form complexes with varied coordination numbers (2-15) of central atom. The complexation of Pb^{2+} , with oxygen donor atoms, forms products with two different bond lengths. While the primary bond exhibit a predominate covalent property, the secondary bonds are to a larger extent ionic and in most cases serving as a bridge for molecules (Davidovich et al., 2009).

Table I-1, gives a summary of cations preference for each of the main functional groups. When there are contradictory results from different researchers, both results are presented.

Functional groups	Carboxylic group	Phenolic	Amine	Sulfur	References
Cations				1	
Al	++	+	n.a	n.a	(Pinheiro et al. 2000; Cabaniss 2011; Marsac et al. 2012)
Са	+ (low pH)	+ (high pH)	+	n.a	(Kinniburgh et al. 1999; Iskrenova-Tchoukova et al. 2010; Cabaniss 2011)
Cd	+	n.a	+	+	(Perdue et al. 1984; Hertkorn et al. 2004; Cabaniss 2009, 2011)
Cu	+ (pH < 7)	++	++	n.a	(Kinniburgh et al. 1999; Torbjörn et al. 2006; Cabaniss 2011)
Fe	+	+	++	n.a	(Pédrot et al. 2011; Marsac et al. 2013; Muñoz and von Sonntag 2000).
Mg	++	+	n.a	n.a	(Lu and Allen 2002; Iglesias et al. 2003; Ahn et al. 2008; Korshin et al. 2009; Yan et al. 2015).
Na	+	n.a	n.a	n.a	(Iskrenova-Tchoukova et al. 2010)
Pb	+	++	++	n.a	(Cabaniss, 2011, 2009).
Zn	+	n.a	++	+	(Karlsson and Skyllberg 2007; Cabaniss 2009, 2011).

Table I-1: Cations preference for the main functional groups of NOM. N.A = Data not available

2.4 Complexation equilibrium of NOM

Metals (s, p and d blocks) as well as ligands have their preferences that are largely determined by their electronegativity, a factor of both the size of the atom and number of valence electrons. Generally, p-block and d-block elements have higher affinities compared to s-block elements. Hence, very electronegative ligands prefer less electronegative metals (and vice versa). Further, there is a stronger adsorption between metal cations and acidic functional groups when the acid is weaker and cation valence is higher. The complexation strength for lighter metals increases with charge to size ratio of the binding ion (Skyllberg and Magnusson 1995; Kalinichev and Kirkpatrick 2007). Metal ions can be bound either as counterions (due to electrostatic forces) or bound specifically to reactive groups. The competition between metal ions may be largely due to the chemical nature of the bound ions. Depending on the deprotonation state and type of cation, the cation-NOM interaction can be specific or unspecific which may be indirectly influenced by the non-polar moieties (Tipping and Hurley 1992). Unlike cation exchange mechanism where there is a fixed and permanent negative charge, the ion exchange behaviour shown by humic substances is not in general charge equivalent (Kinniburgh et al., 1999). The cation-proton exchange depends on the polyelectrolyte structural charge density, the degree of ionization, the type of cation and the total cation concentration in solution. The release of protons during dissociation can either be due to competition from cations for the same binding sites or a decrease in the intrinsic deprotonation constant of non-dissociated groups due to the binding of cations at neighbouring polymeric sites (Mota et al., 1996).

The low ionic charge and relatively large size makes alkali metal ions form complexes faster than other ions on the periodic table, though formed complexes are generally weak. When they react with multidentate ligands, the rate of reaction depends to some extent on the nature of the chelating agent and the differences in the stabilities of the complexes are seen in its dissociation rate. Alkaline earth ions however demonstrate considerable variation in reaction rate depending on the element involved. For example, the greater charge density on Mg^{2+} makes its bonding considerably faster and stronger compared to Ca^{2+} . The rate of reaction is therefore dependent largely on ionic size. The rate of Ni^{2+} is however, about a power of 10 slower than that of Mg^{2+} although they have similar ionic radii. Likewise, is the rate of Cu^{2+} adhesion much higher than that of Ni^{2+} . The rate of reactions for trivalent metal ion reaction is slower compared to divalent ions but they form stronger bonds (McAuley and Hill 1969).

From the sections 2.3, it can be established that the complex and ill-defined nature of NOM place a huge limitation on our ability to measure and interpret the complexation equilibria of

NOM (Filella 2008). In particular, Town and Filella (2000), in a review of trace metal complexation parameters for natural waters highlighted the variation in the results of each work, which was probably, due to the different experimental conditions and techniques used for each study. The use of varied methods for the determination of cation complex with functional groups makes it challenging to quantify and assign a unique definition to 'strong' or 'weak' affinity. For instance, while on the one hand, Cabaniss (2011), used *a priori* model to define binding strength/affinity ("weak" log $K_{ML} < 5$, "moderate" $5 < \log K_{ML} < 10$, and 'strong' log $K_{ML} > 10$), based on the conditional formation constant (K_{ML}) for complex 'ML' from 'M' and 'L', Iskrenova-Tchoukov et al. (2010), on the other hand, used bond angles and lengths (radial distribution functions and potential mean force), to define the strength of affinity.

3. Impact of complexation on physico-chemical characterization of NOM

Complexation reactions modify the basic fractioning constituents of the NOM; concentration, polarity, size, solubility, absorbance properties and acid, base, and neutral characteristics (Yan and Korshin, 2014). This session highlights some of the possible modifications due to NOM-metal complexation and their impact on NOM characterization.

3.1 Morphology (size, hydrophobicity, charges)

Factors that are affected by the cation-NOM binding include molecular size, composition and configuration of the organic matter, pH and ionic strength of the solution and the chemical properties of cation and cation/NOM composition (Ritchie and Perdue 2003; Kalinichev and Kirkpatrick 2007). In analysing the effective MW distribution of the different fractions of NOM in the presence and absence of ions using light scattering techniques and high performance size exclusion chromatography (HPSEC), it can be observed that the size of NOM is significantly reduced when the ionic strength (for Na⁺) of the solution is increased. This is probably due to coil-to-globule conversion and polyelectrolyte interaction that caused the NOM conformation to change from linear to spherical. However, the effect on the size would also be dependent on the cation involved, since the size of NOM is rather increased when the cation under consideration is Ca^{2+} instead of Na⁺. This is possibly due to the aggregation or chelating between humic acids and Ca^{2+} (Xi et al., 2004).

Bowers et al. (2015), in using ⁴³Ca NMR, X-ray diffraction and helium ion microscopy to study cation binding and dynamics in flocculated NOM, however observed no changes in the floc morphologies with the type of NOM fraction, solution pH, or solution ionic strength, though admittedly certain pH and ionic strength were needed to induce flocculation.

In varying the pH at which NOM complexes with cations, Ritchie and Perdue (2003), observed a downwards drift in pH above 6.5 even when aliquots of NaOH was added. This suggests that the slow reactions of some ligands generate additional acidity. Proton association and dissociation from a ligand is influenced by its molecular structure through polar substituents and resonance effects. In comparing formic acid (HCOOH) to acetic acid (CH₃COOH), though they both have an –OH group, the former is seen as a stronger acid because of the ability of the hydrogen atoms to attract electrons more strongly, by displacing electrons from the –OH group in the direction of the O atom. An increase in the distance from the acid group decreases the

influence of the polar components present. Likewise, saturated hydrocarbons groups are electron-repelling while unsaturated ones are electron-withdrawing (Tipping 2002).

3.2 Electronic spectral changes

The optical properties (absorbance and fluorescence) of DOM have been greatly explored in studying its characteristics. This is because of the presence of chromophores and/or fluorophores in the ligands of NOM. Optical properties of DOM are highly variable and controlled by different physico-chemical parameters (pH, photoreactivity, redox conditions) and biological processes (Jaffé et al., 2008). When chromophores or fluorophores absorb light in the UV-vis region, electrons in the ground state are excited to higher energy states. The nature of the transition is mainly $\pi\pi^*$ and $n\pi^*$. The molecular structure therefore determines the absorption maximum and intensity. As conjugation increases in a structure, the maximum wavelength moves to longer wavelength. Classical optical spectroscopy techniques are widely used to study DOM and its interaction with metal cations. Indeed, absorbance or fluorescence measurements are inexpensive, easy, relatively rapid and do not require sophisticate pretreatment.

UV-vis spectroscopy can provide some structural information about the organic matter in water. Spectral features obtained for a complex mixture of molecules can be generally considered as the average of individual compounds that form the solution. Specific UV absorbance (SUVA), ascertained as the UV absorbance of a solution at a given wavelength normalized with DOC concentration, is strongly correlated with the degree of aromaticity of DOM (Weishaar et al., 2003). This indicator of DOM aromaticity is often calculated at 254 nm (SUVA₂₅₄) (Kim et al., 2018; Lee et al., 2018). Numerous studies have shown that the variation of the SUVA₂₅₄ descriptor is directly linked to metal complexation. Studies on copper, zinc, cadmium and nickel complexation with DOM on surface waters, isolated using reverse osmosis, have highlighted important positive correlation between the absorbance indices and the metal binding affinity pointing out that, in those waters, aromatic humic substances are the predominant metal chelators (Baken et al., 2011). This result was also confirmed for iron in natural fresh water (Fujii et al. 2014; Kikuchi et al. 2017). In anthropogenically influenced waters however, nickel is mainly complexed to EDTA and can account for about 87% of all EDTA sites bonded (Baken et al. 2011). The complexation of Na⁺ and Mg²⁺ with tocopheroxyl radicals results in an increase in UV-absorbance (Mukai et al., 2012). Trivalent cations (Al, Fe, Cr) complexes with anthocyanins and its derivatives resulted in a stronger decrease in absorbance than Mg²⁺

(Sigurdson et al., 2016). Whereas an increase in absorbance intensity could be due to band broadening resulting from the superposition of un-associated and chelate form of the NOM, a decrease in absorbance intensity could also be due to a decrease in the energy needed to cause light-induced electron transition in a chromophore, shifting the absorption maximum to a longer wavelength (Fedenko et al. 2017). The spectral modification observed in the absorption spectra upon metal complexation is very difficult to explain in term of binding sites. This is why it is possible to have a more detailed structural information by using absorption spectroscopy coupled with quantum chemistry methods to determine the preferential binding site on a multisite ligand that constitutes a model molecule of humic substances (Cornard and Lapouge, 2004). The difference in the metals' effect on spectral properties of NOM is due to the individual preference of metals for certain binding sites and pH (Langford and Khan 1975; Cabaniss 1992).

However, the spectral changes observed in the UV-visible absorption spectra remain limited and difficult to interpret. Those observed in fluorescence emission are much more important and especially allow to highlight finer phenomena and further analysis of the data. The presence of metals can serve as quenchers or enhancers of fluorescence intensities due to the high affinity between metals and aromatic moieties though metals can sometimes be associated with oxygenated aliphatic carbons (Cabaniss, 2011). During complexation with metals, the properties of the fluorophores are modified making it possible to compare the free and the complexed ligands.

Fluorescence quenching, on the one hand, occurs by the fluorophore donating electrons to the quencher. Generally, quenching can occur without any permanent alteration of the molecule or photochemical reaction (Lakowicz, 2006). The complexation of Cu^{2+} and Ni^{2+} for instance, result in fluorescence quenching while that of Al^{3+} enhances the fluorescence of phenolic compounds at pH 5.0 and quenches at pH 7.5 (Cabaniss, 2011). A change in pH affects both the shape and magnitude of the quenching spectrum of Cu^{2+} ; at pH < 6, Cu^{2+} is more bonded to the carboxylic functional groups while at pH > 8, it is more bonded to the phenolic groups of fulvic acid. Likewise, Fe^{3+} quenches fluorescence very weakly when complexed with poly-acid ligands at pH 7.5 due to the formation of iron oxyhydroxides which sequester much of the Fe^{3+} though a much stronger quenching is observed at pH 5.0 (Cabaniss, 1992). On the other hand, fluorescence enhancement, occurs by a modification in the NOM complex through; (a) the movement of fluorophores into solvent regions (b) an increase in the rigidity of the molecular structure which consequently increases the fluorescence quantum yield (Lakowicz and Masters,

2008). Due to the indirect nature of optical measurements, quenching or increase in fluorescence may not necessarily be due to metal binding but also the presence of marginal aromatic ligands surrounded by abundant aliphatic ligands in the environment (Cabaniss, 2011).

Fluorescence excitation-emission matrix (EEM) coupled with parallel factor analysis (PARAFAC) allows categorizing NOM samples into a combination of several groups of fluorophores with similar optical properties. This method is widely used to characterize DOMmetal binding (Stedmon and Bro, 2008; Xu et al., 2013). The fit of fluorescence quenching by models, (for example, the Ryan-Weber model) enables the characterization of the fluorophore affinity for a given metal cation and the estimation of a complexation constant (McIntyre and Guéguen, 2013; Yuan et al., 2015). Recently, two dimensional fluorescence/Fourier transform infrared correlation spectroscopy allowed the localization of functional groups in different fractions of humic substances and thus to observe the ability of these groups to complex metal cations (Tang et al. 2019). Because molecular spectroscopy methods alone can be limiting in information about the coordination, and the structure of the metal ion binding site as well as conformational changes that occurs on the ligand sites, they may require other supporting techniques (Cornard and Merlin, 2002). One of such techniques that can accompany spectroscopic techniques to provide additional information on the different fractions of NOM with respect to their molecular weight is the high performance size exclusion chromatography (HP-SEC) (Her et al., 2002). The advent of ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) has equally helped to advance knowledge on NOM characterization. FT-ICR-MS can resolve simultaneously thousands of individual molecules from complex organic mixtures like NOM and subsequently provides the molecular formulae for most of the resolved ions (Koch et al., 2008). One major challenge however with this technique is the difficulty in separating noise from analyte peaks (Riedel and Dittmar, 2014).

4. Impact of cations-NOM interaction on water treatment efficiencies

The main goal of the drinking water industry in characterizing NOM is to predict its behaviour in the different treatment processes. This section looks at the impact of the presence of NOMcation complexes on treatment efficiencies of the most common techniques used in drinking water treatment plants.

4.1 **Coagulation and flocculation**

Coagulants can be inorganic salts, organic polyelectrolytes or inorganic-organic composites. Although baseline coagulation targets to remove turbidity, advanced coagulation also targets the removal of NOM as the dose and pH conditions are optimized. Several other factors such as coagulant type, mixing conditions, temperature, properties of NOM, as well as the presence of cations, influences the efficiency of coagulants to remove NOM (Matilainen et al. 2010). When the dominant fraction of NOM has a high molecular weight, the removal mechanism is mainly by charge neutralization and the required coagulant dose is lower, while when the dominant fraction has a low molecular weight, the required dose is higher as the removal mechanism is mainly by adsorption onto metal hydroxide surfaces (Matilainen et al. 2010). The most common salts used in coagulation are aluminium sulphate (alum), ferric chloride and ferric sulphate. These coagulants are added to water with the aim of forming coagulant-NOM complexes to remove mainly NOM by two main mechanisms; adsorption and charge neutralization (Davis and Edwards, 2017). The main NOM removal mechanism for ferric salt is by the adsorption of NOM onto ferric hydroxide flocs (Davis and Edwards, 2014). At a low Ferric/NOM ratio however, NOM has the capacity to interfere with the hydrolysis of a low dose of Fe-based coagulant by forming Fe-NOM complexes, which subsequently influences the size, reduce zeta potential, morphology and identity of the Fe precipitates, thereby limiting the coagulation efficiency of the iron salt. The presence of other cations such as Ca^{2+} can neutralize NOM thereby making enough Fe ions to be available for coagulation. Thus, the efficiency of the low dose iron-based coagulant is restored. When the Ferric/NOM ratio is high, irrespective of the presence or absence of Ca^{2+} , the coagulation efficiency is not reduced as there is sufficient Fe for coagulation (Davis and Edwards, 2017). Generally, ferric-based coagulants are seen to be more efficient in removing NOM than aluminium-based coagulants (Budd et al. 2004; Chow et al. 2009; Golob et al. 2005; Matilainen et al. 2010). Though the overall removal efficiency

of both salts is very close (70 and 67% respectively for ferric and aluminium based coagulants), ferric salts have an advantage of being able to remove more (25%) intermediate molar masses (1000 - 4000 Da) than aluminium salts. Aluminium salts however have a higher turbidity removal efficiency (Matilainen et al. 2005).

For alum, even at low concentrations of 0.5 mg and 1.0 mg Al L⁻¹, there is the formation of small Al(OH)₃-humic substance colloids, and the size of the precipitate colloid formed grows with an increase in the alum concentration (Wang et al. 2014). The presence of Ca^{2+} improves the coagulation of alum and equally increases the pH range for effective removal of even the low molecular weight acids especially at pH 7 and 8 (Davis and Edwards, 2017; Duan et al., 2012). The zeta potential of DOM in the presence of Al^{3+} was observed to increase with increasing addition of Mg^{2+} indicating that unlike Ca^{2+} , the presence of Mg^{2+} may suppress the binding between coagulant Al³⁺ and DOM (Zhou et al., 2017). In using aluminium coagulant at low dosages, residual aluminium concentrations after treatment can be relatively high because less humic substances are removed (and vice versa). High aluminium residual has been attributed to the presence of dissolved Al-NOM complexes formed when a high proportion of aluminium monomers is present in the aluminium-based coagulant (Yan et al. 2008; Kimura et al. 2013). Dissolved residual aluminium is complexed with almost all MW ranges (500 Da -100 kDa) (Kimura et al., 2013). The use of aluminium-based coagulants is currently of concern because aside the presence of residual aluminium in treated waters affecting the aesthetic properties, it may also cause health risk as it is linked to adverse neurological effects such as Alzheimer's disease (Flaten, 2001). Although an adjustment of the pH can minimize the aluminium residue from aluminium coagulants, this is often not done as another post pH correction is needed to prevent corrosion in distribution channels.

4.2 **Precipitation**

4.2.1 Lime softening

The main targets of softening are calcium ions, even though this method may also remove a limited portion of the hydrophobic NOM fraction (Semmens and Staples, 1986). Caustic soda (NaOH) and calcium hydroxide (lime) are added to increase the pH of the solution (> 9.5) and cause the formation of insoluble CaCO₃ and Mg(OH)₂ which would precipitate out of solution (USEPA, 2011). Unlike CaCO₃ which has an overall negative charge on its surface (Stumm, 1992), Mg(OH)₂ precipitates as positively charged particles with high surface area and amorphous structure which aids the sorption by NOM onto its surface (Thompson et al., 1997).

Russell et al. (2009), however observed that at very high pH above 11.5, both CaCO₃ and $Mg(OH)_2$ have positive charges with a great affinity for NOM adsorption. In addition, a limited NOM fraction is removed through precipitation, co-precipitation or both (Thompson et al., 1997). At high concentrations, (no specific values given in literature) NOM can complex with the Ca²⁺ cations and cause them to precipitate in a different phase (organo-calcium salt) from the CaCO₃ if the aqueous solubility of the complex is exceeded, thus inhibiting the formation of CaCO₃ and making the lime softening method inefficient (Liao and Randtke, 1986; Russell et al., 2009). In a jar test experiment of a modified lime-soda softening process to enhance NOM removal by the addition of MgCl₂, lime-soda ash removed about 43% of DOC in the presence of the magnesium salt (7.5 mg/L) compared to 13% when no magnesium was added (Bob and Walker, 2006). Polyaluminium chloride is also proposed to increase the formation of Mg(OH)₂ precipitates thereby increasing the removal efficiency of NOM at relatively low pH <10 (Yan et al. 2008).

4.2.2 Chemical precipitation

Chemical precipitation is one of the principal technologies used to remove dissolved metal pollutants from wastewater streams. This process, which is dependent on the metal concentration and solution pH, is achieved by modifying the solution chemistry through the addition of precipitating agents like Ca(OH)₂, Mg(OH)₂, NaOH and Na₂CO₃. The agents are added to raise the solution pH and form insoluble complexes with target metals (Wang et al. 2005). Unlike the lime-soda softening technology that mainly focuses on the major cations, chemical precipitation can target a wide range of trace metals. The presence of simple but strong complexing ligands like EDTA in wastewater, however, reduces the efficiency of this process. The formed metal-EDTA complexes are highly soluble, poorly biodegradable and extremely stable over a broad pH range (Wu et al. 2013). In quantifying the speciation of EDTA in influents and effluents of sewage treatment plants, it is estimated that Fe(III)EDTA represents the dominant EDTA species comprising 20-90% of the total EDTA in the effluents. While no significant EDTA is removed by biological or chemical processes during sewage treatment, nitrilotriacetic acid is degraded to a large extent (> 90%) (Kari and Giger, 1996). At higher pH (>7), Fe(III)EDTA is not stable and so other metals present like Zn can compete for binding sites (Nowack, 2002).

4.3 Membrane Separation

During membrane filtration (tight ultrafiltration, nanofiltration, reverse osmosis), NOM is removed principally through size exclusion, charge repulsion and hydrophobic interactions (Metsämuuronen et al., 2014). NOM is also known to be one of the main factors responsible for the fouling of low-pressure membrane in natural waters with the biopolymers and hydrophobic fractions being the largest contributors. This fouling is primary caused by the adsorption of NOM onto membrane pore walls, which blocks the pore spaces. The adsorbed NOM further interacts with other substances in the solution, making the solution chemistry an important factor for fouling (Tian et al., 2013). The presence of cations in solution would therefore influence fouling. Divalent cations in particular, partially neutralize the carboxyl functional groups of NOM and consequently reduces the repulsion effect on NOM. The partially neutralized NOM complex maybe easily adsorbed onto the membrane surface forming a densely packed fouling layer. The presence of Ca²⁺ increases the humic acid (HA) fouling of ultrafiltration (UF). Ca²⁺ probably forms Ca-HA complexes which are larger in sizes and consequently reduce the membrane pore sizes, sometimes reaching the cut off values for acids and humics (Amy 2001; Galjaard et al. 2004; Comerton et al. 2009). Li and Elimelech (2004), likewise, observed a decrease in the nano-filter membrane permeate flux of 49% and 16% when 1 mM of Ca²⁺ and Mg²⁺ were present, respectively. On the contrary, monovalent Na⁺ could mitigate HA fouling. The mechanism is probably due to Na⁺ ability to form neutralized Na-HA complexes with a size below the pore cut off size (Hong and Elimelech, 1997; Tian et al., 2013). Hence, both the nature of the ions as well as the NOM constituent have a considerable effect on the treatment process (Xi et al., 2004). The effect on fouling has also been attributed to high ionic strength which reduces the electrostatic double layer thicknesses of both membrane surface and NOM resulting in fouling (Ahn et al. 2008). But even at the same ionic strength, Ahn et al. (2008), observed that Na⁺ and Mg²⁺ cause less fouling of UF polyethersulfone membrane compared to Ca^{2+} due to the strong interaction of Ca^{2+} with negatively charged carboxyl groups of NOM (Kalinichev et al. 2008; Iskrenova-Tchoukova et al. 2010). However, this statement has been controverted, or cannot be applied to all types of membranes as Tang et al. (2014) observed that monovalent cations caused more fouling of polyvinyl chloride UF membrane by forming a gel layer, which blocked pores compared to divalent cations, which formed a porous caked layer structure on the membrane surface.

Aside cations complexing with NOM to increase fouling, they can equally form ionic bridges (stronger for Ca^{2+}) between the negatively charged sulfonate functional groups of the

membranes and the carboxylic groups of NOM which increases the adhesion between the NOM ligands and the fouled membrane surface, forming a very compact fouling layer (Iskrenova-Tchoukova et al. 2010). Ahn et al. (2008), however, suggested that divalent cations (Ca^{2+} and Mg²⁺), which are strongly associated with the carboxylate groups of NOM, may boost the aggregation of NOM rather than forming ionic bridges between NOM functional groups and a polyethersulfone membrane surface as they are weakly attracted to the membrane sulfonyl groups. This suggests that the aggregation of NOM in the presence of divalent cations could be a higher contributor to membrane fouling as partially neutralized NOM molecules are easily adsorbed on membrane surface. Moreover, the large size of organic colloids complexed with trivalent cations like aluminium (Al(OH)₃) could also easily block membranes pores (Leenheer and Croué 2003; Wang et al. 2014). Whereas divalent cations result in irreversible fouling, monovalent cations are only responsible for reversible fouling. The irreversibility of Ca-HA fouling was however, observed to decrease in the presence of Na⁺, as added Na⁺ is able to reduce bond strength of Ca-HA and between HA and the membrane, due to electrostatic shielding. A similar trend was observed when bovine serum albumin (BSA) was used as a surrogate for the protein fraction of NOM, though a mixture of both organic molecules (BSA and HA) resulted in more severe fouling suggesting a synergistic fouling effect (Tian et al., 2013).

Backwashing with demineralized water instead of the normal permeate can effectively control fouling of UF membrane caused by NOM-Ca complex. This is because UF permeate contains same multivalent cation concentration as feed water, while the absence of cations and NOM in demineralized water reduces the ionic strength and the Ca-bridging effect between NOM and membrane surface. Backwashing with demineralized water, then, substantially reduces the rate of increase in the transmembrane pressure. Another approach may be to raise the pH of the feed water in order to keep the carboxyl functional groups deprotonated, thus preventing their deposit onto the negatively charged membrane surface (Hong and Elimelech, 1997).

4.4 Activated Carbon

Activated carbon (AC) is mainly used to remove organic substances or micro-pollutants by adsorption. The carbon surface, which is predominantly hydrophobic, contains functional groups, which are formed during the activation process (Kilduff et al. 1996). When the activated surface is further oxidized, electrochemically or chemically (nitric acid and ozone), the number of oxygen-containing acidic functional groups, such as carboxylic and phenolic groups, on the surface increases, which, in effect, increases its sorption capacity for cations at near neutral pH. Uptake for cations equally increases with increasing pH due to the dissociation of the weakly

acidic oxygen-containing groups (Rangel-Mendez and Streat, 2002). The presence of humic substances can compete with target synthetic organic compounds and reduce the efficiency of the AC (Kilduff and Karanfil, 2002). There can likewise, be competition between cations for adsorption sites confirming that the solution chemistry can also alter the surface charges of the carbon (Sengupta, 2001). For instance, the lower ionic radius of Cu^{2+} (0.70 Å) makes it a better adsorbate than Pb^{2+} with a higher ionic radius of (1.12 Å) as higher ionic radius would result in rapid site saturation and steric overcrowding (Faur-Brasquet et al. 2002). Moreno-Castilla et al. (2004), observed an increase in the uptake of Cd^{2+} ions on AC pre-adsorbed with organic compounds (tannic acid). The capacity of AC to adsorb gallic acid also slightly increased in the presence of Cr³⁺. The adsorption of organic molecules on AC increases with increasing ionic strength and divalent ion content (Ca^{2+}). The increase in adsorption may be either due to an adsorption of larger molecules or an effective adsorption of the smaller molecules, as new adsorption sites are created through cationic bridging. Partial neutralization of the surface functional groups reduces intramolecular charge repulsion and molecular expansion. The ionic strength further influences the electrostatic interactions between the adsorbate and adsorbent and/or the solvent (Kilduff et al. 1996). This has been attributed to the neutralization of its functional groups when complexed with the metal ion, making the adsorbate more hydrophobic and increasing the adsorption capacity (Ferro-Garcia et al., 1998). The effect of NOM-metal complex on AC efficiency is thus due to both the properties of the AC (specific surface area, micropore volume, functional groups) and that of the metal ion (ionic radius and electronegativity) (Faur-Brasquet et al. 2002).

On the other hand, the presence of a chelating agent alters the adsorption properties of a metal ion as the chelates themselves are adsorbed onto surfaces (Nowack, 2002). This may affect the efficiency of adsorption depending on the targeted compound. Although tri-ammonium functionalized mesoporous silica has proven to remove about 96% of Cu(II)-EDTA chelates from aqueous solution with initial concentration of 0.1 mM, the maximum (> 97%) copper adsorption was achieved in acidic condition and low ionic strength (0.01 M) (Wu et al., 2013).

4.5 Oxidation processes

The presence of metal cations in water can influence (positively or negatively) the formation of disinfection by-products by changing the oxidation mechanism of the NOM by chlorination, depending on the cations involved and the NOM composition. For instance the presence of copper oxide reduces the formation of trihalomethanes (THMs) because the metal oxides consume chlorine while the reverse is observed for Cu^{2+} ions (Li et al. 2007; Navalon et al.

2009). The formation of trihalomethanes has been mostly seen to be reduced by the presence of metals from chlorination surrogate compounds such as phenol, 2,6-dihydroxybenzoic acid or tannic acid in the presence of diverse metals (Ca^{2+} , Mg^{2+} , Mn^{2+} , Fe^{2+} , Cu^{2+} and Al^{3+}) (Blatchley et al., 2003; Navalon et al., 2009; Liu et al., 2017). However, considering the chlorination of humic substances, the reverse is usually found. For instance, the increase of THM formation from humic substances in the presence of Mg^{2+} and Ca^{2+} . This has been attributed to the affinity of the metals for carboxylate functional groups of the substrate which consequently increases their reactivity towards chlorination (Navalon et al., 2009; Liu et al., 2017). Also, Cu^{2+} apparently activates certain components of humic substance that are otherwise inefficient precursors and so consequently enhances the formation of chloroform from humic acids during chlorination (Blatchley et al., 2003).

On the opposite, the presence of metal ions has been shown to enhance the formation of haloacetic acids (HAAs) from tannic acid through the production of strong oxidative radicals during chlorination (Liu et al. 2012). Furthermore, complexation of metals to the active sites of organic substrates resulting in a difference in electron densities depending on the type of cation, the change in electron density, affects the reaction activity of electrophilic substitution by chlorine with the corresponding carbon (Liu et al., 2012). The respective inhibitory or enhancement efficiency of the metals is directly proportional to their concentrations (Liu et al., 2012).

When the oxidation is by ozonation however, the presence of NOM can affect the stability of ozone by either directly reacting with the ozone or indirectly by scavenging OH radicals. In natural waters, the presence of NOM can act as inhibitors to stop ozone chain reactions by entrapping superoxides after reaction with OH radicals (Buxton et al., 1988). The protonation and complexation of amine functional groups of EDTA, nitrilotriacetic acid, decrease their reactivity towards ozone in water treatment. During complexation especially with transition metals, the rate constants are lower because the metals are able to interact with the nitrogen groups. For instance, the complexation of Fe³⁺ reduces the reactivity of EDTA to ozone by a factor of 500 since EDTA is mono-protonated at nitrogen (Muñoz and von Sonntag, 2000). This explains why EDTA is not removed from natural waters by ozonation. This interaction can however be weakened by a substitution of the ligand with OH⁻ (Muñoz and von Sonntag 2000; Gunten 2003). To limit the negative effects of NOM complex on treatment process, advance oxidation processes such as UV/H₂O₂ or chlorine, Fenton oxidation, ozonation, TiO₂ photocatalysis and photoelectrocatalytic oxidation, discharge plasma oxidation followed by alkaline precipitation have been proposed as a pre-treatment step to degrade NOM-metal

complexes (Wang et al., 2018). These techniques however either do not work for all metal complexes such as Cr(III) complexes or performs averagely. Photo-Fenton process and Fenton derived process combined with precipitation has been shown to exhibit an efficient removal of Cr(III) complexes (Ye et al., 2017, 2018) and Ca-phosphonate complexes (Sun et al., 2019).

4.6 Ion exchange

Ion exchange is used in water treatment to remove dissolved ions from waters and more recently to target NOM removal (anionic resins) (Li et al. 2014). The resin surface loaded with loosely bound ions exchanges the ions with similar charged ions in the solution (Bauman and Eichhorn, 1947). The presence of NOM or chelating agents can affect the efficiency of this method, as the formed NOM-metal complex is not in the ionic state to be exchanged. For anionic exchange resins, the presence of higher affinity chelating agents like EDTA can compete with target weakly acidic functional groups for exchange sites. Aside neutralizing cations, organic compounds and other solids present in water can foul exchange resins with anion-exchange resins being more susceptible than cation exchange resins. Fouling which occurs when very strong ions are held onto resins' surface or when the inner surface of resins are occupied by hydrophobic substance can be reversible or irreversible depending on the affinity of the ions involved (Dorfner, 2011). Fouling increases the frequency and volume of backwash requirement, increases the sensitivity to flow rate and temperature, increase conductivity of solution and reduces the overall efficiency by increasing volume of desorption concentrate (Abrams, 1982). Liu et al., (2017) recently improved the capacity of polymeric anion exchanger towards the removal of Cu(II)EDTA with nanoscale zero-valent iron deposition.

4.7 Research challenges for water treatment

The variation in the quantity and quality of NOM in the environment is likely to continue due to the effects of climate change. The variations in DOC trends and its complexation with trace elements may not only significantly impact water treatment process selection, design and operation but may also increase the tendency of many treatment facilities to violate water quality regulations on NOM, trace metals or DBPs. The description of the phenomena in real matrices remains a challenge. The coupling of size exclusion chromatography with an elemental detector such as ICP-MS looks promising but lacks at the moment a quantitative measurement validation (Piatina and Hering, 2000; Rathgeb et al., 2016). Other methods based on for example electrochemistry (Moutcine and Chtaini, 2018) are useful in elucidating complexation

in real matrices but applications in the field of water treatment are missing. Progress in many different areas, from high-resolution mass spectrometry to quantum chemistry modelling, could each bring their own valuable information to the field. Also, fluorescence detectors are more and more used on-line to also study the diel variability of NOM (Trubetskoj et al., 2018; Ignatev and Tuhkanen, 2019). This fast measurement will give valuable data on NOM sources, origins and dynamics in the environment and are useful for characterizing water sources. As trace metals and major elements could modify the response of probes, researchers should endeavour to take into consideration this possible effect. NOM-metal complexes impact on ozonation is an important aspect to cover in the next years especially considering the increasing use of ozonation for the treatment of wastewaters. The high concentration of metals coupled with the high level of organic matter make those effluents particularly exposed to such interaction.

5. Conclusion

The complex NOM structure, which contains several functional groups, controls the bioavailability and biogeochemical cycling of trace elements in water. The two main functional groups, *i.e* carboxylic and phenolic groups show their contributions varying with their deprotonation with pH, inducing a higher contribution of carboxylic acids at lower pH due to their lower pKa values compared to phenolic moieties. The type of bonding that occurs between NOM and cations is mainly coordinate covalent bonding as electrons are donated only by the ligand(s) involved. This coordination modifies the physico-chemical properties of the newly formed product, like morphology and spectral characteristics.

The NOM-metal formed complex impacts water treatment efficiencies. In coagulation, the complexation between the coagulant salts and NOM helps to remove NOM from solution. This process is further enhanced when Ca^{2+} is added. The impacts however are not always positive as for example the presence of high NOM concentration complexing with free calcium causes it to precipitate in a different form thereby reducing the efficiency of lime softening method. NOM-metal complexes are equally responsible for membrane fouling as cations partially neutralize NOM making the complexes easily adsorbed onto membrane surface. In addition, the presence of metals influences the formation of disinfection by products by modifying the mechanism in which chlorine reacts with NOM either by increasing or decreasing DBP formation or changing the species formed. The modification of rate constants during ozonation process has also been observed, although limited to model compounds (*e.g.* EDTA and Tannic acid) which are not representative of NOM behaviour.

Acknowledgement:

This work was supported by Interreg 2 Seas program via DOC2C's project. Authors also thank the Region "Hauts-de-France" and CPER Climibio.

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Chapter II

Methodology and experimental conditions

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1. Description of NOM samples

The samples used in this study can be classified into two main groups: water samples and concentrated NOM standard extracts. The water samples were obtained either from surface waters or from partner companies (PWN, SWW and DWG) at various stages of their full-scale treatment lines and pilot plants.

1.1 Origin of water samples

1.1.1 PWN-Andijk Site

Puur water en natuur technologies (PWNT), which is in the Netherlands, is wholly owned by the Dutch water utility company, PWN. PWN supplies drinking water to about 1.7 million inhabitants in the North-Western part of the Netherlands constituting more than 780,000 households, companies and institutions in the province of Noord-Holland. About 106 million cubic meters of drinking water is produced every year, sourced mainly from surface waters in Andijk, Heemskerk and in the dune area of North Holland. At the Andijk site (Figure II-1), the company extracts more than 25 million m³/year of water from the Ijsselmeer. The source of the Ijsselmeer is the Ijssel River (averagely 70%) which is a branch of the Rhine River.



Figure II-1: Water source treatment (a) Ijsselmeer lake (b) treatment plant in Andijk, Image source Google maps ©2009 (b) Friso Bouwgroep ©2016.

The main chloride source is the Ijssel (or Rhine) River while the exchange between Ijsselmeer Lake and the North Sea increases the salinity of the water especially in the summer when the boat locks are opened more frequently for passage. The average chloride content in the lake is about 108 mg/L with a prediction to increase to as high as 250 mg/L in 2050 if global temperature rises by 2°C (Bonte and Zwolsman, 2010). The average DOC and NO₃⁻

concentration of the lake is about 6 mgC/L and 6.5 mg NO₃/L respectively (Galjaard et al., 2005; Martijn, 2015). This average DOC concentration corresponds to that observed by PWNT after more than a decade of monitoring (Figure II-2).



Figure II-2: Concentration of DOC in the raw water of PWNT-Andijk treatment plant from 2007-2019. Red dotted line represents the yearly average concentration.

Water samples were obtained from two different lines of a pilot plant at the Andijk site. The first treatment line called 'SIX-CeraMac' consisted of suspended ion exchange (SIX) and ceramic filtration (CeraMac) processes. The principle of the SIX which was developed by PWNT involves the addition of resins with chloride affixed on its surface to the water before it enters the SIX contactors. These adsorbed chlorides are subsequently exchanged with the naturally negatively charged pollutants in the water. Therefore, SIX processes often precede membrane filtration to remove DOC, sulphate, nitrate and phosphate from water, thus, to improve membrane performance. Air is fed into the system to enhance the ion exchange process and keep the resins in suspension. The resin is separated from the treated water in a lamella settler, and then regenerated with a highly concentrated salt solution and reused.

CeraMac (microfiltration), also designed by PWNT, consists of ceramic membranes. Ceramic membrane is a type of artificial membrane made from inorganic materials like aluminium oxide (Al₂O₃) and titanium dioxide (TiO₂). The catalytic properties of the oxide (mainly TiO₂) nanoparticles facilitates the degradation of membrane foulants under oxidizing conditions (Kim and Van der Bruggen, 2010). For CeraMac, about 190 ceramic membrane modules are all housed in a single stainless-steel vessel. Water is fed into the microfiltration system from the bottom up (dead-end mode) with the help of a pump.

The second treatment line was the 'UF-RO' which consisted of coagulation, ultrafiltration and reverse osmosis (samples not taken) techniques. Figure II-3, shows both processing pathways.



Figure II-3: Flow path of water treatment processes. Sampling was done after each treatment step except for reverse osmosis.

1.1.2 SWW-Plymouth Site

South West Water (SWW) is a British utility company which was established in 1989 and located at Ernesettle Ln, Plymouth, UK. It provides drinking water to about 1.7 million people across Devon, Cornwall, Bournemouth and parts of Somerset and Dorset in the United Kingdom. About 127 million m³ of drinking water is supplied each year. Its raw water is mainly sourced from rivers and reservoirs (95%), notable among are Tamar River and Burrator reservoir respectively.



Figure II-4: (a) Burrator Reservoir (b) South west water treatment plant at Plymouth.

Burattor Reservoir, which was built around the 19th century, is surrounded by mixed woodlands which impact the water quality. According to SWW, the concentration and quality of DOC in

the reservoir has been increasing over the past years which is partly due to reduced acid deposition, elevated temperature, changes in land use, and events associated with climate change (Figure II-5).



Figure II-5: Concentrations of TOC in Burrator Reservoir for a decade (Galjaard et al., 2018).

Water samples were received periodically from the Plymouth treatment plant consisting of samples from Tamar River, Burrator Reservoir, SIX treatment step, In-line coagulation (ILCA) and CeraMac. The SIX and CeraMac techniques are the same as that of the Andijk treatment plant.



Figure II-6: Flow path of Plymouth drinking water treatment process.

1.1.3 DWG-De Blankaert site

De Watergroep, the largest drinking water utility company in the Flandres region of Belgium manages about 180 communities in West and East Flandres, Flemish-Brabant and Limbourg. The company produces about 130 million m³ of drinking water annually which serves about 3 million customers and more than 100 companies. About 72% of this quantity is sourced from ground water with the rest coming from surface waters. The De Blankaert site (Figure II-7) which is located at the west of Flanders produces about 40,000 m³ of water per day, about 80% of which is sourced from the Ijzer River. The river is a lowland stream of about 76 km which rises in France. A reservoir of about 3 million m³ stores and improves the quality of the water

before treatment. The surrounding area is mainly agricultural with intensive farming and cattle breeding. The quality of the reservoir is thus impacted by pesticides, potassium, boron, nitrate and sewage resulting in eutrophication challenges (Baert et al., 1996; Decleer et al., 2000). For the year 2002 alone, it was estimated that about 9 tons of orthophosphate, 424 tons of nitrates and 4,737 tons of suspended matter were washed into the lake Blankaart (Decleer and Denys, 2008).



Figure II-7: *De watergroep treatment plant at De Blankaert.*

Three sampling campaigns were carried out on the full-scale drinking water treatment plant in deBlankaart in the spring and summer of 2018 and another on the pilot plant in autumn 2018. Sampling was done at each treatment step of the full-scale plant namely; raw water, nitrification (biological ammonia oxidation), coagulation (ferric chloride), rapid sand filtration and intermediate chlorination, dual stage granular activated carbon filtration (GAC) and final chlorination (Figure II-8). Samples from a pilot plant which consisted of 4 different ion exchange resins were also received. Details of this pilot plant have been discussed in chapter IV.



Figure II-8: *Flow path of the full-scale treatment process of DWG-De Blankaert.*

1.2 Origin of NOM references and standards

NOM extracts were obtained from the international humic substances society (IHSS). Natural organic matter, fulvic acids and humic acids extracted from the Suwannee River were obtained from the IHSS. Suwannee River rises in the Okefenokee Swamp in South Georgia (USA) and flows southwest to the Gulf of Mexico. The main source of its organic matter comes from the degradation of peat and 'black' water and so its composed of a mixture of degraded tannins, lignins, carbohydrates and fats (Averett et al., 1994; Leenheer et al., 2000). This reference was selected because of its extensive use in the study of NOM characterization as well as NOMmetal complexation (Arakaki et al., 2010; Majzik and Tombácz, 2007). The aqueous-sourced standards were obtained using the resin adsorption method. Under this method, the dissolved organic matter is fractionated according to its polarity and solubility where the hydrophobic fractions are preferentially adsorbed onto the XAD-8 resin. The hydrophobic fraction that has been adsorbed is subsequently extracted with an alkaline solution of NaOH, precipitated at low pH to obtain humic acids and then desalted to obtain fulvic acid (Figure II-9). Thus, both the humic and fulvic acids isolated from aqueous-phase sources contain only hydrophobic organic acids (Aiken, 1985). Detailed elemental composition of the Suwanee extracts has been presented in Table V-3 of Chapter V.



Figure II-9: Extraction procedure for aqueous-phase source NOM extracts.

1.3 Sample preparation

Samples were immediately filtered onsite when possible with a 0.45 μ m polyether sulfone (PES) filter and a glass syringe. PES filters were chosen over cellulose filters to reduce carbon

leach from filters. The syringes were rinsed for at least three times with Milli-Q (18.2 M Ω ·cm at 25 °C) water before reuse on the field. The first 3 ml of the filtrate was discarded to prevent possible contamination from new filters. Small size glass-wares (syringes, TOC tubes) were cleaned by baking at 450 °C for 6 h (Sharp et al., 2002) to eliminate residual organic compounds that may have remained after washing. Samples were transported in opaque glass bottles. When large volumes of samples were received from partners, filtration was done on the same day with a vacuum pump. For samples that needed to be analysed later, 0.5 mM sodium azide was added to reduce biological activities that could degrade NOM before storing at 4 °C.

2. Analytical methods for DOC characterization

The choice of analytical approach for this study was influenced by the historical research experience acquired by our laboratory (LASIRe) over the years and the innovative apparatus we have at our disposal. This study therefore selected in addition to the bulk techniques, one of the state-of-the-art chromatographic techniques for NOM characterization. Hence, the main analytical tool used for this study was a high-performance size exclusion chromatography (HPSEC) coupled with UV absorbance, fluorescence and inductively coupled plasma mass spectrometry (ICP-MS) detectors.

2.1 Bulk techniques for NOM characterization

Due to the complex nature of NOM, different tools and techniques have been developed over the years to measure the optical properties or aromaticity (UV-visible and fluorescence spectrometry), total or dissolved organic carbon (DOC analyser), and the functional groups (GC-MS, FTIR, NMR, EPR) of the bulk NOM (Her et al. 2003; Laborda et al. 2008; Matilainen et al. 2011).

2.1.1 Dissolved organic carbon (DOC)

The DOC concentrations of samples were measured with a Shimadzu TOC-VCSH. Prior to measurement, the samples were filtered through a 0.45 μ m polyether sulfone (PES) filter. The measurement was done by combustion catalytic oxidation at 680 °C. The samples to be analysed were first sparged by adding a small amount of an acid (pH < 3) and a small amount of the carrier gas. This converts the inorganic carbon (IC) present in the samples into carbon dioxide which was subsequently removed in addition to the purgeable (volatile) organic carbon. Thus, the total carbon measured using this method is referred to as non-Purgeable Organic Carbon (NPOC). The CO₂ generated through oxidation process was detected using an infrared gas analyser, nondispersive infrared (NDIR) sensor. The CO₂ concentration measured was proportional to the integrated surface area of the peak and helps to estimate the DOC concentration initially contained in the sample after calibration. Potassium hydrogen phthalate was used for the calibration at a range of 0 to 20 mgC/L. For each sample, the result given was the average of at least three satisfactory measurements.



Figure II-10: Schematic diagram of a non-purgeable organic carbon measurement.

2.1.2 UV absorbance

Offline Cary 100 or 300 Bio (Varian) UV spectrometer was used in this study for wavelengths between 200 and 800 nm. The spectra range of the instrument was between 190 and 900 nm while the spectral resolution was 0.2-4 nm. The detector was a photomultiplier tube with Deuterium and Tungsten as source lamps for the UV and Visible regions respectively. The scan rate was 600 nm/min, the data interval was 1 nm and the average scan time was 0.1 s. The baseline was corrected before each analysis. Quartz cells of 10 mm, which had been thoroughly cleaned with Hellmanex III solution were used for the measurement. In the analysis of water, UV absorbance is used to semi-quantitatively characterize organic matter. This is because when light beam of certain wavelength (200 to 800 nm) passes through a sample, some of the energy is absorbed by the sample due to the presence of light absorbing components (chromophores) in the sample (Figure II-11). Thus, the concentration of the sample at a certain wavelength is proportional to the absorbance and can be calculated using the Beer–Lambert Law (equation 1). The choice of wavelength is made with respect to the type of chromophores contained in the sample or the targeted compound. UV absorbance at 254 nm is used as a surrogate for NOM characterization (Swietlik and Sikorska, 2006).

 $A = \varepsilon cl$ Equation (1)

Where 'A' represents absorbance, ' ε ', molar absorptivity (L/mol/cm), 'c', concentration (mol/L) and 'l', path length (cm)



Figure II-11: Light absorption properties of NOM.

2.1.2.1 Specific UV absorbance (SUVA)

SUVA offers an easy approach to determine the hydrophobicity of a sample. It is the ratio of ultraviolet light absorbance at 254 nm to the concentration of DOC in water. High SUVA (> 4) value means the enrichment in hydrophobic components while a low SUVA (< 2) means the dominance of hydrophilic fraction. SUVA has gained popularity because of its relation to the aromatic content of waters, origin of NOM, molecular weight of NOM, DBP formation and the amenability of DOC removal during drinking water treatment (Fram et al., 1999; Hua et al., 2015). The ratio between two different wavelength have also been identified as a way of improving characterization (Her et al., 2008). Kim and Yu 2007, for instance found that the ratio of A253/A203 correlates with the formation of DBPs.

$$SUVA \left(\frac{L}{\text{mg.m}}\right) = \frac{UV \text{ absorbance } @ 254 \text{ nm} (m^{-1})}{DOC (mg L^{-1})}$$
Equation (2)

2.1.3 Fluorescence

Aside compounds that absorb light, samples may also contain compounds that subsequently emit light (fluorophores). Fluorescence measurements were performed with a FluoroMax-3 from Jobin Yvon, Horiba. This instrument had a spectral excitation range of 240-600 nm and an emission range of 280-850 nm while the spectral resolution was 0.1-15 nm. The detector used was a photomultiplier tube with a Xenon lamp as the source. The excitation wavelength range for this study was from 240 to 440 nm while the emission wavelength was set at a range of 300-500 nm. An increment and slit of 5 nm respectively were applied to both ranges. As the electrons in an atom or molecule are irradiated, it becomes excited and moves to higher energy

level (S₁, S₂). As the electron returns to the ground state, such energy is released (emitted) resulting in fluorescence (Figure II-12). The wavelength at which absorption (excitation) and emission occurs is specific to the molecule. Part of the energy used in excitation is lost making the emission energy lower (longer wavelength) (Coble et al., 2014). The offline fluorescence spectrophotometer presents data as excitation-emission matrices (EEMs) where the intensities of the individual excitation-emission pair of peaks associated with different compounds classes can be identified (Coble et al., 2014).



Figure II-12: The Jablonski diagram. 'S' represents singlet states while 'T' represents the triplet states. ISC denotes intersystem crossing.

2.1.3.1 PARAFAC Model

Parallel factor analysis (PARAFAC) which is a multivariate modelling technique was used to analyse the EEM matrices. Its employs a three-way method to decompose the fluorescence signature of DOM into individual components and evaluates the contribution of each of the components to the total DOM fluorescence. With the help of this technique, the origin, biochemical composition and role of aquatic DOM is known or can be known. The fluorescence components are compared to the fluorescence characteristics of known reference standards and so are normally referred to as humic-like, fulvic-like or protein-like substances (Bro 1997; Fellman et al. 2010).

Prior to PARAFAC modelling, the EEM of the samples were Raman-normalized by diving the dataset by the integrated area under the Raman scatter peak (excitation wavelength of 350). The EEM of Milli-Q water was subtracted from the EEM of each sample. The fluorescent intensities were reported as Raman Units (RU) (Baghoth et al. 2011). When the outlier test was performed using non-negative constraints, none of the samples were outlier. Several models were tested

and the best selected was confirmed by the split half analysis and validation. All models were validated with split-half analysis after the number of components had been chosen. RandInitAnal was performed to determine the model with the least squared error. PARAFAC components were then compared with the fluoresence spectra database of the Open Access Openfluore to confirm the properties of the components. Due to the dorminance of the humic-like region, the datasets were divided into two groups; protein-like groups (Emission < 350 nm) and humic-like groups (Emision > 350 nm) (Coble et al., 2014) to prevent the protein-like region from being masked. Each group was then modelled differently and the components obtained were compared to that obtained before group segregation.

2.2 Auxiliary functions for bulk techniques

The complex nature of NOM makes it difficult to extract much needed information when characterizing it with only bulk techniques. The missing information makes it difficult to design and choose the best drinking water treatment technique for optimum NOM removal. For instance, the efficiency of membrane filtration is influenced largely by the hydrophobicity and size of NOM. While UV absorbance and SUVA may be able to express the former, the later needs other advance techniques. One of such advanced techniques which can fragment the bulk NOM into several fractions is the high performance size exclusion chromatography (HPSEC). When bulk detectors are used in tandem with fragment techniques like HPSEC, the operational conditions of the detectors may be slightly modified although its principle remains unchanged. In this study, an HPSEC was coupled with UV and fluorescence spectrometer, ICP-MS (discussed later under section 3) and an organic carbon detector (OCD).

2.2.1 UV absorbance

The type of UV detector coupled with the HPSEC was a 1260 infinity diode-array detector (DAD). The main advantage of a DAD over a variable wavelength detector is that, all selected wavelengths are measured simultaneously by the different diodes in array (1024 diodes). It uses a cartridge cell (max-light) with optofluidic waveguide technology to increase its performance. The cartridge cell increases the light transmission by the principle of total internal reflection along a non-coated fused silica capillary. This design thus minimizes the baseline perturbation usually caused by refractive index or thermal effect. The detector had a path length of 60 mm (high sensitivity cell). A Deuterium lamp was used as its light source and could operate at a

wavelength range of 190 to 640nm. The optical slit was at 4 nm by default. Although several absorbance wavelengths were monitored, only UVA₂₅₄ is discussed (Table II-2).

2.2.2 Fluorescence

The online fluorescence detector used in this study was an Agilent 1260 Infinity Fluorescence Detector Spectra (FLD). It had a low limit of detection with a Raman S/N > 3000 (using dark signal noise reference). The radiation lamp source was a Xenon flash-lamp. The 3 μ s flash produced a continuous spectrum of light from 200 nm to 900 nm. Since the online FLD allowed for either a fixed excitation wavelength with varied emission wavelength or vice versa, four methods were developed and applied to each sample. Thus, two of the methods targeted Humic-like substances while the other two targeted Protein-like substances. The wavelengths used in the four method development was influenced by the observed main peak regions in an excitation-emission spectrum of aquatic dissolved organic matter, as recorded in literature (Table II-1).

Component	Ex (nm)	Em (nm)	Peak labels ^{a(b)}	Description ^c
Tyrosine-like	270–280	300–320	Β (γ)	Amino acid, free, bound to proteins, probably from a degraded peptide material.
Tryptophan-like	270–280	320–350	Τ (δ)	Amino acid, probably from a less degraded or intact protein.
Humic-like	330–350	420–480	C (α)	Very aromatic humus with high molecular weight and quite widespread.
Marine humic-like	290–320	380–420	Μ (β)	Low molecular weight humus mostly found in marine environment though can be found in wastewater, wetlands and agrarian areas. It is associated with biological activity
Humic-like	240–270	380–480	Α (α')	Less aromatic humus with high molecular weight.

Table II-1: Some common labels of identified regions of fluorescence peaks in an excitation—emission spectrum,	, observed in
aquatic DOM (Coble, 1996)ª, (Parlanti et al., 2000)ª (Fellman et al., 2010a) ^c .	

Using the most common NOM fluorophore regions as a guide, the fixed excitation and emission wavelengths for both methods were selected. Where several wavelength maxima are recorded in literature, a test was performed to choose the optimum wavelength. For instance, although protein-like substance is reported in literature to have two excitation maxima (230 and 280 nm), 230 nm was chosen for this study because of the decrease in signal intensity observed at 280 nm. The parameters of the two methods are summarized in Table II-2.

Target components	Method	Detectors		
		UV (nm)	Fluorescene	ce (nm)
			Excitation	Emission
Humie like substance (III.)	Multi-Excitation		230- 310	430
Humic-like substance (HL)	Multi-Emission		244	360- 450
	Multi-Excitation	205-275	230-280	345
Protein-like Substance (PL)	Multi-Emission		230	305-390

Table II-2: Parameters of the online detectors.

2.2.3 Dissolved organic carbon

Online DOC measurements were outsourced to HET water Laboratorium, Germany where an SEC-OCD-OND system has been developed following the method of Huber et al., (2011). At the inlet of the organic carbon detector (OCD), the solution is first acidified to convert the inorganic carbons to carbonic acid. The working principle involves 3 main measurements;

- i. a small amount of the samples bypasses the column and its directly measured by the detector as total organic carbon (TOC)
- ii. a small amount of sample is filtered online over a $1.2 \,\mu m$ glass fibre filter before being measured by the detector after bypassing the column (DOC)
- iii. 5 ml of the sample is made to pass through the column before entering the detector to be measured as chromatographic dissolved organic carbon (CDOC).

3. High performance size exclusion chromatography (HPSEC)

3.1 **Principle of an HPSEC**

Chromatography refers to the process of separating a mixture between two phases: a stationary and a mobile phase. The sample to be analysed is injected into the mobile phase stream being pumped and subsequently carried into the stationary phase (column) to be fractionated. Generally liquid chromatography (liquid mobile phase) is a preferred technique for water analysis because it does not require any major sample preparation (Krstulovic, 1982; Laude and Wilkins, 1987; Wen et al., 2018). Figure II-13, shows the working principle of an HPSEC. Once the sample arrives at the stationary phase, it is separated based on its interaction with the stationary phase properties. Liquid chromatography can thus further be named according to the specific type of separation at the stationary phase, size exclusion in this study. After samples have been separated at the stationary phase, they are eluted to be identified by a detector. The choice of a detector depends on what sample component is of interest. For this study, the HPSEC was coupled with two spectral detectors (UV and fluorescence), an elemental detector (ICP-MS) and an OCD (outsourced to HET water Laboratorium).



Figure II-13: Working principles of an HPSEC.

3.1.1 Operational parameters

Phosphate buffer was selected as the mobile phase for this study because it does not absorb in UV, it's optimum for neutral pH analysis and it's equally economical. The different compositions of phosphates (K₂HPO₄ and KH₂PO₄) were chosen based on the method of (Song et al. 2010; Zhou et al. 2000). To achieve an optimum ionic strength, the concentration of the phosphate buffer was about 3 g/L. However, due to the coupling of the HPSEC with ICP-MS, the concentration of the salt in the mobile phase needed to be reduced significantly (≤ 1 g/L), to prevent salt deposit on the sampler cone of the ICP-MS and consequently reduce the signal intensity. The method was thus adapted by mixing 0.5 g/L of each of the phosphate compounds together to obtain a 1 g/L buffer solution. This solution had a pH of about 6.6 and an ionic strength of 0.039 M. Although this buffer concentration had a weaker ionic strength, it was still used because test on other buffers like ammonium acetate resulted in poorer peak resolution and separation. Other parameters of the HPSEC included injection volume (100 ul), flow rate (1.0 ml/min), pressure (81 bar) and temperature (23 °C).

3.2 Size exclusion chromatography (SEC)

This study was performed with a high performance size-exclusion chromatography (HPSEC). SEC is a technique used to separate the different components in a sample based on their molecular size. The column or stationary phase for SEC is often made up of porous gel. The porosity of this gel makes it selective, allowing smaller molecules to penetrate while blocking off larger molecules, which would subsequently be eluted earlier by the mobile phase or eluent. Thus, during the separation process, the high molecular weight (HMW) fractions are eluted first and so have a shorter retention time with the low weight fractions following in order of decreasing size (Amy and Her, 2004; Allpike et al., 2005). Figure II-14, illustrates graphically the principle of a SEC column where fractions are eluted based on their size as identified by a detector. The stationary phase for this study was an Agilent Bio SEC-5 (100 Å, 7.8*300 mm) column with spherical, high purity, porous silica and a hydrophilic polymeric coating. Although the column is ideally inert, the separation of various complexes is not always due to size exclusion because the chromatographic behaviour of solutions can be influenced by their hydrophobic and charge-exclusion interactions with the column resin (Piatina and Hering 2000). This could sometimes falsify the retention time resulting in a false fraction classification.



Figure II-14: Principles of a SEC.

3.2.1 Column characterization

The SEC column had a length of 300 mm, internal diameter of 7.8 mm and an exclusion limit of 100 - 100,000 Da. Thus, its total volume can be calculated using the volume of a cylinder formula $(\pi r^2 h)$ which gives a total volume of 14.3 ml. The void volume (volume of mobile phase in the column) was calculated using data available from the manufacturer. According to the manufacturer, Agilent, the maximum filling capacity of an HPSEC column is about 63%. Thus, in the SEC column, it is estimated that 63% of the total volume is occupied by the particles (silica gel) and 37% is the void volume (interstitial volume or inter-particle) which corresponds to 5.3 ml. This value corresponds with the manufacturer's test using the experimental approach (Agilent, 2015). The total permeation volume (inclusion or intrapartum volume) can further be calculated. The 63% fill density occupied by the particles includes the pore volume inside the particles. Typically, SEC particles have a higher pore volume than other types of chromatography stationary phase; often up to 60%. A small (< 100 Å) molecule should therefore be able to penetrate the total pore volume, which is 60% of the remaining 9 ml in the column. Thus, there was 5.4 ml of pore volume per column in which the SEC separation could take place. A molecule too large (> 100 kDa) to fit the pores will therefore elute with 5.3 ml of the eluent while the smallest molecule would elute later at 10.7 ml (5.4 + 5.3) which corresponds to a retention time of 10.7 min, at a flow rate of 1 ml/min. If a molecule elutes after the point of total permeation, it means that its fractionation was not purely based on its molecular size, hence an adjustment in the conditions of the mobile phase is required to prevent this type of non-specific interaction. A change in column tubing connection and pressure could also alter these volumes.

3.2.2 Column calibration

Since the retention time in SEC corresponds to a molecular size, the molecular weight (MW) calibration of the column was done using Suwannee River humic and fulvic acids from the IHSS. The MW of these standards are already reported in literature (Her et al., 2002). Furthermore, samples were requested from PWN (Ijsselmeer) to calibrate the column and identify the different NOM fractions found in natural waters according to the method of Huber et al., (2011). These same samples received from PWN, were sent to the HET water Laboratorium where they were analysed by both UV-visible and organic carbon detector (OCD) with a column already calibrated. This approach was chosen because according to the calibration by Her et al., (2002), using poly(ethylene glycol) (PEG) standards detected by both UV and OCD, slight variations were observed in the molecular weight depending on the detector (UV and OCD) especially for the humic acids. The column used by HET was a GPCkolom Toyopearl HW-50S, 30 µm (250 x 20) (hydroxylated methacrylic polymer). The chromatogram and apparent MW determined by HET for each of the fractions are shown in Figure II-15a. To compare the chromatogram obtained using our column to that of HET, the retention times were normalized and the UV chromatograms of the same sample were superimposed on each other (Figure II-15b). The various peaks obtained with our column were then matched to that of HET with respect to retention times and MW. Our column appears to have a better resolution than that of HET water Laboratorium as more peaks were observed under the various fractions.



Figure II-15: (a) Chromatogram showing the different NOM fractions and their apparent MW as determined by HET laboratorium. (b) Comparison of our column (LASIR) with that of HET laboratorium using chromatograms of the same sample.

The retention times of the standards and fractions as recorded with our SEC column and its corresponding apparent molecular weight are summarized in Table II-3.

Sample	Origin	Retention time	Apparent MW (Da)
		(min)	
SRHA	шее	5.1	~3,305ª
SRFA	IHSS	5.3	~2,114ª
Humic substance		5.9	~1,200 ^b
Building blocks	Ijsselmeer (NL)	6.7	~400 ^b
Low molecular weight		7.3	~300 ^b

 Table II-3: The retention times of standards and fractions used for column calibration as determined with our SEC column.

 (Her et al., 2002)^a (Huber et al., 2011; Metcalfe et al., 2016)^b.

The data obtained was used to plot an exponential curve. The x-axis represents the retention time while the y-axis represents the MW. From the calibration curve, it can be observed that, there is a regression error at high MW (> 3.3 kDa). The regression error is probably due to the proximity to the void volume of the column. Molecules with such high MW are most likely to be biopolymers. The difficulty in measuring accurately the very high MW fractions may be why HET Laboratorium estimated the size of biopolymers as >>20 kDa.



Figure II-16: SEC-Column MW calibration curve.

Since the elution volume decreases linearly with the logarithm of the molecular hydrodynamic diameter or molecular mass, there is a logarithmic relationship between molecular mass and the retention time (Mori, 1999; Huber et al., 2011). The average molecular weights of each peak was calculated using the formulae (Chin et al. 1994);

$$Mw = \frac{\sum_{i=1}^{N} hiMi}{\sum_{i=1}^{N} hi}$$
 Equation (3)

Where h_i is the signal height of the sample at retention time t_i and M_i is the molecular mass recorded at retention time t_i hitherto determined from the MW standard calibration curve.

After the column calibration, natural water samples were analysed and the various NOM fractions identified following the method of Huber et al., (2011).

3.2.3 Size fractionation of NOM

According to the method developed by Huber et al., (2011), NOM can be classified into 5 main fractions based on their size after they have been separated by a size exclusion column. The fractions are; A: Biopolymers, B: Humic substances, C: humic hydrolysates/Building blocks, D: Low molecular-weight acids E: Low molecular-weight neutrals. Figure II-17, shows the various fractions observed for NOM analysed with a SEC-UV.



Figure II-17: SEC-UV chromatograph showing the different fractions of NOM and their MW(Da) in a water sample.

- I. Fraction A: Biopolymers, have a high molecular weight (> 20 kDa) and elute close to the exclusion limit of the column which indicates that they may be hydrophilic (no hydrophobic interaction with the column). They are not removed by ion exchange resin and have very low absorbance in UV. Their components are likely to be a chain of polysaccharides with some protein containing molecules.
- II. Fraction B: humic substances, elutes at the same retention time as standard humic and fulvic acids with good agreement in peak shape and detector ratios.
- III. Fraction C: building blocks are mostly found in natural waters but absent in pure extracts. They are formed by the hydrolysis of humic substances and are least removed by flocculation. At high SEC resolution, sub-fractions of this fraction can be observed though their response to UV can be highly variable.
- IV. Fraction D: low molecular-weight acids elute at the 'salt boundary peak' and its due to the ion chromatographic effect. Thus, all LMW anions are forced to elute here due to the repulsion between the anions and the negative charges on the stationary phase (weak cation exchange resin) at neutral pH. An example of this fraction is acetic acid.
- V. Fraction E: low molecular-weight neutrals have low ion density. Since they do not elute at the 'salt boundary', it presupposes that they are not anions and they would have eluted later if they were cations. They are hydrophilic to amphiphilic and elutes close to the permeation volume of the column. They have poor absorbance in UV and are likely to be composed of alcohols, aldehydes, ketones, sugars, formaldehyde and amino acids.

Huber et al., (2011), further observed that the high hydrophobic fractions of many natural water samples result in a difference between the DOC value measured in the column bypass and that obtained for the hull curve of the chromatogram. This fraction is called the hydrophobic organic carbon (HOC) and its strong retention is due to hydrophobic interaction with the column.

3.2.4 Chromatogram peak deconvolution and integration

In order to semi-quantify each fraction, the peaks under the chromatogram were integrated. The chromatogram peaks were deconvulated as the sum of Gaussian peaks as defined in equation 4 (Laborda et al., 2009).

$$h = h_{o} + \frac{A}{w\sqrt{\pi/2}} exp\left(-2\frac{(t-t_{r})^{2}}{w^{2}}\right)$$
 Equation (4)

Where h signifies the signal measured at time t, h_0 refers to the baseline signal, t_r refers to the retention time of the peak maximum, w is the peak width at half its height and A, the peak area. The peak fitting option of the software <u>Origin 2017</u> was mainly used. The confidence level of the iterations was generally above 0.95. To integrate the peaks, the base line, retention time and the peak width of each expected peak was fixed.

4. Techniques for Characterizing NOM-metal Complexes

4.1 HPSEC-Inductively coupled plasma mass spectrometry (ICP-MS)

ICP-MS is one of the most powerful and frequently used techniques to quantitatively measure metals. It can detect both metals and non-metals at trace levels. The sample to be analysed is first ionized with inductively coupled plasma (generated by heating a gas with electromagnetic coil) before using a mass spectrometer to quantify the ions generated. Upon reaching the mass spectrometer, the ions are separated based on their mass-to-charge ratio and the signal received by the detector is related to its concentration. The instrument was calibrated with reference standards (single or multi-elements). In this study, Agilent 7900 ICP-MS was used as an online detector after size chromatographic separation since it is a fast multi-element technique with high sensitivity, selectivity and robustness. The coupling was done by connecting the column outlet of the HPSEC (An Agilent 1260 Infinity II Bio-inert SEC system) column directly to the crossflow nebulizer of the ICP-MS through a commercial polytetrafluoroethylene (PTFE) connector. The advantage of the bio-inert SEC system is that it is made up of metalfree components in the sample flow-path, thus the absence of iron and steel in the solvent delivery path ensures that trace metal contamination is eliminated. The column used was an Agilent Bio SEC-5 column with an exclusion limit of 100 to 100 kDa. The mobile phase consisted of 1 g/L phosphate buffer with a flow rate of 1 ml/min. As required, the needed tuning was done daily to adjust the ionic lens. Due to the concentration of the phosphate salt, the flow was diluted with an argon gas. The experimental parameters of the setup are summarized in Table II-4.

Instrument	Agilent 7900 ICP-MS
RF power	1550 W
Sampling depth	9 mm
Carrier gas	0.99 ml/min
Integration time	100 ms

Table	II-4:	ICP-MS	instrument	parameters
Tubic		101 1015	motrament	purumeters
4.2 Quantitative method development

The quantitative method developed for this study used EDTA to quantify the concentration of metals complexed to each NOM fraction at neutral pH. EDTA was chosen because it is a strong complexing compound and widely used for metal complexation studies. The complexometric titration was done by spiking known metal concentrations with EDTA at a neutral pH of 7. The pH was adjusted with an automatic titrator using 0.05 M NaOH and 1% HNO₃. Since the stoichiometry of EDTA complexation with metals is in the ratio of 1:1, the metal addition was done to ensure that there was excess EDTA at the final metal concentration. The solution was allowed 30 min for complexation reaction to be complete before analysis (Falantin, 2017).

4.2.1 Column care conditions

Before use, the column was thoroughly cleaned with 2% v/v Nitric Acid (67-69%, Optima, Fisher chemical) for at least 20 column volumes. The pH stability of the column is 2 - 8.5. This procedure was performed to enhance the mobility and elution of any retained trace metal in the column, especially those possibly precipitated as metal hydroxide, since the functional groups on the column surface could retain metals ions. Metal oxide nanoparticles are partially or completely retained in columns compared to their corresponding free ions. These oxidized metals can consequently block column pores (Zhou et al. 2016). The adsorption capacity of columns are in the order of amino columns > carboxyl columns > silica column (Zhou et al. 2016). Thus, our column which was made up of silica gel had a relatively lower adsorption capacity for metals. The column was subsequently rinsed with 0.5 mM EDTA and then with Milli-Q water at similar column volumes. The column was equilibrated before use with the eluent for about 30 mins (1 ml/min).

4.3 Method validation

The optimized method was validated using NOM standard extracts from IHSS. SRHA, SRNOM and SRFA standards were complexed with the 3 metals (Cu, Mn and Zn) of interest with the metals-EDTA solution as a calibrating standard. The concentrations of the metals were varied, and the repeatability of the results tested.

4.4 Complexometric titrations

NOM-metal complexation reaction can modify the basic properties of NOM; polarity, size, solubility, absorbance properties and acid, base, and neutral characteristics (Yan and Korshin, 2014) and further affects treatment processes such as coagulation, membrane filtration, disinfection by product formation and activated carbon. To study the effect of metal complexation on the spectral properties of NOM, a circulatory set up was used (Figure II-18). This set-up consisted of a beaker containing the solution to be analysed. With the help of a peristaltic pump, the solution was circulated between two flow cuvettes (10 mm) placed in a UV and fluorescence spectrometers respectively. An automatic burette was used to readjust the pH of the solution to pH 7. As both cuvettes were not touched until the end of the analysis coupled with the almost fully automated nature of the system, this set up had the advantage of largely eliminating potential human errors. After each metal addition, the solution was left for 30 min while stirring for the complexation reaction to be complete, after which it was pumped to be analysed.



Figure II-18: Set-up of the complexometric titration.

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Chapter III

Combining the physical and spectroscopic properties of NOM fractions to characterize DOC in drinking water treatment.

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1. Introduction

Many bulk techniques or parameters have been developed to study the complexed nature of NOM in water. Among them, UV absorbance at 254 nm (UVA₂₅₄) is mostly used. This method which has been used in some instances as a proxy to TOC analyser to quantify NOM, targets the light absorbing components (chromophores) of NOM. The SUVA parameter has equally been developed as an indicator of NOM's composition and reactivity. These bulk techniques are popular because they are relatively cheap and are able to measure the largest composition of NOM (Gao et al., 2020; Thurman, 2012). Another bulk parameter for NOM characterization which is attractive for its high sensitivity and rapid data acquisition is fluorescence spectrophotometry using fluorescence excitation emission matrices (FEEM). This technique uses the excitation and emission wavelength pairs to identify the light emitting components (fluorophores), in order to study the fluorescence of NOM in different matrices (Liu et al., 2019; Nagao et al., 2003). The fluorescence peaks are grouped under two main categories (humic-like and protein-like) and their wavelength maxima traced on a FEEM contour plot. Peaks with shorter emission wavelength (< 350 nm) are considered as protein-like substances while those with longer emission wavelength (> 350 nm) are considered as humic-like substances (Fellman et al. 2010). Methods like partial least square regression (PLS), peak picking and multi-way data analysis using parallel factor analysis (PARAFAC) have been developed to ascertain the characteristics of the different components that make up these two large groups (Persson and Wedborg 2001; Murphy et al. 2006; Stedmon and Bro 2008; Coble et al. 2014; Yang et al. 2015). PARAFAC especially, has been widely used to decompose FEEMs into different components in order to predict their primary sources (Coble et al. 2014; Fox et al. 2017).

However, the use of these bulk parameters alone may provide limited information about the characteristics of NOM. For instance, waters with similar DOC concentrations or UVA₂₅₄ does not indicate same characteristics in molecular weight or reactivity (Baghoth et al. 2011). These additional characteristics that are not provided by bulk parameters could be integral in

determining the efficiency of water treatment processes. For example, the use of coagulants is mostly effective for very hydrophobic (high SUVA) compounds with high molecular weight. Unfortunately, the latter characteristics cannot be determined by only bulk techniques. The same applies to filtration techniques where knowledge of the size of NOM composition is important in limiting the fouling rate of filters. Thus, understanding both the quantity and quality of NOM is important in improving the efficiency of water treatment processes to remove NOM.

High performance size exclusion chromatography (HPSEC), which classifies the components of NOM into different fractions based on their molecular sizes, has been widely used in characterizing NOM in aquatic system. The different fractions of NOM are biopolymers (polysaccharides, polypeptides, proteins and amino acids), humic substance (humic and fulvic acids), building blocks (hydrolysates of humic substances), low molecular weight (LMW) acids and neutrals (alcohols, amino acids, aldehydes and ketones) (Huber et al., 2011). These fractions can subsequently be detected using bulk techniques like UV, fluorescence and organic carbon detector (OCD) to gain detailed and specific characteristics of the different components of NOM. This chapter seeks to explore the advantages of using bulk detectors in tandem with fractionation techniques to characterize DOC in drinking water treatment. The aim is to bridge the knowledge gap in using only bulk detectors and possibly incorporate this combined approach into online monitoring at water treatment facilities, to accurately predict the evolution of NOM and optimize its removal during water treatment processes.

2.3D Fluorescence excitation-emission matrix (FEEM)

The wavelength at which a molecule or its moiety absorbs and emits light is unique to its chemical structure and can be used as a characterization tool. A contour map simplifies the complex FEEM data into two general signals: protein-like fluorescence and humic-like fluorescence (Li et al. 2016). According to peak-picking method, the protein-like substances could have two excitation maxima and fluoresce at excitations/emission wavelength of ~225(~280)/~305 (Peak B) and ~225(~280)/~350 nm (Peak T) (Carstea et al., 2016). Peaks B and T represent the tyrosine-like and tryptophan-like substances respectively. Humic-like substances (Peaks M, C and A) however fluoresce at longer emission wavelengths (> 350 nm). It can be observed from the contour map that the humic-like signals are dominant (Figure III-1a). The dominance of the humic like signals coupled with the proximity of peak T to the humic-like region means that what is observed at peak T may not necessarily be due to the presence of tryptophan but to an overlap of the humic-like signal (Aiken, 2014). To ascertain the real composition of peak T and the origin of the various fluorophores presents, PARAFAC analysis was performed.



Figure III-1: (a) 3D FEEM contour view of NOM in a raw water sample (b) A graphical representation of the most prominent fluorophore in aquatic NOM. The description of the letters A, C, M, N, B and T are detailed in chapter II, section 2.2.2 (Table II-1).

2.1 PARAFAC components extracted from fluorescence FEEM

A FEEM dataset of 41 water samples was used for the PARAFAC analysis. Both the FEEM contour plot of the 5 PARAFAC components as well as the loadings have been presented in

Figure III-2. The excitation and emission loadings of the components indicate their characteristic excitation and emission spectra while the component scores represent the relative concentrations of groups of organic fractions represented by the components (Stedmon et al. 2003). When the outlier test was performed using non-negative constraints, none of the samples were outlier. All models were validated with split-half analysis after the number of components had been chosen. For PARAFAC modeling, determining the right number of components is very important for correctly resolving the spectra (Bro, 1997). Among all the number of components tested for the model, 5 were found to be the best which was confirmed by the split half analysis and validation. RandInitAnal was performed to determine the model with the least squared error. Since the number of datasets used was relatively small, the models obtained were compared to the fluoresence spectra database of the Open Access Openfluor (www.openfluor.org) to identify the properties of the different components. Components from other studies which were comparable to ours were obtained for all 5 components. The characteristics of the components have been summarized in Table III-1.

Component	Excitation maxima (nm)	Emission maximum (nm)	Description
1	270	315	Tyrosine-like
2	285	350	Tryptophan-like
3	250(335)	455	Terrestrial humic-like
4	< 240(295)	385	Autochtonous humic-like
5	270(350)	500	Terrestrial humic-like

Table III-1: Summary of the spectral characteristics of the five PARAFAC components identified in this study

<u>**Component 1**</u>: This protein-like component which has Ex/Em maxima of < 260/315 nm and has been referred to as tyrosine-like fluorophore (Yamashita et al., 2011). This component is often not resolved as they can be quenched by energy transfer to nearby tryptophan in protein-like structures (Lakowicz, 2008).

<u>Component 2</u>: This autochthonous component has been identified as a protein-like component which is likely derived from microbial origin (Stedmon et al. 2003). This component has been referred to as peak T in other studies and represents trytophan-like fluorophore (Coble 2007) as it shares similar properties to the indole ring structure of tryptophan.

<u>**Component 3:**</u> This ubiquitous fulvic acid-like component has Ex/Em maxima of < 260 (320)/440 nm and its termed traditionally as terrestrial humic-like components (Chen et al. 2017). It is mostly found in waters with high organic matter content especially those within

forested regions as well as wastewater treatment plants although it has equally been found in an antartic lake where no higher derived organic matter input was obvious (Cory and McKnight 2005). It has an emission spectrum identical to syringaldehyde (4-hydroxy-3,5dimethoxybenzaldehyde, produced in the breakdown of lignin) (Murphy et al.2014).

<u>**Component 4:**</u> The spectral properties of this component is similar to other reported microbial humic-like PARAFAC components (Cory and McKnight, 2005; Yamashita et al., 2010) and it resembles peak M which is identified as microbially transformed autochthonous DOM (Fellman et al. 2010).

<u>**Component 5:**</u> This humic-like component which has been identified in many previous models has been hypothesized to be derived from terrestrial materials (Søndergaard et al. 2003; Murphy et al. 2008). Both component 4 and 5 have been observed to have consistent relationship which suggest thay they represent a common ubiquitous signal from their source (Murphy et al. 2014).

PARAFAC modelling has revealed the presence and identity of the different components of NOM and their possible origins, which has been validated by comparing with the Openfluor database.

Notwithstanding, HPSEC-FLD with multi-excitation/emisson scan can provide direct information about the prevalence of multi-peak fluorophores in FEEM thus serving as an effective chromatographic surrogate for determining the proper number of PARAFAC components (Li et al. 2013) as well as providing information about the physical characteristics of NOM.



Figure III-2: 3D FEEM contour plots of the 5 Components and their normalized loadings identified by PARAFAC model.

3. NOM detection after fractionation

Considering the complex nature of NOM, its characteristics and properties are better understood when it is fractionated. This section looks at the spectral characteristics of NOM using online UV and FLD after it has been fractionated according to size by HPSEC. FLD measurements were done at a fixed excitation wavelength for a range of emission wavelengths (vice versa). The possibility of using such handy online devices to evaluate the evolution of NOM during treatment processes was equally assessed.

3.1 Comparison between SEC-UV and SEC-OCD of NOM fractions

UV absorbance detectors until recently have been used in several online studies as one of the accurate detectors for estimating organic carbon concentration. However, the recent development of an organic carbon detector (OCD), which can be coupled with a SEC, has and continue to gain popularity as it has further exposed some of the lapses in using UV. For instance, the bulky fraction of NOM referred to as biopolymer although present, absorbs less in UV, thus it may be underestimated when detected at UVA₂₅₄.

3.1.1 Challenges of using SEC-OCD

Aside the cost of this novel technique which makes it difficult for many samples to be analysed, the OCD is not fully quantitative (compared to the classical total organic carbon analyser) and its quantitative property further reduces when coupled with a liquid chromatography. On the one hand, some OCDs rely on UV for the oxidation of the organic carbon present. Thus, the biopolymer fraction and the very hydrophilic components of NOM are susceptible to underestimation due to poor oxidation. On the other hand, there is a portion of NOM called hydrophobic organic carbon (HOC), which is retained in the column, thus making the DOC value measured in the column bypass differ from the value obtained for the hull curve of the chromatogram. The percentage of HOC varies greatly depending on the sample characteristic. For instance, for a sample treated with an IRA410 resin, HOC accounted for about 19% of DOC though for most of the samples, the percentage contribution was around 12%, making SEC-OCD not entirely quantitative as may be assumed. In some instances, the chromatographic fraction of organic carbon (CDOC) was even about 20% higher than the DOC. For example, the CDOC of Burrator River was 20% higher than its DOC and 14% higher than its TOC while the CDOC of Tamar River sample was 12 and 10% higher than its DOC and TOC respectively.

This denotes that, the HOC which is retained in the column could possibly be released to overestimate the results of subsequent analysis.

3.1.2 Surrogate for OCD detector

As a substitute to SEC-OCD, many studies resort to the use of UV detectors for semiquantitative studies mainly because of economic reason. Even though UVA₂₅₄ has been reported in these studies to have a good correlation with DOC, this is dependent on the sample source and NOM composition. For instance, whereas allochthonous NOM has high aromatic carbon and low nitrogen content (high SUVA), autochthonous NOM has low aromatic carbon and high nitrogen content (low SUVA value). Furthermore, most of these correlation analyses were performed with conventional UV spectroscopy and TOC analyser which considers the entire bulk NOM. There was the need therefore, to test this correlation on chromatography techniques and to ascertain which NOM fractions are significant in the correlation. Likewise, considering the chromatography challenges in SEC-OCD during sample fractionation as highlighted in the previous section, it was necessary to establish if SEC coupled with spectral detectors could still be a valid surrogate for OCD.

3.1.3 Correlation between SEC-UV and SEC-OCD chromatograms

In this study, 14 different samples obtained from DOC2C's project partners were analysed with both SEC-OCD and SEC-UV. After size fractionation, the peak areas which correspond to the DOC concentration in SEC-OCD and the hydrophobic component in SEC-UVA₂₅₄ were compared. Even though a portion of the HOC was retained in the column and analysed, there was still a strong correlation between both detectors with an R² value of about 0.88 (Figure III-3a). The main fractions that contributed to this strong correlation were the humic substance and building block. When only these two fractions were correlated, the R² value increased slightly to 0.90. This highlights the importance of fractionating NOM before detection, as it allows focusing on only the most significant fractions. During treatment processes however, the composition of NOM changes and this can affect its optical properties. The poor correlation of the biopolymer and LMW neutral fractions with UVA₂₅₄ was due to their highly hydrophilic nature which results in a low absorbance of UV. The good correlation between these two detectors indicates that, in the absence of SEC-OCD, SEC-UV could still be used for accurate semi-quantitative analysis especially for the humic substance and building block fractions.



Figure III-3: Correlation between SEC-OCD and SEC-UVA₂₅₄ (a) all NOM fractions (b) humic substance and building block fractions. 14 samples from DWG, SWW and PWN were used for the correlation. LMW=low molecular weight.

3.2 UV absorbance of NOM fractions

The impact of treatment processes such as nitrification, coagulation, sand filtration and activated carbon on the UV absorbing components of NOM were monitored for three different campaigns (summer 2018) at DWG in deBlankaart, Belgium. Online UV measurements were performed at 254 nm after NOM had been fractionated into different sizes by an HPSEC. The description of the different NOM fractions has been detailed in Chapter II, section 3.2.3. From the chromatograms obtained for campaign 1 (Figure III-4), there was no visible peak observed in UV for the biopolymer fraction. A similar occurrence was observed for the other two campaigns. This was not surprising considering the low absorbance of this fraction in UV. When SEC-OCD was used to analyse the same water source but at a different period (autumn 2018), the biopolymer concentration was about 1.6 mgC/L. The peak for the LMW neutrals was poorly resolved in UV for almost all treatment steps and so the removal efficiency was not calculated.



Figure III-4: UVA₂₅₄ chromatogram of samples from the different treatment lines of DWG during campaign 1 after separation by size exclusion.

3.2.1 Correlation between NOM fractions and DOC

From the SEC-OCD results, the biopolymer fraction is made up of only about 14% of the total DOC concentration. Thus, since the bulk (> 80%) fraction of NOM is made up of UV absorbing components, UVA₂₅₄ has widely been accepted for semi-quantitative measurements, to estimate the concentration of DOC in a sample. This was confirmed by the high correlation ($R^2 = 0.92$, n = 30) between the peak areas of UVA₂₅₄ chromatograms and DOC of waters from different sources (Figure III-5a). The integrated chromatogram can therefore be reasonably approximated, to represent the concentration of DOC (Lechtenfeld et al. 2011). Comparing the peak areas under each NOM fraction for the treatment steps, it was observed that in all campaigns, there was relatively no difference between the raw water and the nitrification step. A similar observance where there was no significant change in DOC further confirms that even the aliphatic carbons were not removed at the nitrification step (Figure III-5b). It appears therefore that the nitrification process at this stage of the treatment line serves little importance as far as DOC removal is concerned. The percentage change in UVA₂₅₄ observed at the treatment processes target more the hydrophobic fraction.



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Figure III-5:(a) Correlation between the total peak area under UVA₂₅₄ chromatogram and DOC concentration for SWW, DWG and Centre African Republic, Banqui (CAR) Samples (b) The DOC values (mqC/L) after each treatment step of deBlankaart water treatment plant for all three campaians (c) Integrated area obtained under UVA254 chromatogram for the different treatment step. C 1, C 2 and C 3 represent campaigns 1, 2 and 3 respectively (see Chapter II, section 1.1.3).

3.2.2 Evolution of NOM fractions during treatment

filtration

For all campaigns, a positive removal efficiency was observed at all treatment steps (except nitrification step) when the sum of all NOM fractions is considered (Figure III-6a). According to the DOC concentration for all campaigns, coagulation removed on the average about 60% DOC while GAC removed about 20%. Campaign 1 recorded the maximum DOC removal after coagulation, which was 64%. Considering the UVA₂₅₄ chromatogram, out of this percentage of DOC removed, about 63% were aromatic carbons that formed the humic substance fraction while only 14% formed the building blocks. This was expected as coagulants target mostly HMW substances including biopolymers. The reduction of about half in the mean contribution of HMW fractions by coagulation was confirmed by SEC-OCD (Baghoth et al. 2009). The

bridging of coagulant by biopolymers causes an increase in the sizes of flocs when they are present (Su et al., 2017). The DOC removal efficiency of sand filtration fluctuated, depending on the campaign, which followed the same trend as the UVA₂₅₄ for the humic substance fraction. Whereas DOC increased in about 14% for campaign 1 (increase in 15% UVA254 for humic substance), it reduced in about 3% (decrease in 30% UVA₂₅₄ for humic substance) for campaign 2 after sand filtration (Figure III-6b). This occurrence was probably due to fouling of the filters (Touil et al., 2014). There was however a positive removal of the building blocks (9%) and low molecular weight acids (10%) with sand filtration. It must be noted that, there was an intermediate chlorination step after sand filtration which should be considered when interpreting the UVA254 results at this step, as chlorination affects UV absorbance. When water containing NOM is chlorinated, the UV absorbance of the matrix decreases at all wavelengths (230-480 nm), because the chromophores in NOM are much more susceptible to oxidation and ring cleavage (Li et al. 2000). Treating both SRHA and SRFA with ClO₂ and HOCl resulted in comparable and prominent decrease in the electron donating capacities of the DOM leading to a slight decrease in UV absorbance (220-300 nm) (Wenk et al., 2013). When UVA₂₅₄ chromatogram was normalized with their respective DOC, coagulation process was the most efficient in DOC removal. GAC was efficient in removing more of the LMW acids compared to the building blocks while the humic substance increased. This result implies that the difference in adsorption efficiency of GAC is due to the size of the fractions and not aromaticity which agrees with previous studies (Shimabuku et al., 2017).



Figure III-6: Removal efficiency of aromatic carbon for (a) sum of all fractions (b) individual NOM fractions for all 3 campaigns. Nitri= Nitrification, Coag= Coagulation, Chlori & SF= chlorination and sand filtration, GAC=Granular activated carbon, Clean=final chlorination. Numbers 1, 2 and 3 attached to treatment steps represents campaign 1, 2 and 3 respectively.

3.3 Fluorescence of NOM fraction

The evolution of the two major fluorophores (humic-like (HL) and protein-like (PL)) during the treatment processes was monitored using the online FLD. The 3 humic-like components in our PARAFAC model emitted at 385, 455 and 500 nm and excited at two wavelength maxima for each. Thus, to choose a fixed excitation and emission wavelength for FLD, an average emission wavelength of 447 nm and excitations of 250 or 326 nm would have best represented all components. However, these wavelengths did not represent the highest chromatographic resolution and intensities. The online signal for the HL signal was therefore fixed at 244 nm for excitation and 420 nm for emission. The PL signal was monitored at excitation at 230 nm and emission at 345 nm. The choice of this signal was backed by the same reason for the HL signal selection.

3.3.1 Evolution of the peak maxima during treatment

PARAFAC modelling was not possible with the 2D-FLD dataset since only a fixed emission or excitation wavelength is permitted for measurement. However, the excitation time-maps (Figure III-7) can be used to track the evolution of the peak maxima during treatment processes. The peak maxima of the raw water and nitrification step for instance shifts after coagulation from the humic substance (around 6 mins) to the building blocks (around 7 mins). This trend equally corresponds to the contributions of the fractions to DOC. Since the components of NOM have unique absorbance and fluorescence properties, this map helps to visually identify which NOM fraction is contributing to a specific spectral signal. The UVA₂₅₄ chromatogram area under each fraction was correlated with the total area under the HL and PL chromatograms. Among all the NOM fractions, the humic substance correlated best with the HL fluorescence signal. This was expected considering the hydrophobic nature of this fraction. The correlation for DWG samples was however higher (95%) than for SWW (79%) (annex SIII-1). Even though the DOC composition and the treatment techniques of both source waters were different, the correlation was still strong. This indicates that the HL signal could serve as a good indicator for predicting NOM evolution in water treatment processes. The building block fraction was the second most contributing fraction to the HL signal with 63% correlation for DWG and 60% for SWW samples.

Among the 5 different NOM fractions, the biopolymers, LMW acids and neutrals contain more protein materials. It was therefore expected that these fractions would contribute most to the PL fluorescence signal. On the contrary, the humic substance fraction was observed as the highest

contributor to the PL signal, especially for DWG samples. This confirms the possibility of having an overlap of the HL fluorophore on the tryptophan emission region due to its high intensity and the high sensitivity of the properties of tryptophan to its local conditions (Lakowicz, 2008). If tyrosine is associated with humic substances, its emission energy would be transferred to the humic substance and not observed as a protein-like fluorophore (Yamashita and Tanoue, 2003) while Maie et al. (2007), observed that phenols associated with tannins were likely responsible for the fluorescence signal of tryptophan. Even though the highest R² (68%) correlation of the PL signal of SWW samples was observed for the biopolymer fraction, this correlation was not as high as that observed for the HL signal and the points were not as evenly distributed, compared to the correlation between the building blocks and the PL signal. The uneven distribution of points was probably due to the presence of biopolymer fraction mainly in the raw water samples, thus an increase in dataset may increase this correlation.



Figure III-7: HPSEC excitation time-maps of samples at each treatment step: (a) humic-like fluorescence signal, (b) Proteinlike fluorescence signal effluents. The Ex-axis denotes the excitation wavelength, by which humic-like peaks (Em @ 420 nm) and protein-like peaks (Em @345 nm) can be distinguished. The Time-axis represents the retention time while the color variation represents the fluorescence intensity in arbitrary units.

3.4 Calibration of spectral detectors for semi-quantitative analysis

Like UVA₂₅₄, the HL fluorescence signal showed a good correlation with DOC (annex, SIII-2). For SWW samples for instance, the correlation of both UV and HL signal with DOC were much similar with R^2 of a little above 0.8. This is because the fluorescence detector is more sensitive and selective than total organic carbon analysers. The sensitivity of fluorescence is likewise about 10-1000 times that of UV absorption spectroscopy (Henderson et al., 2009). Thus, with the help of pure compounds, these detectors can be calibrated to serve as a proxy to TOC analyser, for semi-quantitative studies.

3.4.1 HL and UV signal

Since humic like substances can exhibit different fluorescence maxima, the fluorescent signals of the three primary humic-like peaks A, C and M were determined (Fellman et al. 2010). Peak 'A' represents aromatic humus with HMW while peak 'M' represents LMW humus and peak 'C', very aromatic humus with HMW (Coble et al. 2014). The responses of these peaks to fluorescence in 2D-FLD were measured with a SRNOM standard. Considering the fluorescence signals of SRNOM at varied concentrations (expressed as mgC/L), peak C showed the steepest slope and the highest response followed by peak A (Figure III-8). The difference in slope became obvious with emission wavelength variations of more than 40 nm. The difference in fluorescence intensities observed for emissions at 400 nm and 420 nm, with excitation wavelength of 244 nm was insignificant. However, natural water samples showed better resolution at Peak A maxima compared to Peak C and M. Since DOM generally has a broad, featureless fluorescence spectra, they do not always exhibit an absolute peak maxima in the regions outlined in literature (Korak, 2014). Peak A was thus selected for the calibration.



Figure III-8: The fluorescent maxima of the primary humic-like peaks determined with SRNOM standard by (a) FLD and (b) FEEM of Suwanee river of 2.5 mgC/L.

The area under the chromatogram of the standard, excited at 244 nm with an emission at 420 nm was integrated for a concentration range of 0 to 10 mgC/L. The same excitation wavelength was used for the UV calibration. From the calibration curve, it was observed that all signals showed good linearity with concentration ($R^2 > 0.99$). The linearity was however reduced at higher concentrations (> 5 mgC/L) especially in UV absorbance. Specific peak intensity is sensitive to deviations from linearity. At higher concentrations (above the linearity threshold), the intensity decreases and may no longer be considered a compositional metrics. The

magnitude of deviation from linearity depends on both the concentration and composition of the sample (Korak, 2014). To stay within the range of linearity, natural water samples to be analysed were diluted to achieve DOC concentrations < 5 mgC/L. Another reason was to reduce microbial activities in the column due to high carbon load thereby prolonging its durability.



Figure III-9: Calibration of HL signal of FLD with SRNOM as the calibration standard. Calibration was done in both (a) UV and (b) fluorescence.

3.4.2 PL Signal

The PL signal of the FLD was calibrated with a standard Tryptophan (standard DL-tryptophan, 99% from Alfa Aesar). The calibration concentration range was between 0 and 2.5 mgC/L. From the FEEM contour view of the standard Tryptophan, two excitation maxima were observed (Figure III-10a). Li et al. (2013), also observed protein fluorescence excitation peaks at 230 and 280 nm which correspond to the excitation maxima observed for our PARAFAC component 2. Although it was evident from the contour view that excitation at 230 nm had the highest intensity, both excitations were still compared to see their response in the 2D-FLD. The emission wavelength of all pure fluorophores is independent of excitation wavelength but rather solely due to the width of the peak (Coble et al. 2014). FLD confirmed the observation in FEEM where the fluorescence intensity observed at excitation at 230 nm was about 23% higher than that observed at excitation at 275 nm. Absorbance at 230 nm was equally higher (39%) than at 275 nm (Figure III-10c). Aside the standard samples, natural water samples also showed higher absorbance at 230 nm and an emission wavelength of 345 nm.



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Figure III-10: (a) FEEM contour view of standard DL-tryptophan at 1 mgC/L used for calibrating PL signal. Comparison of signal intensities of standard tryptophan at (b) fixed emission at 345 nm but different excitations at 230 nm and 275 nm and (c) at different absorbances at 230 nm and 275 nm.

From the calibration curves of both (HL and PL) signals, the equation of each calibration line can be interpreted as;

$$Fs = m * Xs + C$$
 Equation (1)

Where 'Fs', represents the fluorescence intensity of the standard at the chosen excitation and emission wavelength for calibration at a particular concentration, *X*s, is the concentration of the sample and 'm' represents the gradient of the slope corresponding to a change in intensity per unit (mg/L) of the standard. The measured fluorescence intensities were then divided by the gradient of the calibration curve, to obtain the concentration of HL or PL, signal referred to as SRNOM unit (SRU) or Tryptophan Unit (TU).

3.5 Quantifying NOM fluorophores during treatment processes

3.5.1 HL concentration - SRU

The SRU was used to calculate the concentration change during treatment. The concentration of SRU calculated was very similar to that of DOC for all campaigns. Whereas the average DOC in raw waters for all campaigns was 7.5 mgC/L, the average SRU observed was 7.2 mgC/L. The average concentration observed for nitrification was very similar to that of the raw water. Coagulation averagely removed about 6 SRU which corresponds to about 70% removal. After coagulation, the HL signal of the humic substance fraction remained relatively stable meaning the other treatment steps had little efficiency in removing this fraction (annex SIII-3). There was however a slight increase (about 0.2 SRU) in the signal intensity for the LMW fraction after coagulation also corroborating the possible release of trapped smaller fractions.



Figure III-11: (a) Concentration of HL fluorophores derived from the calibration curve and represented as SRU and (b) Change in SRU concentration during treatment processes. Nitri= Nitrification, Coag= Coagulation, Chlori & SF= chlorination and sand filtration, GAC=Granular activated carbon, Clean=final chlorination. C 1, C 2 and C 3 represent campaigns 1, 2 and 3 respectively.

3.5.2 PL concentration - TU

LMW acids, which are characterized to be made up of amino acids, showed strong PL signal intensity at each treatment steps with the highest (46% on the average) being observed after coagulation. However, TU was generally about 60% lower in intensity than the SRU and its removal efficiency was relatively steady. The low signal intensity represented low concentrations. The raw water of campaign 3 had the highest TU of 136 μ gC/L (annex SIII-4), which was about twice the concentrations observed for the two preceding campaigns. Considering the concentration ranged used for the calibration, this is below the first

concentration point making this semi-quantitative measurement weak. It was however not possible to calibrate the FLD at lower concentrations (μ g/L) as this is below the detection limit resulting in a low signal to noise ratio. During the treatment processes, nitrification showed the least removal efficiency of an average of 12% and a negative removal observed for campaign 2. Coagulation showed the highest removal efficiency of between 38 and 56% followed by GAC which showed a removal efficiency of between 32 and 53%. When TU was normalized with the DOC, coagulation showed a negative removal for all three campaigns. This is probably due to the release of smaller trapped fractions after coagulation. These smaller fractions released may form part of the low molecular weight acids and neutrals since they fluoresce in the PL region. There was a poor correlation (65%) of the PL signal with UV absorbance at 254 nm. This was expected as proteins do not show maximum absorbance at this wavelength. This further reinforces the need to target and monitor specific NOM fluorophores since absorbance at 254 nm is not totally representative.

4. Biopolymer fraction of NOM

The largest molecular size fraction of NOM fraction called biopolymer is known to be primarily responsible for the fouling of membrane and significantly impact other water treatment processes. These fouling experiments are performed with a pure biopolymer fraction whose composition and structure are largely known. However, the actual composition and properties of this fraction in natural waters appear not to be unanimous across literature. For instance, some have attributed the UV absorbance of the very HMW NOM fraction to be likely due to the presence of inorganic colloidal materials (silicates, iron oxyhydroxides and aluminium) and sulphur species (elemental, colloidal or partially oxidized), associated with organic matter which scatter the UV light rays (Huber and Frimmel 1994; Allpike et al. 2005). Even though an organic carbon detector (OCD) can detect this fraction, its cost makes it inaccessible to all. Moreover, the low absorbance of this fraction in UV means that the fraction can be underestimated by OCDs which rely on UV for oxidation. This section looks at the possibility of using the sensitive online 2D-FLD spectral detector to better understand the composition of this fraction.

4.1 Spectral properties of the biopolymer fraction

After size fractionation of NOM, the PL signal of the biopolymer fraction became more visible in fluorescence although it hardly absorbed UV light. This was not just restricted to only one sample source. When samples from different sources and continents were analysed, a fluorescent peak with a substantial intensity, was always observed at the biopolymer retention time even when UV absorbance intensity was low. Water samples from Ubangi River (Central African Republic) and Burattor Reservoir of SWW showed well defined peaks in both UV and fluorescence while DWG and Tamar River samples showed little absorbance in UV but high fluorescence peaks (Figure III-13). This was probably due to the high sensitivity of the FLD. Comparing online FLD and UV detectors coupled with HPSEC, Nagao et al. (2003), observed that, the FLD is about one order of magnitude more sensitive than the UV detector. Another reason could be the UV wavelength, since measuring at 254 nm is not ideal for the components of biopolymer. To verify this, the absorbance measurements were performed at 230 nm which is the excitation wavelength of the PL signal. It was observed that, UVA₂₃₀ chromatogram for the biopolymer fraction was similar to that of UVA₂₅₄ for all samples. Thus, the low absorbance of the biopolymer fraction at UVA₂₅₄ was not due to the wavelength but rather the sensitivity of the detector. This is likely because whereas absorbance measures the difference in intensity

between light passing through a bright reference and the sample, fluorescence measures directly (relative to a dark background) without comparing to a reference beam (Lakowicz, 2008). Thus, the PL signal of the FLD can be used to study this structure. BR and RT would be discussed in detail to represent globally all samples which have higher absorbance in UVA₂₅₄ and fluorescence and those with only high fluorescence respectively for the biopolymer fraction.



Figure III-12: PL signal (Ex@ 230 nm and Em @345 nm) of biopolymer fraction in natural samples compared with UV @ 230 and 254 nm of Samples from (a) Bangui water treatment plant (Central African republic), (b) Burrator reservoir (c) Dewatergroupe (d) River Tamar.

4.1.1 Variation in UVA and fluorescence (PL) of the biopolymer fraction

The samples studied showed varied response in UV absorbance but similar response in fluorescence. Even though no peak was observed at around 5.4 min in the UV chromatogram

of RT sample, it does not necessarily represent the absence of the biopolymer fraction (Figure III-13d). The UV chromatogram of BR sample however shows a shoulder peak around 5.4 min (Figure III-13b). Thus, the biopolymer composition of both samples can be said to be containing different chromophores. The energy needed to excite an electron depends largely on the type of orbital and electron conjugation (delocalization). There is a large energy gap between the ground and excited states of σ -bond orbital. This energy gap is significantly reduced (longer wavelengths) in π -bond orbitals facilitating the ability of electrons to delocalize. The energy needed to excite a non-bonding electron, n, is lower than that required for both bonding orbitals (Valeur, 2002). Thus, the chromophores that are contained in the biopolymer fraction of BR may contain more π and n electrons (lower energy difference) or more aromatic molecules compared to that of RT. In the use of UV-visible spectroscopy to study organic molecules, conjugated compounds are primarily detected as the spectrum generated represents a specific bonding arrangement in the molecule (Aiken, 2014; Silverstein and Bassler, 1962). The fluorescence chromatogram of both samples however showed the presence of a peak at a retention time of about 5.4 min. This confirmed the high sensitivity of FLD and further showed that, HPSEC multi-excitation/emission chromatography can provide informative fingerprint on isolated DOM fractions. It can further be used to verify the prevalence of multi-excitation properties of fluorophores (Li et al. 2014). DWG samples behaved similarly to that of RT where no absorbance was observed at both 230 and 254 nm. CAR sample however showed a welldefined biopolymer peak in both UV and fluorescence compared to BR. The PL signal disappeared after coagulation process.

4.2 Composition of biopolymer fraction

The observed fluorescence peak of the biopolymer fraction was more likely due to the presence of protein-like fluorophore moieties and not light scattering. At excitation range of 230 nm to 310 nm and fixed emission at 430 nm and at emission range of 360 nm to 450 nm and fixed excitation wavelength of 244 nm, no peak was observed at 5.4 min (Figure III-13). This range corresponds to the humic-like fluorescence peak region as fluorophores with emission wavelengths above 350 nm represent humic acid-like substances (Chen et al. 2003; Lakowicz 2006; Coble et al. 2014). If the absorbance was due to the presence of colloids or light scattering, a peak should nonetheless have been observed irrespective of the emission wavelength although at different intensities because the intensity of the light scattered increases with decreasing wavelength (Marmer and Hurtubise, 1996). The first order Rayleigh scatter line is centred at the emission and its equal to the excitation line, while the second order

Rayleigh, scatter at an emission wavelength which is equal to twice the excitation wavelength (Rinnan et al., 2005). This scattering phenomenon effect was not observed in the SEC excitation/emission-time maps.

The absence of a biopolymer peak at emission wavelengths above 350 nm indicates that the components of this fraction are not of humic-like in nature.



Figure III-13: Fluorescence chromatogram of Burrator reservoir samples observed at (a) different excitation wavelengths and fixed emission wavelength @ 430 nm and (b) different emission wavelengths and fixed excitation wavelength @ 244 nm.

However, at excitation wavelength of 244 nm and emission wavelength of 360 nm, a very small peak is observed at the retention time corresponding to the biopolymer peak. This lower emission wavelength represents the fluorescence region of protein-like substances. Tyrosinelike substances have an excitation wavelength range of 200-250 nm and an emission wavelength range of 240-330 nm while tryptophan-like substances have excitation wavelength range of 200-250 nm and an emission wavelength range of 330-380 nm (Chen et al. 2003). As we reduce further the emission wavelength, more into the PL substance region (< 360 nm) for the same sample, the peak intensity increases (Figure III-14). The increase in signal intensity below emission < 360 nm confirms that this peak is not due to a spectral overlap from the closest high intensity humic-like substance present since it has emission maxima of 385 nm (component 4). The match of the component 2 of the PARAFAC model to the tryptophan-like fluorophore further confirms the existence and composition of this peak. At lower emission wavelength of 305 nm however, the peak intensity begins to decrease again, as the signal to noise ratio decreases, making it difficult to observe the peak. Among the three aromatic amino acids (phenylalanine, tyrosine, and tryptophan), tryptophan dominates in aquatic NOM (Lakowicz, 2008). This is because the energy absorbed by the other two amino acid residues is transferred to the tryptophan residues in the same protein. Hence tyrosine residues in proteins and peptides do not emit fluorescence in the presence of tryptophan (Lakowicz 2006; Li et al. 2014).



Figure III-14: Fluorescence chromatogram of Burrator reservoir samples observed (a) at different excitation wavelength and fixed emission wavelength @ 345 nm and (b) different emission wavelength and fixed excitation wavelength @ 230 nm.

Since the biopolymer fraction is composed of nitrogenous compounds like proteins and amino acids, the organic nitrogen content of this fraction was monitored using the organic nitrogen detector (OND). It was observed that the correlation between the organic carbon and the organic nitrogen concentration of the biopolymer fraction was highly dependent on the sample source and composition. This was expected as autochthonous NOM has high nitrogen content than allochthonous NOM. When dataset from different locations (SWW, DWG and PWN) were combined, there appeared to be a strong correlation ($R^2 = 0.94$), but the uneven distribution of the correlation points means that the correlation was a false positive (annex SIII-5). Indeed, when the isolated points were removed, the correlation decreased to 54%.

4.3 Fluorescence maxima of the biopolymer components

After base line correction, the fluorescence maxima of this fraction were extracted at various wavelengths. At fixed emission wavelength of 345 nm, the fluorescence intensity for the excitation wavelength was in increasing order of 280 nm, 235 nm, 244 nm and 230 nm respectively. At fixed emission wavelength of 430 nm, which falls within the humic-like region, the fluorescence maxima were extracted at 230 nm, 244 nm and 280 nm which were expectedly low. At fixed excitation wavelength of 230 nm, the highest biopolymer peaks were observed both at 305 nm and 345 nm which corresponds to tyrosine-like and tryptophan-like components respectively (Coble et al. 2014). Species with varied emission wavelength are more

predominant in protein-like substance (Li et al. 2015). This occurrence is due to the quick dissipation of excited electrons which moves them to the lowest vibration levels of the first excited state (Lu et al., 2009). Thus, from the iso-absorbance plot (Figure III-15), the highest excitation and emission wavelengths were observed at 230 nm and 345 nm respectively, meaning the dominant component in the biopolymer fraction indeed bares a tryptophan-like signature. Tryptophan-like fluorescence may represent the intact form of proteins and the less degraded peptide (Fellman et al. 2010). This is very similar to the peak maxima of the component 2 of our PARAFAC model, confirming that the absorbance and fluorescence of the biopolymer fraction is not due to light scattering, but the presence of protein-like fluorophores. Baghoth et al. (2011), in using FEEM and PARAFAC to track NOM in a drinking water treatment plant, equally observed that tryptophan-like and tyrosine-like components correlated positively (r = 0.78 and 0.75, respectively) with the biopolymer fraction. In the SWW sample, a 68% correlation was observed between the biopolymer fraction (UVA₂₅₄) and the fluorescence PL signal. Trubetskoj et al. (2018), likewise observed the presence of protein-like fluorescence in the highest and medium molecular size fractions, while humic-like fluorescence was detected mostly in the low molecular size fractions of SRNOM. Although the fractions were not named, their molecular weight (> 30 KDa) indicates that they were possibly biopolymers. Thus, the use of the online 2D-FLD does not only identify biopolymer fraction as composed of protein substance but also the type of protein-like signature.



Figure III-15: Excitation/emission iso-map constructed from extracted fluorescence maxima of the biopolymer fraction for Burattor Reservoir sample at different excitation and emission wavelength.
4.4 Heterogeneity of NOM fraction

Aside the biopolymers, the low molecular weight acids and neutrals are also known to be composed of protein molecules in the form of amino acids (Huber et al. 2011). It can be deduced from the various chromatograms that, even the humic substance and building block fractions contain molecules that fluoresce in the PL region, even though their intensities are weaker. Likewise, there are substances that fluoresce in the HL region present in the biopolymer, low molecular weight acid and neutrals fractions. The fluorescence intensity of the humic-like signal was however high across all fractions. This confirms that NOM is a heterogeneous aggregation of hydrophobic and hydrophilic components such as proteins, carbohydrates, amino acids (Her et al. 2003). Their polarity is influenced by protonation and deprotonation processes and in some instances PL fluorophores can be joined with humic substance giving them similar hydrophilicity (Li et al. 2014). Li et al. (2016), for instance observed that, the alleged protein-like fluorescence observed in FEEMs does not only represent proteins but also the contribution of humic substances. There is also the possibility of having a spectral overlap between the fluorescence signals of humic-like substance and background fluorescence assigned with lower molecular weight molecules and the signals for proteins (Aiken, 2014). When NOM was fractionated by flow field-flow technique, humic-like fluorescence was found in both the high and low molecular size fractions at 350/450 nm (Zanardi-Lamardo et al., 2002). A good correlation between the biochemical oxygen demand and the fluorescent intensity of these peaks indicates that, they are associated with living and dead cellular materials, as well as microbial activities (Bridgeman et al., 2013). The fluorophores of these peaks originate from autochthonous sources and their signals are from a mixture of free and combined amino acids. The fluorescence emission is dependent on the hydrophobicity of the site surrounding the amino acid moiety (Coble et al. 2014).

4.5 UV absorbance ratio index (URI) - A₂₁₀/A₂₅₄

The ratio of peak heights of SEC-UV absorbance measurements at 210 nm and 254 nm has been proposed by Her et al. (2008), for the characterization of biopolymers in natural water samples, as certain biopolymers contain different proportions of chromophores and sp²hybridized carbon. The URI is, therefore, an index for the dominance of nonhumic/autochthonous over humic/allochthones NOM and so is inversely proportional to the SUVA. The interest in the use of A_{210}/A_{254} in the current study was to gain additional insight into the chemical composition of the biopolymer fraction, with respect to the relative proportions of non-aromatic and aromatic components. For the biopolymer fraction, the URI values were higher than all the other fractions except the LMW acids. This means that this fraction was less aromatic and dominated by saturated aliphatic carbons. The URI value of Tamar River biopolymer fraction was about 44% higher than that of Burrator reservoir (Table III-2). This could mean that the Tamar River biopolymer fraction contained protein-like substances, saturated carbon bonds and/or simple amino acids, which further explains why it absorbed less in UV. The protein-like substance concentration is however not expected to be high, judging from the fluorescence signal intensity. The biopolymer fraction of the Burattor reservoir sample probably contained some conjugated bonds, that gave it little absorbance at UVA₂₅₄. This is confirmed by its higher SUVA value. This URI is likely to be impacted by seasonal changes as NOM composition varies.

 Table III-2: URI values of the biopolymer fraction obtained by UV scans at 210 nm and 254 nm for Burattor Reservoir and

 Tamar River samples compared to that of standard SRHA and SRFA (Her et al. (2008)^c).

NOM Fraction	UVA210	UVA254	URI
Burattor Reservoir	36.15	16.56	2.18 ^a
Tamar River	9.05	2.33	3.88 ^b
SRHA ^c	0.223	0.140	1.59
SRFA ^c	0.180	0.096	1.88

5. Relative quantum yield of the biopolymer fraction

Since the biopolymer fraction is known to have a low UV absorbance but can fluoresce, its fluorescence lifetime and quantum yield (QY) which gives the probability of the excited state being deactivated by fluorescence rather than a non-radiative mechanism could be used to measure the efficiency of its fluorescence process. QY, is a ratio of the number of photons emitted to the number of photos absorbed (equation 2), and can be used to deduce the photophysical behaviour of a compound (Fery-Forgues and Lavabre, 1999).

$$\Phi = \frac{\Gamma}{K_{nr} + \Gamma} = \frac{\text{number of photons emitted}}{\text{number of photons absorbed}} \qquad \text{Equation (2)}$$

Where K_{nr} is the radiationless decay and Γ represents the radiative decay. The closer the quantum yield number is to 1 (*Knr* < Γ), the brighter the emission. However, because of Stokes losses, the energy yield of fluorescence would always be less than unity.

Since the measurement of absolute quantum yield is critical and requires special equipment, relative fluorescence QY of samples were rather determined, by comparing their integral emission spectra to that of a standard obtained under similar conditions (Fery-Forgues and Lavabre, 1999; Lakowicz, 2008; Würth et al., 2013) as showed in Equation 3. Tryptophan and quinine sulphate were chosen as reference samples for protein-like and humic-like substance respectively because their absorption and emission bands are closer to those of the unknown samples. Both the standard and the unknown were measured at the same slit and gain setting. The spectra of standards were repeated to ensure repeatability and measurements were carried out in one session to avoid drift in the detector settings.

$$\Phi f(x) = \left(\frac{As}{Ax}\right) \left(\frac{Fx}{Fs}\right) \left(\frac{\eta x^2}{\eta s^2}\right) \Phi f(s)$$
Equation (3)

Where Φ f is the fluorescence quantum yield, A is the absorbance, F is the area under the corrected emission curve (intensity of the FLD in this case), η is the refractive index of the solvents used. Subscripts s and x refer to the standard and unknown samples, respectively. The refractive index (η) was neglected since both reference and sample were injected into the same phosphate solution. The fluorescence quantum yield of pure tryptophan is reported in literature to be 0.14 (Bianco et al. 2016). When pure tryptophan was used as a standard and a sample (concentration of 1 mgC/L), the intensities cancel out (value of 1), resulting in a relative QY value of 0.14 as reported in literature. However, the objective of this study was to determine

the relative fluorescence quantum yield of only the biopolymer fraction which is detected after fractionation. Since this fraction is determined by online detectors, there was a need to calibrate these detectors.

5.1 Calibrating the 2D-FLD with a 3D FEEM

Quantum yield measurement is performed with conventional (offline) UV and fluorescence spectrometers (Grabolle et al., 2009; Würth et al., 2011). To determine the QY of fractions with FLD, a correction factor was needed to match the signal intensity of FLD to conventional detector. To make this possible, offline UV spectrophotometer (Cary 100) and FEEM were used to calibrate the FLD. The fluorescence QY of tryptophan is about 0.14 and has two strong fluorescence excitation maxima at 230 and 280 nm respectively, while the emission maxima is always at 350 nm (Yang et al. 2017). Since FLD measures at a fixed emission and excitation wavelengths, measurements on the offline fluorescence detector were also performed at fixed wavelengths. Thus, fluorescence measurements were performed at excitation wavelength of 230 nm and emission wavelength of 345 nm on both detectors. Absorbance and fluorescence measurements for the tryptophan standard were performed at concentrations of 0, 0.5, 1, 1.5, 2 and 2.5 mgC/L on both offline and online detectors. To avoid re-absorption effect, absorbance in the 10 mm cuvette used was kept at 0.1 at the excitation wavelength. The fluorescence and absorbance signals were then plotted against each other (Figure III-17). The linearity between fluorescence intensity and absorbance illustrates the absence of inner filtering effect.



Figure III- 16: Calibration of both classical and online UV and Fluorescence detectors using pure tryptophan standard of concentration range 0-2.5 mgC/L on (a) Online detectors and (b) offline or classic detectors.

5.2 Effect of the HPSEC mobile phase (buffer)

Tryptophan is highly sensitive to its local environment making it a good gauge for protein conformational changes. The effect of the buffer on tryptophan was analysed, since the pure tryptophan used for the QY measurement was prepared in Milli-Q water. In HPSEC, the standard was injected into a stream of phosphate buffer eluent. The polarity of the surrounding environment or solvent can affect tryptophan emission because its emission is sensitive to the hydrogen bonding to the imino group. In a completely apolar environment, a decrease in emission wavelength (blue-shift) is observed. As the residues become hydrogen bonded or exposed to water, the emission shifts to longer wavelengths and lower energies (Lakowicz, 2008).

5.2.1 Tryptophan

To assess the effect of the buffer on the spectral properties of the standard, absorbance and fluorescence measurement of tryptophan prepared in Milli-Q water and tryptophan prepared in Milli-Q water but subsequently diluted in the phosphate buffer, were recorded with offline spectrophotometers at the same concentration range used for the calibration. The dilution factor for the lowest concentration was above 150, to ensure that it corresponds to the dilution in the column of the HPSEC after injection. Measurements were done on both offline and online spectrometers at concentration of 0.5, 1, 1.5 and 2.5 mgC/L respectively. From the spectral obtained at each sample condition, it was observed that both tryptophan prepared in Milli-Q water and that subsequently diluted in phosphate buffer were very similar (Figure III-17). The area under the spectra showed that the difference between both conditions was less than 2%. The buffer could not modify the spectral composition of tryptophan because the pH was kept at 6.5~7.5 which is the pH range for the strongest fluorescent intensities of tryptophan (Yang et al. 2017). In neutral aqueous solution, the amino group of tryptophan is protonated (NH_3^+) while the carboxyl group is ionized (CO₂⁻) (Gudgin et al., 1981; Jameson and Weber 1981). Aside the pH, the bond distance, polarity and the charged distribution surrounding the indole ring and the amide groups influence the quenching intensity. From the results obtained, it can be concluded that, the eluent had insignificant impact on the spectral properties of tryptophan, thus both online and offline spectrophotometers can be compared with high certainties.

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Figure III-17: Effect of eluent on the spectral properties of pure tryptophan as recorded by classical detectors. (a) Spectral obtained at different concentration and (b) area under the spectra.

5.2.2 Quinine Sulphate (QS)

Quinine sulphate $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O)$ was chosen as the reference for humic substance and was prepared in 0.05 M H₂SO₄ (Nawara and Waluk, 2017). The effect of the buffer on the spectral properties of quinine sulphate was determined, by measuring the absorbance and fluorescence of the standard prepared in the acid solution, and that subsequently diluted in the phosphate buffer, to mimic the effect of injection into the eluent of the chromatographic system. Measurements were done with offline spectrophotometers at different concentrations. QS was excited at 244 nm and emitted at 420 nm. The spectral properties of both QS prepared in only acid and that diluted subsequently in phosphate buffer were very much different (decrease in fluorescence), as the eluent modified the pH of the solution. Quinine is a diprotic weak base and possesses pKa values of 8.5 and 4.1 at 20°C. Thus it appears as a free base (deprotonated form on an amine) at high pHs and as one of its ionized forms (quinine-H⁺ or quinine-2H⁺) at lower pHs (Moffat et al., 2011; Strauch et al., 2012; Troy, 2005). The fluorescent properties of many molecules change following ionization. Phenol and its moieties for instance show strong fluorescent maxima in acidic conditions but show no signal in alkali solutions. The reverse is true for naphthol and its moieties. For transition and fluorescence to occur, the symmetry of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) should be similar (Wang et al., 2006). A change in pH may have affected the symmetry properties of the functional groups of QS. The QY for the humic-like fluorescence was thus not determined.

5.3 Normalizing the fluorescence detectors for QY determination

Since it was established that the mobile phase composition had no effect on the spectral properties of tryptophan, the FLD online detector was normalized by the offline FEEM. The normalization was done by modifying equation 3, to incorporate the gradient of the calibration curve of both standards into the formula for calculating QY.

$$\Phi f(x) = \Phi f(s) * \frac{As}{Ax} * \frac{Fx}{Fs} * \left(\frac{\eta x^2}{\eta s^2}\right) * \frac{Gradient (SEC)}{Gradient (Classic)} \qquad \text{Equation (4)}$$

Where Φf represents fluorescence quantum yield, A represents absorbance at the excitation wavelength, F, the peak area (intensity in this case), n, the refractive index of solvent and subscript s and x for standard and sample respectively.

However, because the chromatogram obtained for FLD is for a specific excitation and emission wavelength, the peak intensities of the 3D FEEM was taken instead of the area (Cawley et al. 2015). There is currently no precedence where QY has been calculated using FLD making this method novel. The quantum yield values of the biopolymer fractions of natural water samples were on the average very low (3.42×10^{-6}) (Table III-2) compared to that of the pure tryptophan (0.14) which was expected. It was difficult ascertaining if the low relative Φf values of the samples, were because the proportion of excited molecules that were deactivated by emitting a fluorescence photon was low, or it was due to the normalization of both detectors. The former could be true considering the very low signal intensity observed in both FLD and FEEM. Furthermore, Bianco et al. (2016), in investigating the photochemical behaviour of tryptophan in cloud aqueous phase found that, the concentration of free and complexed tryptophan which was up to 10^{-7} M had a Φf of about 8.37x10⁻⁴ after 4h of irradiating 10 μ M of pure tryptophan solution. Thus, in a natural environment where pure tryptophan can be easily degraded, it may be normal to find such low Φf . Even at similar fluorescence intensities and DOC, the difference in absorbance resulted in varied QY, thus even though samples generally had different chromophores, they had similar fluorophores. QY generally decreased during treatment as fluorophores were removed. Since actual QY has never been determined with online detectors after fractionation, further studies are needed to verify the normalization of the two detector types.

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	Samples	PL	UVA ₂₃₀	QY
Campaign 1	Raw	3.59E-02	1.66	7.68E-07
	Nitrification	3.44E-02	1.59	7.68E-07
Campaign 2	Raw	2.69E-02	0.43	2.25E-06
	Nitrification	2.90E-02	0.07	1.52E-05
Campaign 3	Raw	4.15E-02	3.96	3.73E-07
	Nitrification	5.76E-02	1.71	1.20E-06

 Table III- 3: Fluorescence Quantum yield of the biopolymer fraction of DWG campaigns and its change during treatment processes. Signals are expressed in intensities. PL signal - (Ex@ 230 nm and Em @345 nm).

The values of the apparent QY of the biopolymer fraction (raw water and after the nitrification step) show that, the experimental values could vary within 2 orders of magnitude. In campaign 1, despite the change in UV and fluorescence signal, the apparent quantum yield was constant. On the contrary, the QY values changed for the other two campaigns, increasing by 1 order of magnitude.

These results are difficult to explain since there is no reference in existence for comparison, hence, this methodology would need further research in order to confirm the range of observed apparent quantum yield. Also, ultrafast spectroscopy which is used to determine real quantum yield could be useful in providing further insights into the experimental values and boost confidence in the results. This work could be performed in LASIRe laboratory in the future in collaboration with the inner transitory spectroscopy platform. Nevertheless, this technique could be interesting to further characterize the behaviour of NOM. Another standard compound would be needed to be able to apply this methodology to the humic substances.

6. Conclusion

Even with the application of multi-variant tools, data obtained from NOM characterized using only bulk techniques often lack some valuable information needed to enhance NOM characterization and optimize water treatment processes. However, using bulk techniques in tandem with fractionating techniques, provides a synergy effect which largely fills in the information gap. SEC-OCD is currently the most quantitative chromatography detector available for measuring organic carbon, although SEC-UV/FLD have demonstrated their ability to be used as a surrogate to ODC, especially for characterizing the humic substance and building block fractions. These proxy detectors were calibrated with reference samples for accurate semi-quantitative measurements. The concentration of SRU calculated was very similar to that of DOC for all campaigns. Coagulation which was the most efficient process in removing mainly the HMW fractions of NOM averagely removed about 6 SRU which corresponds to about 70% removal. TU was generally about 60% lower in intensity than the SRU and its removal efficiency was relatively steady.

The combination of the physical and spectral properties of NOM equally provided more insights into the characteristics of the biopolymer fraction in natural waters, which although affects some treatment techniques, is less known. The protein-like property of this fraction became more visible in fluorescence after fractionation. The visibility of this peak only at lower emission wavelength which corresponds to the protein-like region, confirmed that the peak observed had a tryptophan-like nature, and not due to light scattering. This identity was further confirmed by the iso-absorbance plot and component 2 of the PARAFAC model, which showed an excitation and emission maxima at 230 nm and 345 nm respectively for this fraction. URI, which is only possible after fractionation, provided further insights into the chemical composition of this fraction without interference from other NOM fractions. This parameter could be useful in tracking the seasonal variation of this fraction, which appears to increase during summer periods at least in the PWN source waters. Even though the column used in this study is expected to be inert to NOM, it was no possible to verify if the HOC are also retained in the column, since UV and FLD are not quantitative detectors.

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Chapter IV

Employing the spectral properties of NOM for the prediction and monitoring of precursors responsible for the formation of disinfection by products

Part of this chapter is based on the following published papers:

- MacKeown, H., Adusei Gyamfi, J., Schouttete, K., Dumoulin, D., Verdickt, L., Ouddane, B., Criquet, J., 2019 "Formation and removal of disinfection by-products in a full-scale drinking water treatment plant". Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2019.135280
- MacKeown, H, Adusei-Gyamfi, J., Delaporte, M., Schoutteten, K., Verdickt, L., Ouddane, B., Criquet, J., 2020 "Removal of disinfection by-product precursors by ion exchange resins". Environmental Science: Water Research & Technology - submitted

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1. Introduction

As it has been detailed in the previous chapter, when techniques that have been developed to characterize the physical and spectroscopic properties of natural organic matter (NOM) are combined, they produce a synergetic effect, which enhances the characterization of DOC in drinking water treatment. The monitoring and subsequent removal of DOC is important as it serves as a precursor for the formation of disinfection by-products (DBPs). Chlorination remains the most widely used disinfection technique in drinking water treatment, mainly because of its cost effectiveness and it provides residual disinfection capacity to ensure treated water stays safe during transport through distributing networks (Sedlak and von Gunten, 2011). Close to half a century ago, researchers had raised concerns that the DBPs formed by the reaction of NOM with chlorine might be carcinogenic (Rook, 1974; Clark and Sivaganesan, 1998). Further studies have suggested that DBPs are genotoxic and chronic exposure is associated with bladder and colon cancer (Jeong et al., 2012; Pals et al., 2013). Among the identified DBPs, trihalomethanes (THMs) and haloacetic acids (HAAs) are the most prominent groups prompting many countries to set standards to regulate them (Pressman et al., 2010). Generally, the hydrophobic and HMW DOM represent the major precursors of DBPs, although the hydrophilic or LMW DOM which are not removed by many primarily treatment steps, can also contribute to the formation of DBPs (Leenheer and Croué, 2003). For instance, iodine and bromine, formed from the reaction between chlorine and iodide or bromide, are known to be very reactive with the hydrophilic DOM forming more toxic compounds than chlorinated DBPs (Hua and Reckhow, 2007).

The formation of DPBs can be avoided by changing the disinfection technique or eliminating NOM which serves as a precursor. Since the former solution often involves capital and resource investment, the latter is often adopted. Rightly, the De Blankaert treatment plant of DWG which sources about 80% of its raw water from the Ijzer River, which relatively has high DOC

concentrations, has decided to adopt the approach of enhance DOC removal by modifying their water treatment processes. Additionally, the average DOC of the raw water is about 13 mgC/L, thus, high dose of coagulant is needed to significantly reduce the concentration of DOC to ensure the efficiency of further treatment steps. Feasibility studies on the possibility of introducing an IEX treatment step just before the coagulation step, to reduce the amount of coagulant required and improve overall NOM removal has been in operation since 2015. This was informed by the reported ability of IEX to remove charged organic compounds and in effect reduce the dose of coagulant required by 50 to 80% when used as a pre-treatment (Singer and Bilyk, 2002; Fearing et al., 2004; Grefte et al., 2013). IEX is generally a reversible exchange of ions between an insoluble solid phase which carries exchangeable ions and a liquid phase. If the solid phase carries anions (anion exchange resin), they can be exchange with the negatively charged NOM in the water. The ions are exchanged for a stoichiometric equivalent amount of the counter ions. It is estimated that IEX can remove about 60-90% of NOM with the unremoved fraction corresponding to the uncharged species of NOM (Bolto et al., 2004; Levchuk et al., 2018). It has even been demonstrated to be more efficient than coagulation for the removal of charged organic compounds from water (Bolto et al., 2002). The solid phase can be regenerated when all the exchangeable ions have been exhausted using a solution of sodium chloride (Helfferich, 1995).

In this study, four different IEX resins have been tested in a pilot plant for their removal of NOM, the reduction of DBP formation potential and their ability to fit into the current treatment steps. The possible use of spectral detectors to predict the formation of DBPs on the two separate treatment lines are monitored using an HPSEC coupled with online UV and fluorescent detectors. PARAFAC modelling which has been suggested in other studies as a promising tools for predicting DPBs (Yang et al., 2015; Brophy et al., 2019) was also assessed. Finally, the results obtained from SEC-UV were compared with that of SEC-OCD to assess a possible surrogate to DOC for the prediction of DBP formation.

2. Materials and Method

2.1 Samples source

Samples were obtained from two different water treatment lines: a full-scale treatment plant and a pilot scale plant of DWG drinking water company. The full-scale plant consisted of the following steps in the starting order of nitrification, coagulation, sand filtration and intermediate chlorination, granular activated carbon and chlorination. Three different campaigns were undertaken on this plant in the spring and summer of 2018. Samples were collected in a 2.5 L amber glass bottles and transported in less than an hour to the laboratory, after which they were filtered through a 0.45 μ m PES glass syringe and stored at 4 °C. A detailed description of the full-scale treatment process as well as the 3 campaigns undertaken have been outlined in chapter II, section 1.1.3. The pilot plant consisted of flow-through columns for IEX resin test which would be detailed in the sections below. For simplicity and easy reference, the full-scale treatment line would henceforth be referred to as treatment lane A while the pilot test would be referred to as treatment lane B.

2.2 Selected IEX resins properties

Four different commercial resins (Dowex TAN-1, Amberlite IRA410, Amberlite IRA958 and Purolite PPA860S) were tested for this study. IRA958 and PPA860S are macroporous with quaternary ammonium functional groups while TAN-1 and IRA410 have similar matrix or structure. Except for IRA410, the total exchange capacities and water content of the resins are similar. The detailed characteristics of the resins are presented in Table IV- 1.

Trade name	Resin Type	Matrix/ Structure	Functional Group	Mean Particle Size (µm)	Water content (%)	Total Exchange Capacity (eq/L)	Manufacturer
TAN-1	MP	Styrene- DVB	Quaternary amine	420-1200	70-82	0.7	Dowex
IRA410	Gel	Styrene- DVB	Dimethyl ethanol ammonium	600-750	45-51	≥ 1.25	Rohm and Haas
IRA958	MP	Crosslinked acrylic	Quaternary ammonium	630-850	66-72	≥ 0.80	Rohm and Haas
PPA860S	MP	Polyacrylic crosslinked with DVB	Quaternary ammonium	740	66-72	0.8	Purolite

Table IV- 1: Characteristics of the four anion exchange resins selected for lane B.

DVB: divinylbenzene; MP: macroporous; Reference: https://www.lenntech.com/Data-sheets

2.3 Column experiments

The column experiments were performed by DWG and the samples collected at the defined bed volumes sent to us for onward analysis.

2.3.1 Resin pre-treatment

Before the resins were transferred into each column, they were extensively pre-treated by jar pre-treatment. The Jar pre-treatment was performed by running 10% NaCl brine up to 3 bed volumes (BV) followed by decantation. This procedure was repeated thrice followed by triple rinsing with tap water of equal bed volume followed by decantation. The resins were loaded into the columns after the jar pre-treatment and a dummy loading run was performed using 500 BV of filtered raw water at an empty bed contact time (EBCT) of 1.73 min. Subsequently, 10% NaCl at 1 BV and 30 min EBCT was used for regeneration followed by 1 BV rinse of tap water for another 30 mins EBCT. This pre-treatment was necessary to eliminate any residual monomers or polymers present after manufacturing and to prevent the irreversible adsorption of NOM unto virgin resins.

2.4 Breakthrough experiment

The column breakthrough experiments were performed in a fluidized bed modus (Figure IV-1) where the feed water was introduced at the bottom of the column. For fluidized ion exchange, the rate of sedimentation of suspended solids in the raw water is lower than that of the ion exchange resins (Levchuk et al., 2018). Four transparent columns made up of PVC materials

and with an internal diameter of 86.4 mm were used. With the help of a peristaltic pump, the raw water which had been prefiltered over a 25 μ m bag filter (Filtration CVBA, Drongen, Belgium) was introduced into the column from a storage tank at an EBCT of 1.73 min. All resins were run until 500 BV with sampling done at 50 BV intervals, making a total of 11 samples for each resin. From each of the 11 samples, a fixed volume (208 mL) was taken and put together into a 'mix sample', to average out the different water qualities obtained at different runtime. The 4 mixed samples were used for the DBP experiments.



Figure IV-1: Schematic diagram of the IEX column test for lane B.

2.5 Chlorination test

The chlorination test was performed by my colleague, Henry Mackeown. The 7-day chlorination procedure, THM_{7d}, was used to compare the different treated waters for both lane A and B. This chlorination procedure was according to the Standard Methods 5710 (Standard method, 2017) with minor modifications. Samples which have been chlorinated with different doses of 5 g/L stock solution of sodium hypochlorite, were stored in 25 or 50 mL glass reagent bottles with PTFE lined screw caps, buffered with a phosphate solution at pH 7 and stored at 2 °C for 7 days. The experiment was performed to ensure that the free chlorine residuals was between 3 and 5 mg/L at the end of the chlorination test. Since a high dose of chlorine was added to the 7-day test which could influence the DBP speciation and especially iodine speciation, chlorination experiments at lower chlorine dose were performed, *i.e.* 0.3 mgCl₂/L residual after a contact time of 24 h at 20°C referred to as THM₂₄.

At the end of the chlorination test, the free chlorine residual of each sample was measured using the N, N-diethyl-p-phenylenediamine (DPD) colorimetric method (Rodier et al., 2016). The chlorine residuals in samples were quenched using sodium thiosulfate and the samples were stored at 4 °C in the dark until the THMs were analysed.

2.6 Disinfection by-product analysis

THMs were analysed with a sensitivity headspace-trap method. The analytical instrument used was a TraceTM 1300 GC ISQ-LT single quadrupole mass spectrometer (Thermo Scientific) coupled with a TurboMatrix Headspace 40 Trap (Perkin Elmer). For THMs analysis, 10 mL of the water samples was transferred into a 22 mL glass vials containing 4 g of sodium sulphate and were spiked with 1,2-dibromopropane as internal standard (6.4 μ g/L). The vials were immediately sealed and stirred in a vortex mixer until complete salt dissolution.

2.7 Organic carbon characterization

2.7.1 Dissolved organic carbon

The dissolved organic carbon content of the samples was measured following the same protocol as outlined in section 2.1.1 of Chapter II.

2.7.2 NOM size fractionation

NOM size fractionation was performed with an HPSEC coupled with UV, fluorescence and organic carbon detector. The chromatography principles and experimental conditions have been detailed in section 3 of Chapter II. UV absorbance wavelength was fixed at 254 nm while the online fluorescence detector wavelengths were fixed at excitation at 230 nm and emission at 345 nm and excitation at 244 nm and emission at 420 nm for the protein-like and humic-like fluorescence respectively. The SEC-OCD analyses were performed by the HET laboratorium.

2.8 Inorganic ions analysis

The presence of both cations and anions in the raw water and mixed samples and their evolution during the treatment processes were measured for lane B (Table IV-2). The concentration of chloride ions in the raw water was lesser than that of the resin's samples, due to the exchange of sites on the resins. There was an increase in chloride ions as sites on resins were replaced by NOM and other anions (sulphate). While the main target of IEX is NOM, the presence of other

inorganic anions like nitrates, sulphates, bromides can compete with NOM for exchange sites and be removed by IEX (Humbert et al., 2005). In this study, while the concentration of sulphate was significantly reduced by the resins, especially IRA410 (89% reduction), nitrite concentration was relatively stable probably due to the low concentration present in the raw water. Also, the concentration of calcium and bicarbonate ions in the raw water did not change significantly during treatment. TAN-1 and PPA860S resulted in an increase in ammonium.

	mg/L						
	Cl-	NO ₂ -	SO 4 ²⁻	HCO ₃	Na^+	NH4 ⁺	Ca ²⁺
Raw	147	0.01	104	247	105	0.16	71.9
IRA410	264	0.03	11	227	123	0.17	71.3
IRA958	305	0.06	58	243	185	0.17	73.1
TAN-1	270	0.03	52	232	147	0.28	71.9
PPA860S	220	0.05	49	226	112	0.26	72.1

Table IV-2: Concentration of some of the selected cations and anions during the test run

3. Effect of treatment processes on DBP precursors

3.1 Evolution of DOC during treatment processes

Hydrophobic DOC has been shown in other studies to correlate well with DBP formation potential (Wang et al., 2013). The raw water sample of lane A had an average DOC concentration of 8 mgC/L, while that of lane B had average DOC of about 12 mgC/L. The raw water samples from both lanes were from the same source and so should ideally have similar properties. This was however not the case and could be attributed to seasonal variations and anthropogenic factors. For instance, for the raw waters of lane A campaigns, there was about 20% variation in DOC, while comparing the raw waters of both treatments, there was a 30% variation (Table IV-3). Comparing the various treatment techniques, it was observed that coagulation and IRA410 were the most effective in reducing DOC for lane A and B respectively, with little DOC removal after the nitrification step. Many studies have reported coagulation as the most effective technique for removing DOC, especially the hydrophobic and HMW fractions (Matilainen et al., 2005; Davis and Edwards, 2014, 2017). The efficiency of IRA410 resin may be attributed to its high total exchange capacity compared to the other resins. There may however be other contributing factors such as the type of functional groups, since the other 3 resins have similar exchange capacities but reduce DOC at varied degrees.

DOC (mgC/L)	Decrease (%)					
Lane A						
7.6	N/A					
2.9	61.3					
3.2	N/A					
2.6	17.3					
ne B						
6.94	41.09					
9.08	22.91					
7.83	33.55					
7.45	36.77					
	DOC (mgC/L) ne A 7.6 2.9 3.2 2.6 ne B 6.94 9.08 7.83 7.45					

Table IV-3: Evolution of DOC at each treatment step. Data for Lane A represents average campaigns. N/A = < LQ.

Correlating the DOC to DBP formation potential only gives general information and the correlation risks being poor. This is because, the DOC measured corresponds to all organic carbon present whether they are responsible for DBP formation or not. The spectral properties of NOM can reveal both the composition and reactivity of DOC which would help to focus on the main precursors. To do this, an HPSEC was used to fractionate the bulk NOM into different fractions. The semi-quantitative UV detector was compared with the quantitative OCD.

3.2 Contribution of NOM fractions to DOC concentration

The different NOM classes obtained after size fractionation were namely biopolymer, humic substance, building block, low molecular weight acids and low molecular weight neutral. Lane A samples were analysed with SEC-UV while lane B samples (Raw water and mixed samples) were analysed with both SEC-UV and SEC-OCD. According to the SEC-OCD chromatogram, the humic substance was the highest contributor to DOC contributing about 40% (4.92 mgC/L) of the total DOC in lane B raw water. All the other fractions (biopolymers, building blocks and low molecular weight neutrals) contributed about 14% each to the total DOC (Figure IV-2). The low molecular weight acid fractions of SEC-OCD could not be interpreted due to ion chromatographic effect: the stationary phase (weak cation exchange resin) induces anionic repulsion forces between the LMW anions and the resin forcing the LMW acids to elute as a compressed peak (Huber et al., 2011).

In the absence of an OCD for the lane A analysis, the areas under the UV chromatogram measured at 254 nm were used to semi-quantitatively estimate the percentage contribution of

each fraction to the total DOC. This was possible due to the direct proportional relationship between absorbance and concentration, coupled with the hydrophobic fraction representing the bulk part (> 90%) of DOC. The semi-quantitative measurement corroborated that of the OCD, where the humic substance fraction was also seen as the highest contributor to DOC (about 60%), followed by the building block fraction with about 26% contribution for campaign 1. From Figure IV-2a&c, it can be observed that, even though the DOC of the other fractions may not have changed significantly, a reduction in the quantity of humic substances (after coagulation), resulted in a modification of their contribution to DOC. For instance, the contribution of the building block and LMW fractions to DOC became more significant (about 47 and 35% respectively) after humic substance removal, but subsequently remained relatively stable till the end of the treatment. A similar observation was made for lane B, where no major change in the DOC of all other fractions, except the humic substance, was recorded (Figure IV-2b, d). The slight increase in the biopolymer fraction could be due to a release of polymeric IEX material (Cornelissen et al., 2008) though this is in doubt considering the extensive pretreatment that was done.



Figure IV-2: (a) SEC-UVA₂₅₄ Chromatogram for the different treatment steps of lane A (b) SEC-OCD Chromatogram of the raw water and mixed samples of treatment steps lane B. (c) Contribution of each fraction to the total DOC in SEC-UV for the lane A. (d) Contribution of each fraction to the total DOC in SEC-OCD for lane B. Nitri= after nitrification, Coag= after coagulation, sand=after sand filtration and chlorination, GAC= Granular activated carbon, Final Chlo= final chlorination step, LMW=low molecular weight.

Comparing both treatment lanes, it can be ascertained from the chromatograms that, even though the total DOC may decrease during treatment, the elimination of specific NOM fractions was dependent on the treatment techniques used. While the humic substance fraction was targeted by coagulation and IEX, the LMW fraction was the main target during the filtration and adsorption process. It is thus important to know the evolution of NOM fractions during treatment, since the contribution of the fractions to the total DOC and DBP formation potential are also likely to change.

3.3 Evolution of NOM fractions during treatment

For treatment lane A where the average DOC was 8 mgC/L, nitrification step resulted in an insignificant reduction in DOC, with the average humic substance and low molecular weight fractions increasing. After coagulation however, the DOC decreased to about 1.8 mgC/L. Thus, about 96% and 47% of humic substance and building block were averagely removed causing their contribution to DOC to decrease from 63% to 11% for humic substance and an increased from 22% to 49% for building block (Figure IV-2a). Coagulation did not remove much of the LMW fraction which was expected considering the hydrophilic nature of this fraction. A similar occurrence was observed for lane B where the resins averagely reduced DOC from about 12 mgC/L to about 8 mgC/L, representing a 33% reduction. The humic substance was the main fraction removed in both lane A and B. Coagulation removed averagely about 96% of this fraction while the different resins removed about 41-72% of this fraction. While the efficiency of coagulation in removing NOM has been attributed mainly to charge neutralization, that of IEX resin has been attributed to ionic interactions (Cornelissen et al., 2008; Matilainen et al., 2010). Whereas sand filtration removed more of the remaining building block, GAC was efficient in removing the LMW fractions. Even though the biopolymer fraction was not removed by the IEX resin, there is no cause for worry in incorporating the lane B into A as the subsequent coagulation process can greatly remove this fraction (Galjaard et al., 2018).

3.3.1 Comparison of the different resins

The effect of resin on NOM fractions was dependent on the resin type, though generally, humic substance was the main fraction removed by the resins, even for the poorest performing resin (IRA958), followed by the building blocks and the LMW neutrals. The relatively low removal of the other fractions compared to the humic substance is an indication that they were not significantly charged. It has been already reported in literature that neither cationic nor anionic exchange resin are capable of removing the biopolymer fraction (Huber et al., 2011; Winter et al., 2018). Other studies have attributed this phenomenon to the large molecular weight of this fraction which limits it from diffusing into the porous structures of resins (Cornelissen et al., 2008). The LMW neutrals have equally been classified as having hydrophilic to amphiphilic property (Huber et al., 2011). Amberlite IRA410 and Purolite PPA860S were the most effective resins in removing about 43% and 34% of DOC respectively. Considering the different NOM fractions, the most effective resin types for the removal of humic substance was PPA860S (72%

removal) and IRA410 (68%), while IRA410 was the most effective resin for the removal of the building block and LMW fractions (41% and 36% removal respectively) (Figure IV-2b). Thus overall, for both SEC-OCD and SEC-UV, the efficiency of the resins was in the order of IRA410 > PPA860S > TAN-1> IRA958. Although the resin type (macroporous or gel) has been reported to influence its efficiency (Bolto et al., 2002; Tan et al., 2005), it appears this influence was not evident in this study as the DOC removal efficiency of IRA410 (gel structure) was quite closer to the other 3 macroporous resins. Equally, the 3 resins of similar type (macroporous), did not exhibit the same efficiency making it difficult to explain the NOM removal efficiency order of the resins used in this study.



Figure IV-3: Change in NOM fractions during treatment processes (a) lane A with SEC-UVA₂₅₄ (b) lane B with SEC-OCD. HS = humic substance, BB= building blocks, LNW= low molecular weight. Nitri= after nitrification, Coag= after coagulation, sand=after sand filtration and chlorination, GAC= Granular activated carbon.

The removal of NOM fractions in lane B as presented in the preceding section gave the global performance of each resin after 500 bed volumes (BV). However, these average results do not inform us if certain resins would require less or more BV than 500 BV to be effective. SEC-UV was used to assess the performance of each resin at each bed volume (0 to 500 BV).

3.3.2 Evolution of NOM fractions at different bed volumes

The evolution of the different fractions was monitored at 11 different bed volumes. Samplings were done at interval of 50 BV, thus from 0 to 500. The removal of NOM at each bed volume for the different resins are shown in Figure IV-4. Overall, the most effective resin was IRA410 which removed about 41% of the initial DOC. At 150 BV, this resin had removed almost all (> 90%, based on UVA₂₅₄) the hydrophobic humic substance and building block fractions present and a larger proportion of the LMW fraction (87%). The efficiency of this resin remained

relatively stable after 300 BV, except for a little decrease in the removal efficiency for the LMW fractions, meaning that this resin can be effective even for smaller bed volumes. This further shows that, for this resin, more volumes of water (at least up to 500 BV) can be treated without significantly compromising the quality of the effluent. The second most effective resin (PPA860S), also eliminated a larger proportion of the humic substance and building blocks at 150 BV. However, the removal efficiency for the building blocks and the LMW fractions significantly reduced after 150 BV. Thus, even though this resin could be effective at high volumes (> 250 BV), the removal efficiency for the humic substance may be assure but not for the other fractions. The performance of the two most effective resins at the different bed volumes (> 250 BV), where the removal of humic substance by PPA860S was 72% followed by IRA410 at 41%. TAN-1 removed almost all the humic substance fraction, although its efficiency for this fraction seems to have decreased after 300 BV. The increase in some fractions at higher bed volumes could be due to the release of hitherto trapped fractions, as has been highlighted to occur during coagulation process or just a decrease in the performance of the resin.

The least effective resin behaved completely different from the other resins. While the others appeared to be effective even at smaller bed volumes, IRA958 appeared to be more effective at larger bed volumes. At 50 BV only 56, 70 and 54% of the humic substance, building blocks and LMW fractions were removed respectively. The removal of these fractions however increased steadily as the bed volumes increased and at 400 BV, about 97, 81 and 83% of the humic substance, building blocks and LMW fractions had been removed respectively. This efficiency was stabilized until the last bed volume. Thus, contrary to PPA860S, IRA958 seems to be reliably efficient for treating only high volumes of water. It is unclear why the efficiency of this resin increased after higher volume runs. It can be established from these results that all resins tested have a good efficiency for removing mainly the humic substance and building blocks fractions though this is dependent on the bed volumes. The SUVA of the raw water decreased by about 32-62% after IEX treatment which confirms that the resins targeted more the hydrophobic components (humic substance and building blocks). The final SUVA value was between 0.75-1.34 L/mg/m which indicates that the remaining NOM is mainly hydrophilic (Hong et al., 2017). Whereas high SUVA₂₅₄ value (> 4) indicates that NOM is mainly made up of hydrophobic, high molecular weight compounds, low SUVA₂₅₄ (< 2) value indicates that NOM is mainly composed of hydrophilic, low molecular weight compounds (Edzwald and

Tobiason, 1999). Although the LMW fractions are not effectively removed by the resins, they can be subsequently removed by the sand filtration and GAC treatment as demonstrated in the preceding section. Knowledge about the efficiency of the different resins and their ideal bed volumes can help treatment facility to optimize their processes, by knowing when resins need to be regenerated to be more efficient and save resources.



Figure IV-4: Evolution of the different NOM fractions at some selected bed volumes. 200 BV for IRA410 and IRA958 were not available for analysis.

It has been demonstrated that the different techniques remove NOM fractions at different extent. Moreover, the contribution of NOM fractions to DBP formation varies and the fractions reported to be the least precursors are more hydrophilic. The next section assesses the contribution of the different NOM fractions to the formation of DBPs.

4. Effect of NOM fractions on DBP formation potential

4.1 Contribution of NOM fraction to DBPFP

The formation potential of DBPs (DBPFP) depends very much on both the structure and molecular weight of the NOM (Zhao et al., 2016). Results from two campaigns (1 and 3) of lane A, are presented here (no DBPFP performed for campaign 2). Generally, the THM_{7d} formation potential for the of raw water samples for both treatment lanes were very similar; 572 μ g/L and 550 μ g/L for lane A (campaign 1) and B respectively, despite different levels of DOC (8.5 mgC/L for lane A and 12 mgC/L for lane B). The formation potential of campaign 3 was however lower, 487 µg/L and DOC concentration of 6.7 mgC/L. Thus, the composition of the organic carbon is as relevant to the formation of DBPs as its concentration. The various fractions of NOM have different characteristics and would contribute to the formation of DBPs at different magnitude. A good correlation was observed between the humic substance fraction and THMFP for both lane A and B. This fraction was observed to be the main contributor to THMFP especially for the raw water and nitrification steps (Figure IV-5a). The UVA₂₅₄ areas under the chromatograms for this fraction were 60 and 48 a.u for lane A (campaign 1) and lane B respectively. The UVA₂₅₄ area for this fraction in lane A (campaign 3) was lower (38 a.u) which resulted in a reduced THMFP of 487 µg/L. This finding agrees with previous studies which reported the same fraction as the main precursor for THMFP (Golea et al., 2017). After the coagulation step, the humic substance fraction reduced in about 90% which resulted in a corresponding decline in THMFP. The THMFP however continued to decrease even though the humic substance fraction remained relatively stable. This was because the dominant fraction to DBPFP after coagulation process was shifted to the building blocks resulting in a positive correlation with DBPFP (Figure IV-5b).



Figure IV- 5: Contribution of the various NOM fractions to DBPFP for (a) the entire treatment steps of lane A (b) after coagulation step of lane A (c) lane B. For lane A: 1- raw water, 2- after nitrification, 3- after coagulation-decantation, 4- after intermediate chlorination and sand filtration, 5- after GAC filtration, 6- after final chlorination. (a.u.: arbitrary unit). For lane B: a- raw water, b- IRA 958, c- TAN 1, d- IRA 410, e- PPA860S.

4.1.1 Speciation of DBPs

After the 7-day chlorination test on both lane A and B samples, it was observed that, $CHCl_3$ was the main THM formed in all waters accounting for between 54 and 78% of total THM formed. THMs formed are in the order of $CHCl_3 > CHBrCl_2 > CHBr_2Cl > CHBr_3$ (Figure IV-6). It must be noted that the high dose of chlorine used in this protocol does not represent the speciation of THMs in real chlorination conditions. Especially considering the iodinated species, high dose of chlorine enhances the formation of iodate compared to iodinated-DBPs, albeit small amounts of $CHCl_2I$ and CHBrClI were detected in these tests. Whereas $CHCl_3$ was the main THM formed in all waters after the 7-day formation potential test, $CHBrCl_2$ was the main compound formed after the 24-hour test due to the lower chlorine dose used, leading to a

higher bromide to chlorine ratio. The formation potential of the 24-hour test was equally about 2.5 - 3.5 times lower than the 7-day test. Thus, the 7-day procedure should only be used to compare the removal of precursors during different treatment steps. It was further observed that, DBP formation potential decreased upon treatment for all the four resins. The THM_{7d} of the raw water which was about 550 μ g/L, decreased between 30 and 60% after the IEX treatment. The efficiency of the resins in reducing THMFP was in the order of IRA410 > PPA860S \approx TAN1 > IRA 958. This order was the same as what was observed for the efficiency of the resins in removing NOM fraction or DOC, confirming that as the precursors are removed, DBPFP is consequently reduced.



Figure IV- 6: Speciation of the DBPFPs for the different samples of lane B after the (a) 7-day chlorination test (a) 24-hour.

4.2 Effect of treatment processes on THMFP

The most effective approach for controlling the formation of DBPs is the effective removal of their precursors, mainly NOM before disinfection as the aromatic fractions are known to be the major precursors of THMs and HAAs (Gallard and von Gunten, 2002). It was not surprising therefore that for both treatment processes, there was a good correlation observed between THMFP and DOC. The correlation was however reduced when data from both treatment plants was combined. This was because, at the same DOC concentrations, samples had different organic matter composition, which resulted in the formation of DBPs at different magnitude. For instance, while about 7 mgC/L resulted in THMFP of about 460 μ g/L for lane A, the same DOC concentration saw about 2-fold decrease in THMFP to about 200 μ g/L in lane B (Figure IV-7). It is possible that the DOC observed in lane B was due to the contribution of the biopolymer fractions, which although present, contributed less to THMFP. Thus, the
importance of the composition of NOM cannot be underestimated in the prediction of DBPFP. Since the raw waters had similar THMFP concentrations, it presupposes that the difference in THMFP magnitude is as a result of the treatment techniques used, which may have preferentially removed more or less reactive fractions or may have modified the precursors of DBPs. The DOC concentration of the raw water sample for treatment lane A averaging about 7.5 mgC/L, was reduced to about 2.9 mgC/L after coagulation, corresponding to about 60% removal of DOC. This removal percentage is within the range of what has been reported in literature that if the DOC of the raw water is higher than 4 mgC/L, a removal of up to 70% is possible (Volk et al., 2000; Tubić et al., 2010; Vasyukova et al., 2013).

There was equally a strong correlation between the change in THMFP and the change in DOC (Figure IV-7b). The reduction in about 56% of DOC translated into a decrease of about 65% THMFP for just coagulation technique and about 80% for the entire treatment lane A. The average DOC decrease in subsequent steps was quite low with less than 16% of the initial DOC content removed after GAC, resulting in about 34% reduction in THMFP. The progressive decrease in the THMFP/DOC ratio along the entire lane A (about 35% for all campaigns) indicates that, THMFP is more reduced than the DOC during treatment. This is in agreement with what has been found in previous studies where a 50% decrease in THMFP/DOC was observed during treatment (Golea et al., 2017). This reflects a more preferential removal of the highly reactive organic THM precursors by the water treatment process, such as reactive hydrophobic fractions by coagulation-decantation or the progressive transformation of NOM towards less reactive moieties, as the DOC is neither significantly removed during nitrification nor in the filtration steps. The THM4 in the final chlorinated water ranged from 26 to 34 μ g/L, which was below the regulated threshold of 100 μ g/L of the European Union (Premazzi et al., 1997; Nikolaou and Lekkas, 2001).

The THMFP/DOC ratio of lane B saw a decrease of 10-33% (Figure IV-7a), meaning that like observed in lane A, the different resins were also more efficient at removing THM precursors than the overall DOC. For instance, the removal of about 41% DOC by IRA410 resulted in a 60% decrease in THMFP, while the decrease in THMFP for PPA860S was likewise higher (about 49%) than the reduction in DOC (about 37%). A similar trend was observed for TAN1 where the THMFP decrease was 47% compared to change in DOC of 34%. Comparing the close reduction efficiency in THMFP of PPA860S and TAN1 resins, it can be concluded that,

the ability of PPA860S to effectively remove most of the humic substance fraction (about 70%) compensated for its poor removal of the other fractions, unlike TAN1 which removed a fair amount of each fraction. Furthermore, it can be deduced that, the other fractions aside the humic substance equally contributed to THMFP albeit to a smaller extent, which was why IRA410 was the most effective resin in reducing THMFP though it removed similar proportion of the humic substance fraction as PPA860S. Comparing the change in UV absorbing component and the change in DOC to the change in THMFP relative to the raw water, it was observed that, there was higher percentage reduction in UV than DOC indicating that a portion of the precursors do not absorb UV and are made up of more hydrophilic components (Figure IV-7b).



Figure IV-7: Correlation between (a) DOC and THMFP concentration (b) change THMFP concentration vs Change in DOC and UV absorbing components with respect to the raw water.

4.2.1 Enhancement of brominated THMs

Bromide is often present in natural waters at low concentrations (~10 μ g/L to > 2 mg/L). However, when chlorine is added as a disinfectant to bromide containing waters, it can react with the bromide and oxidize it to form hypobromous acid/hypobromite ion (HBrO/BrO⁻). This derivative compound formed usually reacts with aromatic compounds through substitution reactions (Heller-Grossman et al., 1999; Magazinovic et al., 2004; Jiang et al., 2018). The corresponding bromine-containing DBPs formed are of much interest to water treatment plants and regulatory agencies due to their higher toxicity compared to chlorinated DBPs (Jiang et al., 2018). The formation of brominated THMs has been reported to be favoured in the presence of high hydrophilic fractions especially after coagulation (Tubić et al., 2013). Although there is evidence in literature to support that the hydrophilic/LMW fractions of NOM could have

significant potential for THM formation (Marhaba and Van, 2000), the lower reactivity of chlorine with the hydrophilic fraction has made other studies to conclude that this fraction does not significantly affect the formation of THMs (Croué et al., 2000; Chu et al., 2017). In this study, CHBrCl₂ was found to be the principally present brominated THM species (24-h test), being formed at all treatment steps of lane A. It was observed that after coagulation, the contributions of the LMW and building block fractions towards DOC were significantly increased with a decrease in SUVA from about 4 to 2.5 L/m/mg. Thus, it was not surprising that the bromine incorporation factor (BIF) (equation 1) which describes the molar contribution of all brominated species was higher after coagulation, because of the higher reactivity of the hydrophilic NOM with bromine compared to chlorine (Chowdhury et al., 2009; Criquet et al., 2015), coupled with the fact that the decrease in DOC favours the reaction path-way of bromide in forming DBPs.

$$BIF (THM_4) = \frac{[CHBrCl_2] + 2[CHBr_2Cl] + 3[CHBr_3]}{[CHCl_3] + [CHBrCl_2] + [CHBr_2Cl] + [CHBr_3]}$$
Equation (1)

The BIF can range from 0 to 3 with values closer to 3 representing a more brominated THM sample (Gould et al., 1983). Whereas the BIF for the raw water and nitrification step were about 0.15, that of the sample after coagulation was approximately 0.4. The BIF after GAC further increased (from 0.5 to 0.65). The average bromide concentration in the raw water, after nitrification and after coagulation were very much similar, about 155 μ g/L. The bromide concentration however decreased after sand filtration (87 μ g/L), due to the intermediate chlorination step but increased again after GAC filtration (117 μ g/L) indicating that, bromide was released while DOC was slightly removed. Br⁻ is almost inert to GAC filtration and so consequently increases the ratio of Br⁻ to DOC (Chiu et al., 2012). This could also be due to microbial degradation of some brominated compounds adsorbed on GAC.

For lane B, the BIF was relative to the sample composition and resin type. Whereas the raw water and IRA410 had the lowest bromide concentration of about 195 μ g/L, IRA958 had the highest of 228 μ g/L. The BIF was in decreasing order of PPA860S > IRA410 > TAN-1 > IRA958 > raw water. Thus, as DOC was effectively removed, the Br⁻/DOC ratio consequently increased, which favoured the reaction of chlorine with Br⁻ instead of DOC, leading to the formation of reactive bromine species. This explains why the most effective resins in removing DOC had the highest BIF values.

4.3 Correlation between SUVA and THMFP

Since the hydrophobicity of NOM is an important component in determining its ability to serve as a DBP precursor, the correlation between SUVA and THMFP was assessed. In this study, SUVA had a positive correlation with THMFP for both treatment lanes with an R² value above 0.9, which was higher than what has been reported in previous studies (Hua et al., 2010) (Figure IV-7). Whereas the SUVA value for the raw water in lane A was around 4 L/m/mg that of lane B was about 2 L/m/mg. Since their THMFP were very similar, it suggests that although the raw water of lane B was more hydrophilic, the biopolymer fraction may have accounted for its low SUVA value since it absorbed less in UVA254. The significant reduction in SUVA after coagulation step resulted in a corresponding decrease in THMFP concentration. The percentage decrease in SUVA and THMFP were very similar, which implies that the decrease in THMFP was due to the elimination of the hydrophobic NOM fractions by the resins. The NOM composition of the IRA958 (least performing resin) effluent was more hydrophobic than the rest of the resins. Since SUVA considers the different properties and composition of NOM, it serves as an additional technique to compliment the information derived from DOC concentration. The difference in slope between the two lanes may be due to the biopolymer fractions.



Figure IV- 8: Correlation between SUVA and THMFP of lane A lane B. For lane A: 1- raw water, 2- after nitrification, 3- after coagulation-decantation, 4- after intermediate chlorination and sand filtration, 5- after GAC filtration, 6- after final chlorination. For lane B: For lane B: a- raw water, b- IRA 958, c- TAN 1, d- IRA 410, e- PPA860S.

5. Fluorescence of DBP precursors

As it has been highlighted in the preceding sections, the correlation between DOC and THMFP is mostly assured for highly hydrophobic NOM. Thus, understanding the properties of the components of NOM is indispensable in accurately predicting DBPFP. One sensitive technique which could be employed to characterize the composition of NOM is fluorescence. In this study, the 3D excitation and emission matrix of the samples were analysed and a multivariant analysis (PARAFAC) was used to assess the variation of NOM components.

5.1 PARAFAC Components

PARAFAC was applied to the FEEM data of only samples from the various treatment steps of lane A for all campaigns as well as ion exchange resins samples, which were different from the main treatment line. This meant fewer dataset (22 samples) were analysed here compared to what has been presented in section 2.1 of Chapter III. Although there is no cut-off for the minimum dataset that are required for PARAFAC, it is preferable to have model datasets of 20-100 samples although modelling becomes simpler with samples above 100 (Stedmon and Bro, 2008). Thus, the dataset used for this analysis was within the required range, albeit small. The decrease in dataset however resulted in a decrease in the number of components validated (from 5 to 3). When the components were compared with the PARAFAC model fluoresence spectra database of the Open Access Openfluore, it was confirmed that the 3 components were a subset of the 5 components obtained in Chapter III, as they bare similar characteristics. Component 1 and 2 represent humic-like fluorophore while component 3 represents protein-like fluorophore. The difference between the components from the two data sets were their wavelength maxima. The 3 components observed in our model are shown in (Figure IV-7).



Figure IV- 9: 3D EEM contour view of the three Components and their normalized loadings identified by PARAFAC model. The datasets are samplings from the three campaigns at DWG and were normalized to their respective DOC values.

5.2 Identity of the PARAFAC components

After comparison with the fluoresence spectra database of the Open Access Openfluor (<u>www.openfluor.org</u>), 41, 20 and 11 references matched with components 1, 2 and 3

respectively. There were two peaks of excitation maxima for each of the three components but with a single emission maxima, meaning they all had two absorption bands. Dual fluorescence is attributed to the 'Kasha's rule' which states that photon emission (fluorescence or phosphorescence) occurs in appreciable yield only from the lowest excited state of a given multiplicity. Thus when atoms are excited into higher electronic and vibrational levels, they quickly (< 10^{-12} secs) dissipate their excess energy leaving them in the lowest vibration level (S₁) (Lakowicz, 2008; Brancato et al., 2015). The properties of the different components observed in our model are as follows;

<u>**Component 1**</u>: This has excitation maxima at < 240 nm and 320 nm and emission maxima at 420 nm. The fluorophore present in this component is characterised as humic-like peak M (Coble et al. 2014) and shares similar characteristics with the component 4 in chapter III, section 2.1.

<u>**Component 2**</u>: This is linked to the Humic-like C+ fluorophore which are terrestrial and allochtonous. It equally has two excitation maxima at 260 nm and 360 nm and an emission maxima at 475 nm. This component shares similar characteristics with the component 3 in chapter III, section 2.1.

<u>**Component 3:**</u> This component has excitation wavelegth maxima at < 240 and 280 nm and an emission maxima at 335 nm. It is represented by peak T and characterize the tryptophan-like (protein-like) fluorophores, similar to the component 2 in chapter III, section 2.1.

Other PARAFAC models agree with peak-picking methods that Em < 380 nm generally have components with 2 excitation wavelengths and emission wavelength (Carstea et al., 2016).

5.3 PARAFAC components removal during treatment processes

The fluorescence maxima of each component at the different treatment stages were normalised by the raw water sample, in order to observe the evolution of each component along the treatment steps. The majority of fluorophores found in the natural water samples were removed by the coagulation proccess. This trend was similar to DOC, where coagulation removed the most (averagely 60% for all three campaigns). The highest removal by coagulation was observed in component 2 for all campaigns where an average of 74% of this component was removed. This aggrees with literature that this component is largely removed during coagulation, ozonation and biological activated carbon filtration (Bieroza et al. 2009; Baghoth et al. 2011). Ishii and Boyer (2012), argue that although this component dominate in residual DOM of treated water, during water treatment processes, its fluoresence intensity is moderately reduced during reservoir storage and chlroination. In this study, component 2 was not dominant in the treated water even though its fluroescence intensity moderately reduced during chlorination process. The higher removal by coaguation for both component 1 and 2 relative to component 3 confirms that, coagulation targets the more hydrophobic (mostly allochtonous) components and less of hydrophilic components (mostly autochtonous). Thus fluorophore of NOM could be used to assess the character of raw waters and predict NOM removal (Baghoth et al. 2011; Shutova et al. 2014).

Even though campaign 1 had the highest DOC removal during coagulation (64%), it had the lowest fluorophore removal (44-50%) for all components, meaning that some hydrophilic carbons were also removed during coagulation. A similar trend was observed by Shutova et al. (2014), where the DOC removal by coagulation did not necessarily reflect the same percentage removal of component fluorophore. Sand filtration was next to coagulation in terms of fluorophore removal efficiency (about 58%). The tendendcy of a fluorophore to be removed may therefore be more dependent on the treatment process than on the components involved. A good correlation has been observed between DOC removal and fluorophore removal suggesting that, fluoroscence could be used to predict DOC removal by coagulation as it is currently the case for UV absorbance (Shutova et al., 2014).

5.4 PARAFAC components for predicting DBPFP

The different PARAFAC components of treatment lane A did not correlate well with THMFP but for component 1 (0.75) (Figure IV-7). Even for this relatively good correlation, the points were not evenly distributed and there appeared to be no correlation at THMFP < $200 \mu g/L$. This component is a characteristic of the humic-like peak M. Thus, whereas PARAFAC components have been proposed in other studies for monitoring DBP formation potential, the results of this study show that, PARAFAC model may not be able to directly predict DBPFP. Compared with chromatography fluorescence fingerprints (HL signal), the FEEM-PARAFAC model are unable to show variation of DOM species with similar fluorescence but different physicochemical properties (Li et al., 2014).



Figure IV-10: Correlations of THMs formation potential with component 1 of PARAFAC model of lane A.

PARAFAC modeling may however be able to help indirectly. The various PARAFAC components were correlated with the chromatography fluorescence fingerprints (HL and PL signal) using Spearman's correlation method (15 degree of freedom and confidence interval of 95 %). The objective of this was to see if PARAFAC can be used to validate these signals, to serve as a surrogate for online monitoring of DOC and consequently THMFP during treatment processes. HL signal correlated best with both component 1 (r_s = 0.92) and 2 (r_s = 0.80) of our PARAFAC model. This was higher than the correlation with DOC (r_s = 0.88 for component 1 and r_s = 0.76 for component 2) and UVA₂₅₄ (r_s = 0.77 for component 1 and r_s = 0.65 for component 2). This performance of fluorescence compared to UV absorbance was however dependent on the NOM composition since UV correlated better than PL except for component 3, which was mainly made up of protein-like components. As expected, the PL signal correlated best with the component 3 which was microbial based component, also its correlation with component 1 was higher. This confirms the description of this component, that the source of its fluorephores are likely to be autocthonous and microbial oxidized (Cory and McKnight, 2005).

 Table IV- 4: Summary of Spearman's rank correlation coefficients between PARAFAC components score, UVA254, DOC, HL and

 PL signal for all three campaigns.

	DOC	HL	PL	UV
Component 1	0.879	0.915	0.733	0.766
Component 2	0.760	0.799	0.529	0.645
Component 3	< 0.47	0.490	0.635	0.481

5.5 Chromatography fingerprints for online monitoring of DBPFP

From the spearman's rank correlation coefficients, the fluorescence and UV chromatography signals, which showed a good correlation with the PARAFAC components were further explored to see how best they could be used to predict DBPs. It must be reemphasized that even though DOC had a good correlation with THMFP for each of the treatment lanes, the correlation was poor when both treatment lanes were combined due to the variation in the raw water quality. This shows that the use of DOC as an indicator for THMFP may only be valid for individual treatment plants, as the quality and quantity of NOM is dependent on the treatment technique used. It has also been established in chapter III, that the spectral signals could be used as a surrogate for DOC. With the composition of NOM been highlighted as an important parameter for DBP formation potential, this section seeks to apply this proposition by using the chromatography fingerprints to monitor and predict the formation potential of DBPs.

5.5.1 Humic-like (HL) and UVA₂₅₄ signal

Among the different precursors of DBP present in NOM, phenolic structures have been identified as the principal precursors of THMs and HAAs (Gallard and von Gunten, 2002; Chu et al., 2015). This functional group is mainly present with the humic substance fraction. Since the humic-like structure has good absorbance in UV and fluorescence, the peak areas of the chromatograms recorded at each treatment step were integrated and correlated with the THMFP. The HL fluorescence signal was fixed at an excitation at 244 nm and emission at 420 nm. The reasons for the choice of this wavelength has been detailed in Chapter III, Section 4.2.1. It was not surprising that for both treatment lanes, UVA₂₅₄ and the HL signal showed the best linear relationship with THMFP ($R^2 = 0.94$ and 0.91 respectively), even higher than DOC (0.79) (Figure IV-7a-c). The HL and THMFP correlation were higher than that between component 1 and THMFP. The relatively strong correlation of UVA₂₅₄ and HL fluorescence of NOM with THMFP compared to that of DOC as observed in this study was not surprising, although DOC has been widely reported in literature to have a better correlation (Bond et al., 2014). Even though a decrease in \mathbb{R}^2 was observed when both treatment lanes were combined compared to their individual lanes, the correlation was more robust as the points were evenly distributed. Notwithstanding the slight decrease in correlation for the combined lanes, it was comparable to what has been reported in literature (Li et al., 2016). Since hydrophobic carbons when ever present are preferentially removed by any treatment technique, it explains why these signals correlate well with THMFP, coupled with the fact that they serve as the main precursors of DBPs. Thus spectral (fluorescence and UV) data reflects the chemical composition and predicts the reactivity of organic matter (Peleato et al., 2018).

5.5.2 Protein-like (PL) signal

The PL signal (excitation at 230 nm and emission at 345 nm) for lane B was very well correlated (0.98) (annex SIV-1), but not for lane A and so the combined data had a reduced correlation (Figure IV-7d), although in general, the PL fluorescence did not correlate well with THMFP compared to that of the HL signal. Previous studies have also reported better correlation between humic-like components and THMFP than protein-like components (Hua et al., 2010; Li et al., 2016), though the relation reported in this study is nonetheless higher than what has been reported in other studies. The reported studies were however performed on several water sources making those results more robust. The results of the PL fluorophore signal were expected as it rather correlates more with the biopolymer and LMW fractions, which have been shown in the previous sections to be less precursors of DBP. HL fluorescence therefore has more advantage than PL fluorescence for online application because biopolymers are mainly eliminated after coagulation and the residual protein-like fluorescence is mainly attributed to the combined protein-like fluorophores in humic substance (Li et al., 2016). The elution of the protein-like peak with the humic-like peak may be due to the presence electrostatic attraction or hydrophobic effect between them (Tomaszewski et al., 2011).



Figure IV- 11: Correlation between THMFP and (a) DOC concentration (b) chromatography UV absorbance at 254 nm (c) HL signal (d) PL signal.

The signal response of the chromatography fingerprints recorded at each treatment step was further compared with the change in signal response with respect to the raw water (Δ). Since spearman's function is less sensitive to the distribution of the data compared to the default correlation coefficient function in excel (correl), the correlation was slightly improved. From Table IV-2, among all signals, HL showed the best correlation with THMFP followed by UVA₂₅₄. Δ UV absorbance however saw an increase in its correlation, with a slight decrease in the correlation with Δ HL signal. This confirms that, the formation of DBPs is mainly due to the presence of hydrophobic carbons (chromophores) most of which equally fluoresce in the humiclike region and are the main targets during treatment. The Δ DOC resulted in an improved correlation with THMFP. Thus, it is evident that both HL signal and UVA₂₅₄ could be used as a good surrogate for DOC, for online monitoring and prediction of DBPs. In summary it can be concluded from our results that, spectral detectors can be used to accurately estimate DBPFP even with changing water characteristics during treatment processes. These detectors are easyto-use even in remote environments and provides instantaneous reading (Sorensen et al., 2018). This is of much interest to treatment facilities because the frequent monitoring of dissolved organic carbon (DOC) and DBPs is relatively expensive and time-consuming (Henderson et al., 2009). This has resulted in many agencies highlighting the need for a surrogate monitoring of DOC and estimation of DBP formation potential. HL fluorescence has shown a good correlation and potential for online monitoring of treatment plants. Large dataset of samples from different water sources is still needed to evaluate the robustness of this tool.

 Table IV-5: Spearman's rank correlation between surrogate indicators and THMFP. In bracket represent correlation due to the change in signal intensities relative to the raw water.

	THMFP (A THMFP)	DOC (A DOC)	UV (Δ UV)
DOC (A DOC)	0.88 (0.93)		
UV (Δ UV)	0.92 (0.97)	0.86 (0.89)	
HL (Δ HL)	0.93 (0.90)	0.85 (0.76)	0.97 (0.96)
PL (Δ PL)	0.83 (0.78)	0.80 (0.81)	0.67 (0.70)

6. Conclusion

The principal aim of water treatment facilities is to eliminate NOM as much as possible which would in effect reduce the potential formation of DBPs. The availability of very sensitive techniques that has the capacity of both monitoring NOM and predicting DBPFP is thus indispensable in attaining this objective. Although DOC is the most quantitative technique currently in existence for assessing water quality, the information obtained is not enough to make informed decision on treatment processes. For instance, for coagulation process to be effective, NOM in water has to be both hydrophobic and of high molecular weight, such information cannot be obtained with only DOC measurement. The use of an HPSEC coupled with spectral detectors can however fill in this information void and be used for effective characterization of NOM. In this study, we have demonstrated that the use of chromatography fingerprint signals is capable of directly relating DOM fluorescence spectra to polarity and molecular size. These fingerprints can be used to monitor and predict the formation potential of DBPs.

Comparing both treatment lanes, it can be concluded that the raw waters contained a mixture of hydrophobic and hydrophilic components which contributed to the formation potential of DBPs at different extent. The humic substance fraction of NOM which is known to be more hydrophobic, contributed most while the LMW and hydrophilic fractions contributed least. Coagulation removed most of the humic substance fraction and DOC which translated in its highest reduction in THMFP for lane A. For lane B, the most effective resin for THMFP and DOC removal was IRA410. This resin was effective even in removing (35%) the LMW neutrals which has a low ion density. The use of spectral detectors where validated with the DOC signal and proved to be capable of predicting and monitoring online precursors responsible for the formation of DBPs. Using these fingerprints, it was observed that the reduction in THMFP during treatment processes was due to the preferential removal of the highly reactive organic THM precursors and the progressive transformation of NOM towards less reactive moieties. The THM₄ in the effluent was below the regulated threshold of the European Union attesting to the efficiency of the treatment process.

The high concentration of sulphate in the de Blankaert waters can however pose a challenge to the use of IEX resins, due to competition with NOM for exchange site, since its smaller size makes its ion exchange process kinetically faster (Cornelissen et al., 2010). About 44 to 89%

sulphate was removed for all resins certainly reducing the capacity of the resins to remove NOM. Even though N-nitrosodimethylamine (NDMA) has been reported to be found in waters treated with IEX, indicating that there may be the release of organic compounds acting as precursor of NDMA (Fiddler et al., 1977), Kemper et al., (2009), in studying three commercially available resins concluded that, the concentration of N-notrosamines produced by the resins are below the permissible levels before and after disinfections. Further research is required nevertheless to investigate the contribution of the 4 resins used for this study toward the formation of NDMA.

7. Reference

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Chapter V

Quantitative and qualitative method for the distribution of metals in NOM fractions

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V. Quantitative and qualitative method for the distribution of metals in NOM fractions

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1. Introduction

The speciation of metals and their interaction with NOM influences their mobility in natural waters and their bioavailability (Martin et al., 2017). Due to the complexity of natural systems, analysis of metal speciation in natural waters remains a challenge. Studies about NOM-metal binding properties can be classified under two main approaches. On the one hand are studies that focus on the characterization of metal binding behaviours, while on the other hand are speciation studies that provide field evidence of metal binding (Laborda et al., 2008). The former entails complexation reactions between NOM isolates and metals under well-defined laboratory conditions. Information such as the binding capacity and stability constants can be obtained using this approach. The later approach however entails the use of analytical methods to determine the speciation of metals in environmental samples (Ure and Davidson, 2008). Due to the complex nature of natural systems, the later approach is more complicated and requires very sensitive and highly selective methods. One of such methods is the coupling of ICP-MS with fragment techniques like size exclusion chromatography, to study the distribution of metals in the different molecular sizes of NOM (Rathgeb et al., 2016; Zhou et al., 2016). Although some studies use SEC-ICP-MS technique to characterize NOM-metal interactions, most of them do it qualitatively while the few ones that propose quantitative methods are not direct measurements and may require the use of fraction collectors (Schmitt et al., 2001; Sadi et al., 2002; McKenzie and Young, 2013; Kozyatnyk et al., 2016; Zhou et al., 2016). Out of the 18 references reviewed where SEC-ICP-MS was used for NOM-metal analysis, the method of Piatina and Hering (2000), was found to use a direct quantification approach to determine the conditional stability constants of metal complexes with strong organic ligands in competitive equilibration experiments. Their method was however fraught with a lot of challenges. This means that to the best our knowledge, there is currently no robust method for the direct quantification of NOM-metal complexes in aqueous solution using SEC-ICP-MS.

The current inexistence of a robust quantitative method is probably because one of the greatest challenges, is ensuring that the complex is adequately kinetically inert to remain intact, when NOM is fractionated in the SEC column, despite the perturbation of equilibrium conditions that may arise due to the chromatographic conditions (Piatina and Hering, 2000). Therefore, the objectives of this study were to assess the changes in NOM properties that occur upon complexing with metal ion through spectroscopy techniques and to develop a quantitative method for the distribution of metal ions in NOM fractions using SEC-ICP-MS.

2. Materials and Method

2.1 Samples source

Samples were obtained from the various treatment lines of project partners notably PWN (NL) and SWW (UK) drinking water treatment companies. The treatment line of PWN consisted of coagulation, suspended ion exchange (SIX), Ceramic microfiltration (CF) and Ultra filtration (UF) while that of SWW consisted of SIX, in-line coagulation and membrane filtration. A detailed description of the full-scale treatment plants has been outlined in chapter II. Received samples were immediately filtered through 0.45 μ m PES using a glass syringe and stored at 4°C. Standard reference samples (extracts) were purchased from the IHSS and stored at 4°C. Suwannee River fulvic acid, humic acid and NOM were mainly used in this study.

2.2 Chemicals and reagents

Metal standard solutions were prepared by dissolving the required volume of the mono element standard solution (99% Astasol) in deionized water (DIW, 18 M Ω -cm). EDTA solution was prepared with 99.99% trace metals basis reagent grade (Aldrich) while the phosphate buffer for the mobile phase was prepared from K₂HPO₄ and KH₂PO₄ (99.99%, Suprapur).

2.3 Complexation analysis

The effect of metal (Al³⁺, Zn²⁺ and Mn²⁺) complexation on the composition and spectral properties of NOM was studied using a circulatory set up connected online to both UV absorbance and fluorescence spectrophotometer. These metals were chosen based on their affinities for NOM. Raw water samples from PWN were spiked with known concentrations of metals at neutral pH, with the help of an automatic titrator which added required volumes of 0.05 M NaOH. The initial concentrations of Al³⁺, Zn²⁺ and Mn²⁺ in the natural samples were 0.46, 0.78 and 0.43 μ M respectively. These initial concentrations correspond approximately to the first spike concentration (0.5 μ M). After each metal addition, the solution was allowed a reaction time of 30 mins while stirring continuously before it was pumped to be detected by the two detectors simultaneously. This reaction time was chosen following earlier pre-test runs (Falantin, 2017). Same experiment was performed for subsequent analysis by HPSEC-UV-fluorescence. In total, 11 different concentrations (T1-T11) were studied within a range of 0.5-150 μ M for each metal.

Titration	Metal concentration (µM)
T1	0.5
T2	1.0
T3	4.0
T4	8.0
T5	20.0
T6	40.0
T7	60.0
T8	80.0
Т9	100.0
T10	120.0
T11	150.0

 Table V- 1: Concentration of metal ions that were added in series to natural raw water samples during complexometric experiments.

2.4 Total metal analysis

The total trace metals concentration in samples were analysed using a Varian 820 ICP-MS while the major cations were measured using VISTA-PRO CCD simultaneous ICP-OES. Before analysis, samples were acidified with 1% HNO₃ (67-69%, Optima, Fisher chemical). Calibration curves for the metals were generated from a 1 g/L multi-elemental standard stock solution with appropriate dilutions in 1% HNO₃ to the desired concentrations. Anionic measurements were performed using ICS-3000 (Dionex).

2.5 SEC-ICP-MS instrumentation

The SEC-ICP instrumentation protocol has been detailed in section 4.1 of chapter II.

3. Samples characteristics

The characteristics of the natural water samples obtained from PWN, SWW and reference standards are summarized in Table V-2. The raw water sample from PWN had a DOC concentration of 4.4 mgC/L and a SUVA value of 3.68 L/m/mg meaning the sample is relatively hydrophobic. This concentration varied upon treatment. The most abundant trace metal was Zn while the most abundant anion was Cl⁻.

The addition of Aluminium at neutral pH did not result in observable precipitation because pH is not the sole determinant of metals solubility (Cravotta III, 2008). In natural waters, the chemistry of Al is more complicated to predict due to the presence of both organic and inorganic ligands which compete with the hydroxide ion for complexation, speciation mainly depends on factors such as pH, alkalinity, temperature, DOC, dissolved inorganic carbon and anion concentration (Chamier et al., 2015). For instance, the formation of AlSO₄ instead of Al(OH)₃ increase the solubility of Al in waters (Cravotta III, 2008). Considering the higher natural concentration of SO₄²⁻ (50 mg/L) in our samples, it is possible that some Al³⁺ were complexed with dissolved ligands like sulphate which caused it to remain in solution under the experimental conditions. Furthermore, at neutral pH such as in natural waters, Al³⁺ is expected to bond to weak carboxylic groups (Dudev and Lim, 2004; Marsac et al., 2012).

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Table V- 2: Natural concentrations of ions in water samples. Sample names correspond to different treatment steps; Raw
(SIX influent), CFE (ceramic microfiltration influent), CFI (ceramic microfiltration effluent), UFE (ultra-filtration influent), UFI
(ultra-filtration effluent), RT (Tamar River), BR (Burattor Reservoir), SIX (suspended ion exchange), N/A (not
available/measured).

Concentration (mg/L)										
Sample	$\begin{array}{ccccc} SUVA \\ DOC & (L m^{-1} mg^{-1}) \end{array} Ca & K & Mg & Na & Cl^{-} & NO3^{-} & SO4^{2^{-}} \end{array}$) ₄ ²⁻			
Raw	4.4	3.68	33.8	6.0	11.5	88.0	134.0	6.2	49	9.7
CFE	2.4	2.95	36.0	6.1	11.3	96.0	201.0	3.7	4	.7
CFI	1.8	2.99	35.4	6.2	11.4	94.0	200.0	3.6	4	.8
UFE	3.6	3.96	68.0	6.2	12.3	68.0	141.0	9.6	6	5.0
UFI	4.0	3.49	68.0	6.2	12.4	53.0	137.0	9.4	6.	3.0
Concentration (µg/L)										
Sample	Al	As	Со	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Raw	12.4	0.7	0.4	3.1	4.2	16.8	23.6	3.1	0.5	50.9
CFE	< 5.0	0.5	0.4	1.9	3.0	7.3	16.5	1.3	< 0.05	< 1.0
CFI	< 5.0	0.5	0.3	1.7	3.4	0.8	10.4	1.0	0.1	1.2
UFE	< 5.0	0.3	0.2	1.2	5.4	2.4	0.5	1.9	0.1	1.9
UFI	< 5.0	0.3	0.2	1.3	4.3	1.0	0.4	1.9	0.1	< 1.0
RT	N/A	12.2	N/A	N/A	5.3	N/A	6.0	N/A	0.56	5.2
BR	N/A	8.9	N/A	N/A	0.9	N/A	4.9	N/A	0.28	3.9
SIX	N/A	9.1	N/A	N/A	1.0	N/A	4.5	N/A	0.26	3.6
SRHA	23.0	N/A	< 0.02	0.61	0.60	N/A	N/A	0.48	0.14	1.47
SRNOM	10.28	N/A	< 0.02	0.95	1.21	N/A	N/A	0.19	0.24	0.89

The elemental compositions of the IHSS standards published on the supplier's website (http://humic-substances.org) has been summarized in

Table V-3. Although the most recent extracts were purchased; SRHA (3S101H), SRFA (3S101F) and SRNOM (2R101N), the elemental compositions are not yet completely available. In such instance, the most recent available data was chosen and stated accordingly.

Carbon distribution (ppm)										
NOM sample	Aliphatic (0-60)	Hetero aliphatic (60–90)	Acetal (90–110)	Aromatic (110–165)	Carboxyl Carbonyl (165–190) (190–220)		Ratio of aromatic to aliphatic carbon			
SRHA	23.9	13.4	8.9	35.3	12.8	3.9	1.48			
SRNOM	27	15	7	23	20	8	0.85			
SRFA	35	16	6	22	17	5	0.63			
Acidic functional										
groups (mmol g ⁻¹)				Elemental co	ompositions	% (w/w)				
	Carboxyl	Phenolic	H ₂ O	Ash	С	Н	0	Ν	S	
SRHA	9.13	3.72	11.9	4.62	54.59	3.90	40.03	1.50	0.55	
SRNOM	11.21	2.47	5.69	4.01	50.70	3.97	41.48	1.27	1.78	
SRFA	11.17	2.84	11.3	0.78	53.30	3.98	41.81	0.66	0.41	

 Table V-3: Carbon distribution, acidic functional groups and elemental compositions of extracts (SRNOM (1R101N), SRFA (2S101F) and SRHA (2S101H) as reported on IHSS website.

Ratio of aromatic to aliphatic carbon = (percentage peak area of 13C NMR spectrum 110–165

ppm) / (percentage peak area of 13C NMR spectrum 0–60 ppm).

4. Effect of NOM-metal complexation

4.1 Changes in absorbance spectra of NOM

4.1.1 Conventional UVA₂₅₄

The negative charges on the surface of NOM cause it to have a high affinity for cations and capable of carrying an ion (ionophore) especially cations. When the chromophore and fluorophores of an ionophore complexes with a metal ion, their properties may be perturbed resulting in significant changes in both the absorption/emission spectra and a possible shift in wavelengths (Kim et al., 1999). Absorbance spectroscopy has been widely used to study complexations between NOM and metals because of its high sensitivity to the variation of NOM composition and the need for no sample pre-concentration. However due to the often featureless nature of the absorbance spectroscopy of NOM-metal, the concept of differential absorbance spectroscopy (DAS) has been proposed as a useful technique to study the deprotonation of NOM and their reactions with metals (Yan et al., 2013; Gao et al., 2015). In this study the linear differential was calculated using equation (1) as described in previous studies (Yan et al., 2013; Yan and Korshin, 2014; Gao et al., 2015; Yan et al., 2016):

$$\Delta A_{\lambda} = A_{\lambda,i} - A_{\lambda,ref} \qquad \qquad \text{Equation (1)}$$

Where $A\lambda_{i}$ and $A\lambda_{ref}$ represent DOM absorbance measured at the wavelength λ for any selected condition (i) and an applicable reference (background concentration) respectively.

Scanning across a range of wavelength, it was observed that for Al complex with NOM in the raw water sample, there was a decrease in UVA₂₅₄ with increasing metal concentration without any shift of spectra wavelength. Differential spectra elucidated more this decrease with a prominent negative band at ca. 240 nm. Similar decrease in absorbance (225 to 300 nm) and negative bands have been observed at 250 and 280 for the complexation of SRFA and Al³⁺ (Yan et al., 2016), which has been attributed to a bathochromic shift (position shift of a peak or signal to longer wavelength) of absorbance bands of some NOM chromophores when they interact with metals (Chen et al., 2015). The decrease in absorbance intensity could also be due to a decrease in the energy needed to cause light-induced electron transition in a chromophore (Fedenko et al., 2017). Upon the addition of Zn, there was a linear increase in absorbance and a spectral shift at 250 nm towards longer wavelength. The Red shift is probably due to an increase in conjugation in the NOM structure upon metal addition. Thus, the nature of transition was mainly $\pi\pi^*$ which required less energy to move electrons thereby shifting the absorption

maximum to a longer wavelength. A band at ca. 244 nm has been attributed to the deprotonation of phenolic group in SRFA (Dryer et al., 2008; Zhang et al., 2018). Specifically, at 254 nm, the absorbance increased until T6 (40 μ M) and then decreased subsequently upon metal addition. Mn addition resulted in an increase in absorbance for all wavelength range at all concentrations without any shift in wavelength. The change in the absorbance properties of NOM in the presence of Mn was less intense than for Zn. Because of the natural nature of the sample, there could be quantitative competition between added metal and naturally existing cations.



Figure V- 1: Changes in absorbance properties of NOM (raw water) upon complexation with Al, Zn and Mn measured with a classical UV spectrophotometer. Red-dashed arrow represents change in signal response or shift in wavelength.

Like most bulk techniques, the information extracted from conventional UVA₂₅₄ albeit useful is limited. To proceed further on the characterization of NOM-metal complex, a fragment technique (HPSEC) was coupled with bulk detectors to characterize the absorbance and molecular weight signature of each NOM fraction after interacting with metals.

4.1.2 Online DAD

The effect of metal complexation on NOM absorbance was observed with a DAD coupled with an HPSEC at 254 nm since this is the usual wavelength for NOM monitoring (Tian et al., 2018). The interaction of Al and Zn with NOM resulted in a decrease in absorbance in the humic substance and building block fractions, meaning that the metals reacted with the chromophores of these fractions. From the chromatogram, it can be observed that the change in absorbance in Al complex was higher than in Zn complex (Figure V-2), which is not clearly comparable with the observation with classical UV spectrometer. The reactions of Mn with NOM however did not result in any visible change in absorbance as the signal intensities were generally stable. It is possible that the sample may have evolved at the time Mn experiment was performed. The strong modification in absorbance observed with Al complex compared with the other divalent cations could be due to the fact that, although polyvalent cations can aggregate molecules by binding to same or different ligands from the same or different humic molecules through intermolecular bridges, trivalent elements have a high binding capacity than divalent elements (Wu et al., 2004). Among the divalent cations studied, Zn has been reported to have a higher affinity for organic matter than Mn (Ghodrat et al., 2017). From the chromatogram, it was observed that the HMW fractions were the most significantly modified fraction. It is thus possible that, metals either reacted first with this fraction due to preference or metals had greater effect on this fraction, resulting in a more intense modification in absorbance. Several studies have also suggested that metals have a higher affinity with HMW fractions (Schmitt et al., 2001; Wu et al., 2004). For Al for instance, the most significant change in absorbance (> 6%) at the LMW fraction was only observed at concentrations above 40 µM. At 60 µM of Al addition for instance, the first peak at 6.8 min was almost disappeared. A similar observation was made for Zn which has been reported in previous studies to elute with the HMW fraction (Wu et al., 2004). The preference of a metal for a particular NOM fraction is closely related to the binding strength; with high binding capacity metals being distributed more in the HMW fractions and low binding capacity being distributed among the LMW fractions (Wu et al., 2004). The high affinity of polyvalent cations to humic substance has been attributed to the high content of oxygen functional groups like carboxylic, phenolic and carbonyl groups (Simpson et al., 2002; Wrobel et al., 2003). It is most likely that Al was bonded to the phenolic groups among other functional groups, because at pH range of 4 and 8, most of the binding can be attributed to the phenolic sites (Kinniburgh et al., 1999). The LMW fractions did not show a uniform trend for all metals. Whereas, Al caused no significant change in the absorbance of the LMW acids, Zn caused an increase in the absorbance of the LMW acid fraction.



Figure V-2: Effect of metal complexation on the absorbance of NOM determined by HPSEC-UV. Red-dashed arrow represents change in signal response and black-dashed arrow represents change in retention time for (a) AI (b) Zn (c) Mn.

4.2 Changes in apparent size

A change in the apparent molecular size of NOM complex was noticed by a shift in the retention time. Although all metals were observed to be bonded to some extent to almost all fractions resulting in a modification in its spectral properties (absorbance and fluorescence), the change in size was restricted to some specific fractions notably the LMW fractions. The effect of complexation on the LMW fractions was dependent on the type of element. This was expected since different metal ions have unique hydrolyzation efficiencies and binding capacity to NOM
(Yan and Korshin, 2014). The size of both the humic substance and building block fractions did not change upon metal addition suggesting the absence of aggregation at these fractions. For Al and Zn, complexation with the LMW fractions resulted in a shift in the retention times Figure V- 4a. Kozyatnyk et al., (2016), observed that Zn interacts with a broad range of NOM size fractions including LMW compounds. It has been suggested that LMW fractions which have high affinity for metals may contain amino groups (Xue and Sigg, 1999). Thus, even though Zn may not have had a primary preference for the LMW fraction, it still reacted with it to some extent.

4.3 Changes in fluorescence spectra of NOM

4.3.1 Excitation-emission matrices

The interaction between certain ions and conjugated fluorescing ligands are known to affect one or more of the chemical aspects of the ligands resulting in an enhancement or decrease in fluorescence intensity, depending on the influence that the ion has on the non-radiative process competing with fluorescence. Metals for instance are Lewis acids and as such can coordinate to fluorescing ligands and influence the electronic state of the ligand in a similar faction as protonation reactions (Sharma and Schulman, 1999; Tan et al., 2011). The interaction of metals with organic matter often results in static quenching where the potentially fluorescing chromophore, in the ground state, reacts with the quenching species to form a non-fluorescent species. The unpaired electrons of the metal ion interact initially with the π electrons of the ligand, consequently producing a pathway for intersystem crossing from the directly excited singlet state of the ligand to a triplet state and then deactivate the fluorophore to the ground state with loss of heat energy rather than photon emission (Coble et al., 2014). Aside intersystem crossing, charge transfer and electron exchange can also contribute to overall fluorescence quenching (Lakowicz and Masters, 2008).

From the 3D FEEM contour of the sample without any metal addition, two excitation maxima were evident; 275 nm and 315 nm (annex, SV-1). Since it was difficult to extract useful information from the contour maps after metal addition, a fixed excitation wavelength of 315 nm was selected to assess the impact of metal on the fluorescence properties of NOM over a wide emission wavelength (350-550 nm) as proposed by other studies (Provenzano et al., 2004; Tan et al., 2011). This excitation wavelength was selected based on the fact that this wavelength also allows to obtain the maximum emission intensity typically observed for dissolved NOM (400-460 nm) (Fabbricino and Korshin, 2004). The area of the peak under the emission

wavelength range was integrated using 'Origin 2017'. Quenching of fluorescence intensity was observed for all metals although the extent of quenching was high in Al in accordance with what has been reported in previous studies (Wrobel et al., 2003), meaning that the fluorophores responsible for the peaks were involved in metal complexation. At Al concentration of 140 μ M, there was almost no more fluorescence. Similar to absorbance, the fluorescence as a result of Mn addition did not change much while that of Zn appeared to have rapidly decreased with small amount of metals addition, then stabilized above 60 μ M. The variation in the extent of complexation can be attributed to both the affinity of NOM and the electronic configuration of each metal.



Figure V-3: Changes in the fluorescence intensity of NOM (raw water) upon complexation with Al, Zn and Mn at fixed excitation at 315 nm and emission wavelength range of 350 nm to 550 nm.

4.3.2 Online 2D-FLD

The evolution of fluorophore with metal addition was not very clear to interpret from the 3D FEEM contours maps (data not shown). However, from the online fluorescence signal measured at excitation at 275 nm and emission at 460 nm with the FLD after HPSEC, the modification of spectral properties was evident. Excitation at 275 nm was selected because the chromatographic peak resolution was better than at 315 nm. This wavelength maxima has been defined as peak C associated with humic like fluorophore (Coble, 1996). The trend in fluorophore modification upon Al complexation was very similar to the modification in chromophore. There was a steady decrease in the humic substance and building block fluorophores with increasing Al concentration. The LMW neutral recorded a decrease in fluorophore while no change in the LMW acid fluorophores was noticed. Both increase and quenching of fluorescence intensity upon Al addition has been recorded in literature. While enhancement of fluorescence was

attributed to the formation of Al-induced aggregates, quenching was suggested to be due to the loss of material by precipitation (Wrobel et al., 2003). It can be concluded that for Al complexation, the change in chromophore and fluorophore with respect to MW was very similar. There was a significant decrease in the fluorophores of all fractions upon the addition of Zn at concentration above 20 μ M. In fact, above this concentration, there was no more fluorophore present at the LMW neutral fraction. Mn had the least effect on NOM fluorophores which was similar to what was observed for DAD chromatogram. The modification (spectral and size) observed at the LMW fractions of NOM was similar for all metal. This could mean that this fraction is the most 'vulnerable' once the condition of the solution changes. Upon metal addition, weakly bounded cations are displaced to be associated with weaker sorption sites that are mostly abundant in the LMW fractions (Wu et al., 2004).



Figure V- 4: Changes in fluorophore properties of NOM upon complexation with Al, Mn and Zn measured with HPSEC-FLD. Red-dashed arrow represents change in signal response for (a) Al (b) Zn (c) Mn.

Whereas a modification of the spectrum on all the fractions is observed, coupling an elemental detector with HPSEC can define the priority fractions of metals and respond to why some fractions experience significant modification in absorbance than others.

5. HPSEC-ICP-MS Coupling protocol

Studying the binding behaviour of metals with NOM based on laboratory measurements under well controlled conditions can be very useful as has been demonstrated in the preceding sections. The complexation experiments allowed characterizing the behaviour of NOM in the presence of several ions, relying on the changes in their chromophores and fluorophores as benchmarks. These experiments can further provide information about the binding capacities of NOM towards different cations which has led to the development of very useful theoretical models to describe NOM-metal interactions (Wang et al., 2016; Van Cappellen and Wang, 2018). This approach however has its own limitations as their detection limits are too high to simulate a natural environment. In our studies for instance, the last concentration of metal added (T11) was about 300 times above what would be naturally present in the sample. Thus, to mimic the trace metal concentrations found in natural systems, very sensitive and highly selective methods are required. One of such sensitive methods that has demonstrated to be a promising technique for the speciation analysis of trace element in natural waters and was selected for this study is an HPSEC-ICP-MS (Zhou et al., 2016; Martin et al., 2017).

5.1 Detection time correction

When an HPSEC was coupled with the ICP-MS, the capillary tube connecting the column inlet and outlet to the cross flow nebulizer of the ICP-MS had to be changed from a stainless steel to a polytetrafluoroethylene (PTFE) tube to limit metal contamination. The difference in tube dimensions can modify the travel time of the sample to reach the detector albeit not affecting the retention times of the peaks or the peaks separation. To standardize the detection times and compare the chromatograms obtained by SEC-ICP-MS to that of SEC-UV, EDTA was used as a metal carrier since it is a good complexing ligand and absorbs UV light at 254 nm. Different concentrations of Cu were added to EDTA and the peak retention times were monitored with both SEC-ICP-MS and SEC-UV. From the chromatogram of both detectors (Figure V-5), it can be observed that, the peaks of the SEC-ICP-MS chromatograms eluted at about 0.20 min (12.0 sec) earlier with a slight peak broadening compared to the SEC-UV chromatogram. A correction factor of 12 sec was therefore applied to compare the retention times of NOM fractions detected in both detectors.



Figure V-5: Comparison of the detection times in chromatograms obtained in both (a) SEC-UV and (b) SEC-ICP-MS for EDTA-Cu complex in order to obtain a correction factor.

5.2 NOM-metal complexation in natural water samples

One advantage of HPSEC-ICP-MS that has been highlighted already is its sensitivity in measuring trace metal concentrations in natural environmental systems. The raw water sample from PWN was used to test the coupling protocol. The natural metal concentrations of the sample are presented in Table V-2. Based on the elements naturally present in the sample, a multi-element screening was performed to detect the possible complexation of Al, As, Cr, Cu, Fe, Mn, Pb, and Zn to NOM fractions. It must be noted that this technique does not detect free ions but only complexed ions which elute as a peak. This was confirmed by the absence of a peak when metal only solution was injected as a sample. Out of the metals screened, 5 (Cr, Zn, Cu, As, Pb) showed chromatographic peaks which indicated complexes formed. No peaks were observed for Al, Mn and Fe inferring that they were present in free forms. For the complexed metals, the chromatograms were different for each metal (Figure V-6) with each metal being complexed with different NOM fraction(s).



Figure V-6: Chromatogram of NOM-metal complex in raw water sample of PWN analysed by SEC-ICPMS. Sampling was done on 26/04/17.

5.3 Selectivity of metals for NOM fractions

After correcting the detection times, the chromatograms obtained with both UV and ICP-MS were compared, which showed a good fit Figure V-7. Most elements were observed to be complexed to just one fraction while Cu appeared to be complexed with almost all NOM fractions, which was not surprising considering the strong complexing capacity of Cu (Ouddane et al., 1992). It has been observed that at pH 7, Cu and Co show a wide peak and complex with DOM fractions of molecular mass range of 0.2-35 kDa, with a maximum around the humic substance fraction (1.2 -1.5 kDa) (Sadi et al., 2002; Laborda et al., 2008). The complexation of Co with NOM was not considered in this study and so only copper was observed to be complexed with the humic substance fraction of NOM. Even though the number of available sites of NOM was unknown, two possible reasons could account for the behaviour of Cu; on the one hand, among the elements which complexed with NOM, only Zn was higher in concentration than Cu. Nevertheless, Cu can use its higher binding affinity to nullify the higher concentration effect of Zn. The binding capacities of some studied polyvalent elements are in decreasing order of affinity as; $Cu^{2+} > Ni^{2+} > Co^{2+} > Pb^{2+} > Cd^{2+} > Cr^{3+}$. (Mn²⁺, Mo⁴⁺, Zn²⁺) (Pandev et al., 2000; Evangelou and Marsi, 2001; Sadi et al., 2002; Wrobel et al., 2003). Thus, it is possible that Cu complexed with all the available sites of the humic substance fraction, thereby forcing the other cations to complex with the remaining fractions. On the other hand, it could mean that the other cations just had a preferential choice for the other NOM fractions Figure V-7. Zn complexed mainly with the building block fraction and to a smaller extent with the LMW acids. Zn has earlier been found to be complexed to the fraction of SRNOM with size just below 1 KDa (building block in our sample) (Rathgeb et al., 2016) although Vogl and Heumann, (1998), contrarily observed that Zn prefers to complex rather with the high molecular weight acids. It is thus very likely that the changes in absorbance observed in section 4.1.2, was due to preference of metals for the HMW fraction. Although the concentration of Pb was low (0.52 μ g/L), the chromatogram showed that this metal was also complexed with the building block fraction. Previous studies have equally observed both Zn and Pb complexing with the same NOM fraction (0.36 - 1.35 KDa) of a compost extract (Wrobel et al., 2003; Metreveli et al., 2010). This further largely confirms that, the choice of complex sites by Zn and Pb was probably less likely influenced by the presence of Cu. Cr and As formed complexes with only the LMW acids. Metals that are present in the dissolved phase or complexed with the LMW fraction of NOM are considered to be more bioavailable (Cabaniss et al., 2000). Since toxicity of metals is largely linked to its bioavailability, the weaker affinity of ligands in the LMW fractions can cause toxic metals like Cr and As to be released into the environment.



Figure V-7: Preferential binding sites of metals for NOM fraction of PWN raw water sample analysed by HPSEC-ICPMS compared to UVA₂₅₄. PWN samples were received on 26/04/17.

Even though no evidence of Al and Fe complexation were observed in PWN samples, Al and Fe showed evidence of complexation with the biopolymer and the humic substance fractions in SWW samples. This is confirmed in literature where both ions are known to have similar preference for the very HMW fraction of NOM. The test for repeatability on different samples received on different dates showed that the results were reproducible. It has been suggested in literature that, the peak of Al and Fe which elutes at the biopolymer retention time (exclusion volume) may be due to hydroxide colloids of their ions which are adsorbed onto NOM. It has however been demonstrated in section 4.2 of Chapter III that, the absorbance of this fraction is likely to be due to the presence of chromophores and not due to scattering. A similar behaviour of Cu complexing with different fractions was observed for multiples samples received from SWW Figure V-8. For these samples, a slight shift in retention time was observed although this did not affect the identification of NOM fractions.

Comparing the results obtained for PWN and SWW samples, it can be ascertained that the preference of Cu for almost all NOM fractions was similar irrespective of the source especially for raw waters where the metal concentrations are high. Unfortunately, complexation of other metals with SWW sample was not evident due to the high background noise. Once the total metal concentration was low, there was a very low signal to noise ratio of the chromatogram. The finding in this study is very novel, because as much as other studies exist on the complexation of metal with NOM, none exist to the best of our knowledge that looks at NOM complexation at MW < 1.2 KDa especially with respect to drinking water treatment. The fraction of NOM with size < 1.2 KDa are however of great concern to drinking water treatment processes, as they are the main size range that remains during secondary treatment process. It was thus interesting to understand the complexation properties of this low MW range fractions compared to that of the high MW fraction range.



Figure V- 8: Preferential binding sites of metals for NOM fraction of SWW samples analysed by HPSEC-ICPMS compared to UVA₂₅₄. The various reception dates for samples are included in the legend. BR=Burrator Reservoir, RT= Tamar River, SIX= Suspended ion exchange.

5.4 Competition among metals for binding sites

Depending on the functional groups available, it may be possible for metals to exchange binding sites. Competition for binding sites was observed upon the addition of metals. For instance, the addition of 20 μ g/L of Cu was able to completely displace Zn from its binding site (Figure V-9). It was observed that during this displacement of Zn peak, only the building block fraction of the new Cu chromatogram increased. Thus, it is possible that there were no more available sites on the humic substance fraction which caused all the 20 μ g/L of Cu added to complex with the building blocks. There was however no significant modification in the Cu complex when 20 μ g/L of Zn was added as this concentration was not enough to displace complexed Cu ions. This again confirms the higher binding capacity of Cu compared to Zn.



Figure V-9: Cation competition at binding sites (a) natural concentrations (b) addition of 20 µg/L of Cu

More samples from different water sources and NOM composition are needed to confirm the preference of metals for NOM fractions. This would also help ascertain if the preference is relative to the concentration of the metal.

5.5 Evolution of metal distributing during treatment processes

The evolution of metal distribution with respect to elimination of NOM was monitored in two treatment lines of PWN. The first line consisted of raw water, suspended ion exchange (SIX) and ceramic microfiltration (CF). The second treatment line consisted of coagulation (Coag) and ultrafiltration (UF). Details of these two treatment lines have been outlined in section 1.1.1 of Chapter II. The natural concentrations of total metal ions in the samples as summarized in Table V- 2 have been graphically represented in Figure V-10.



Figure V-10: Total metal concentration in samples at each treatment step measured with an ICP-MS. SIX (after suspended ion exchange), CF (ceramic microfiltration), Coag (after coagulation), UF (ultrafiltration), Absence of bar = < LQ.

For the first treatment line, after treatment with suspended ion exchange technique (SIX), there was generally a positive removal of the hydrophobic carbons according to the UV chromatogram, which translated into a positive removal of the metals complexed to these fractions Figure V-11a. There was however a negative removal (about 38% increase) of the LMW acid fraction after SIX which equally explains the increase in the percentage of Cu complexed at this fraction Figure V-11b. This points to a positive correlation between NOM removal and complexed metal removal. The increase in carbon of the LMW fraction could be due to a release of smaller complexed fractions which were hitherto entrapped in the HMW fractions. Since there were fewer sites at the preferred HMW fractions for Cu to be bonded, the increased available sites on the LMW acids became the preferred site for complexation. The increase in the LMW acids fraction after SIX may have also contributed to the increase in Cr and As complexes since they were preferentially bounded to this fraction Figure V-11c,d. Complexed Zn was totally removed after SIX which is confirmed by the total metal concentration. There was no significant change in the hydrophobic carbons during the microfiltration (CF) processes, which may have contributed to the insignificant changes in the complexes of As and Pb as well as their total concentrations. The highest complex modification by this treatment was observed with Cr where about 6% of the complex was removed.

For the second treatment process, even though no significant UVA₂₅₄ carbons were removed, ultrafiltration (UF) process resulted in a positive removal of all metal complexes although the removal was not always significant. This is confirmed from the total metal concentration after UF which showed very little change. It can be concluded therefore that, UF like CF were not effective techniques in significantly removing metal complexes. This ineffectiveness could be due to their inability to significantly remove hydrophobic carbons. Thus, once a treatment technique is effective in reducing NOM, metals complexes would be consequently removed.

It must however be mentioned that, the coupling of HPSEC-ICP-MS is not a fully developed method with some challenges such as elevated background noise especially when analysing treated samples. Thus, these results need to be confirmed especially for the treated samples which are close to the uncertainty of the ICP-MS measurements. Nonetheless, qualitatively, the results seem to be consistent with the removal of the NOM fractions and their associated metals.



Figure V-11: Elimination of hydrophobic carbons (UVA₂₅₄) and complexed metals (b) Cu (c) As (d) Pb (e) Cr from NOM fraction during treatment. Treatment processes are: SIX (suspended ion exchange), CF (ceramic microfiltration influent) and UF (Ultrafiltration).

5.6 Effect of complex removal on MW and metal preference

Globally, it was observed that aside a change in signal intensity, the retention times of the peaks remained the same throughout the treatment steps, meaning the MW of NOM fraction were not

modified. A similar occurrence was observed in the HPSEC-UV-FLD where the only modification in NOM that was observed especially for the HMW fractions, was a change in signal response. This is in agreement with what has been observed in other studies where the removal of polyvalent cations resulted in no disaggregation of NOM suggesting that either the molecular size of NOM was not affected or that SEC is insensitive to the aggregation-disaggregation processes (Schmitt and Frimmel, 2003). The former is likely to be true as changes in retention times were observed at high metal concentrations for the complexometric titrations in section 4.1.2. Another observation was that, although the different treatment techniques could modify the concentration of complexed metals, they did not change the preference of metals for a particular NOM fraction(s). It was not possible to correlate the total metal concentration removed with the change in UV signals because of the presence of free ions.

6. Quantitative method development

It has been demonstrated in the preceding sections that there could be some correlation between the removal of NOM fractions and the consequent removal of metals complexed to these fractions. The challenges associated with this semi-quantitative analysis have been equally highlighted, necessitating the need to develop an online quantitative method that can predict with higher certainty the actual concentration of metals complexed to NOM fractions. In classical ICP-MS, signal intensities are measured which quantitatively translates into concentration with the help of a calibration curve. However, when ICP-MS is coupled with an HPSEC, a different operation mode is used which measures rather the area of a chromatogram. A calibration curve can neither be done in this mode because free metal ions are either retained in the column or eluted, heavily diluted by the eluent and so are undetectable. Studies that have tried to calibrate ICP-MS by directly injecting metal standards while bypassing the SEC column have concluded that, for repeated experiments, although it is possible to have some elements consistent in their elution times, they are less consistent in peak heights (McKenzie and Young, 2013). Another approach that has been proposed consists of using a fraction collector. However, although some sample dilution is unavoidable during chromatography, direct coupling of column to detectors limits such dilution (Piatina and Hering, 2000), compared to the fraction collection technique. Also, the peaks boundaries of NOM in natural samples are not always well defined, making the fraction collection technique prone to errors.

6.1 Calibration method development

To make ICP-MS quantitative, a calibration curve was needed. Since free metals were not detected, a pure grade (metal free) EDTA was selected as the metal carrier. The choice of EDTA was because it is a simple ligand whose molecular structure, concentration, stoichiometry of its complexation with metals and other equally important parameters are known or can be known. The calibration standards were prepared by spiking known concentrations of metals with EDTA at a neutral pH and reaction time of 30 min. The metals chosen for this method development were Cu, Zn and Mn based on the difference in their binding strengths. The neutrality of pH was ensured by preparing solution directly in the phosphate buffer. The calibration curve was done by plotting the integrated peak area under the chromatogram against the known concentrations of metal solution. Figure V-12, shows the calibration curve obtained after adding different concentrations of Zn and Cu to EDTA. Since the stoichiometry of EDTA complexation with metals is in the ratio of 1:1, the titration was done to ensure that there was

excess EDTA at the final metal concentration. Piatina and Hering (2000), observed insignificant changes in Cu recovery for complexes with different Cu to EDTA ratios as high as tenfold excess of EDTA. EDTA is a strong complexant and so almost no decomplexation was expected in the column. This was confirmed by the strong linearity obtained from the calibration curve. Al did not demonstrate good complexation with EDTA as it may have precipitated at neutral pH and consequently poorly complexed. Both Cu and Zn showed good complexation with EDTA with high R² values. Although equilibrium conditions were violated during chromatographic separation of complexes, the complex was sufficiently kinetically inert and stable. Strong 1:1 complexes are kinetically inert under chromatographic conditions (Piatina and Hering 2000).



Figure V-12: Calibration curved obtained from the reaction of Zn and Cu with EDTA as detected by HPSEC-ICP-MS.

6.1.1 Test for repeatability

The repeatability of the calibration method was tested three times to confirm that EDTA complex did not dissociate in the column. There was consistent linearity for all three metals tested confirming that there was a good quantitative estimation irrespective of the metal concentration (Table V-4). The possible fluctuation in signal intensity of the ICP-MS instrument makes it require a recalibration at each run. With increasing metal concentration, the corresponding increase in signal intensity may have drowned the background noise of the chromatogram resulting in reduced deviation.

Sample	Cu	Zn and	Cu replicate	Zn replicate	Mn replicate
	(µg/L)	Mn (µg/L)	(%)	(%)	(%)
	1	10	101 ± 12.9	99 ± 3.3	104 ± 2.6
EDTA	5	30	100 ± 0.2	100 ± 1.9	99 ± 1.3
	10	50	100 ± 0.1	100 ± 1.3	100 ± 0.3

Table V-4: Repeatability of calibration curve determined by SEC-ICP-MS (mean $\pm s$, n=3).

6.2 Column cleanliness – 'Mop' eluent

At the initial stages of the method development, the calibration curve obtained had significant concentration of metals in the blank solution of EDTA despite the high purity of the product. This was thought to be due to the presence of historical trace elements which were retained in the column. For simple ligands like EDTA, the equilibrium speciation can be used to predict the extent of competition between metals. This is however not possible for a very complex matrix like natural waters. The conditional stability constant for ligands in natural waters compared to that of EDTA is lower. The conditional stability constants of Cu, Zn and Mn found in natural waters are within the range of $10^{8.44} - 10^{15.7}$ for Cu, $10^{4.69} - 10^{9.3}$ for Zn and $10^{4.30} - 10^{4.85}$ for Mn at neutral pH (Mantoura et al. 1978; Van den Berg and Dharmvanij 1984; Pesavento et al. 2004; Cheng and Allen 2006). Thus, although the stability constant for Cu-EDTA ($10^{15.4}$) may be within the range (for Cu) in natural waters, those of the other metals studied appears to be out of range ($10^{13.1}$ for Zn and $10^{10.6}$ for Mn) (Davidge et al., 2001; Pesavento et al., 2004; Boija et al., 2014). Thus, for weaker ligands complexed to metals, alteration of the equilibrium conditions in the column may result in dissociation.

To ensure therefore that free metals ions do not remain in the column, an auxiliary mobile phase consisting of EDTA, which would be henceforth referred to as a 'mop' was introduced into the method. The role of the mop was to ensure that any uncomplexed or decomplexed metals (especially from weaker ligands such as in natural samples) were swept away, keeping the column clean and the complexation of subsequent samples not falsified. Thus, at 6 min of run time, the mobile phase flow was switched from phosphate buffer to 0.5 mM EDTA solution. This lasted for 30 sec after which the mobile phase was reverted to phosphate buffer. The total analysis time was then extended to 17 min to ensure that the baseline returns to stability before

the next sample injection. The eluent flow timetable is shown in (Table V-5). The introduction of the mop resulted in a clean and repeatable blank sample.

A (%) – PO ₄	B (%) - EDTA
100	0
0	100
100	0
100	0
	A (%) – PO ₄ 100 0 100 100

Table V-5: Schedule of eluent flow during SEC-ICPMS analysis.

6.2.1 Mop peak - an indicator for checking contamination

Figure V-13, shows an ICP-MS chromatogram displaying the elution peak of both complexed sample and mop peak. A switch time at 6 min was chosen so that the auxiliary eluent did not interfere with the complexation chemistry of our complexed EDTA/NOM to be analysed which eluted from 6 min. The area of the mop peak served as an effective indicator to verify whether the column was 'clean' to receive the next injected sample. The mop peak was also monitored simultaneously for all studied elements, as a control to check possible contamination that may have occurred during sample preparation. It was observed that, the mop peak of Zn remained constant when different concentrations of Cu and Mn complexes with NOM (SRNOM) were analysed. The peak however increased when NOM-Zn samples were being analysed. This was expected because according to the calibration curve, not all spiked metals complexed. Thus, the mop was effective in complexing and consequently ridding the column of free Zn ions that were either uncomplexed or decomplexed in the column. A similar trend was observed with the mop peak for Mn which only changed when Mn complexed were being analysed. However, the mop peak for Cu remained relatively constant even when NOM-Cu samples were analysed. Compared to Zn and Mn where the average increase in mop peak intensity was 45 and 68% respectively when their respective complexes were analysed, that of Cu increased only about 6%. It presumes that there were no significant free Cu ions left in the column. Thus, either all Cu were complexed or the uncomplexed ions eluted with the mobile phase. The latter explanation may be more viable since there was not 100% complexation for Cu added to NOM, neither was there a peak observed when EDTA was injected as a sample after the SRNOM-Cu analysis.

For NOM with unknown ligands and metal affinity, the distribution of metals at various fractions due to dissociation and re-association could be a concern. However, kinetic studies focused on the competition between strong ligands and metals have shown that the dissociation rate constants of NOM-metal complexes are within the range of 10^{-2} to 10^{-5} s⁻¹ while the equilibrium process took hours (Sekaly et al., 1998; Schmitt et al., 2003). This has made previous studies to conclude that, for a column designed to limit hydrophobic/hydrophilic interactions, the residence time of NOM-metal complex in a column is too short for any significant competition between column material and NOM for metal (Sadi et al., 2002; Wu et al., 2004). Furthermore, the neutral pH of the mobile phase and the matrix ensures that there is minimum disturbance of the complex formed (Laborda et al., 2009). It can therefore be concluded that the free ions that were mopped were already uncomplexed at the point of sample injection, but since they are not always flushed out by the eluent, they needed to the mopped from the column.

It must equally be noted that the concentration of metals mobbed was not proportional to the concentration of uncomplexed ions. The mob peak after NOM-metal analysis was subtracted from the blank (NOM only) to obtain a signal intensity, which was due to free metals that stayed in the column. The signal intensity was then converted to concentration with the help of the calibration curve. Unlike Zn and Mn, there was no evidence of mopping of Cu ions by the auxiliary eluent. Upon addition of 10 μ g/L of Zn and Mn to 5 mgC/L SRNOM, only about 2.7 μ g/L and 1.22 μ g/L were mopped respectively. This was not due to the saturation of the EDTA sites as the concentration of ions mopped increased to 9.1 μ g/L and 10.7 μ g/L when 50 μ g/L of Zn and Mn were subsequently added respectively. Thus, it is highly possible that some of the free ions did not even enter the column to be mobbed but were adsorbed onto the walls of the glass vials or were gently eluted from the column contributing to the background signal.



Figure V-13: Chromatogram showing NOM-metal peak (right) and mop peak (left).

6.3 Method validation using IHSS standard extracts

The quantitative method was validated using IHSS standards (SRHA and SRNOM) with mixed ligand strengths as found in natural water samples. Standard NOM extracts of 5 mgC/L (SRHA and SRNOM) were spiked with metals (Cu, Mn and Zn) of known concentrations. The concentration range of Cu was 1-10 μ g/L, while that of Zn and Mn was 10-50 μ g/L. Results between samples of 2 hrs and 24 hrs reaction times showed that complexation was higher for longer reaction times. For instance, the concentration of Cu complexed to NOM increased by almost 50% after 24 hrs. However, this was probably not just due to the reaction time but desorption of metals from the vials as the concentration of Cu in the blank solution (MQ) also increased in about 76% after 24 hrs. Thus, due to possible desorption of metals from vial into matrix, a compromise was made to prepare the samples overnight (about 12 hrs of reaction time). This reaction time has been proposed in previous studies as enough to have an equilibrium reaction between NOM and metals (Sekaly et al., 1999; Kogut and Voelker, 2001; Yan et al., 2019).

6.4 Percentage distribution among dissolved and adsorbed phase of NOM-metal interaction

The chemical speciation of Cu, Zn and Mn were determined using the Visual MINTEQ 3.1 model. This model equally allowed us to verify if the experimental conditions had some possible effect on the complexation mechanism. Two different models were generated; EDTA complexation and NOM extract-metal complexation (Table V-6). For the NOM extracts modelling, NICA-DONNAN model was selected. Whereas NICA model describes specific

association, Donnan model rather describes non-specific association (Kinniburgh et al., 1996). Since the model does not have an input for NOM, the interaction of NOM-metal was calculated using 50% of active site of both FA and HA, labelled as DOM. The results obtained showed that at the experimental conditions of our study, the phosphate buffer did not have any effect on the metal complex especially for Cu and Zn. Whereas, no Cu²⁺ was observed to be complexed with PO4³⁻, only 1.5% of Zn²⁺ was complexed with the eluent. These results did not change significantly with increasing metal concentration. However, about 19% of the concentration of Mn²⁺ added potentially complexed with the eluent even in the presence of HA. Thus, for Mn complexation, a different eluent may be ideal. Even though about 100% of metals were complexed with EDTA, there was no saturation of sites as about 98% of EDTA were in excess. This was confirmed by the linear correlation obtained in our calibration curve. The percentage of metals that were complexed with both HA and DOM were in the order of Cu > Zn > Mn which is equivalent to the order of their binding strengths. The percentages of DOM that complexed with metals was below 2% indicating that the available active sites were not saturated.

The characteristic of NOM extracts from the Suwannee River has been reported to change depending on the sampling period. For instance, whereas the carboxylic and phenolic content of SRHA I are 9.59 and 4.24 mmol g^{-1} respectively, that of SRHA II are 9.13 and 3.72 mmol g^{-1} respectively. This translates into an aromatic to aliphatic ratio of 1.8 and 1.0 for the older SRHA 1 and SRHA II respectively, while the most recent SRHA III has a value of 1.5. The possible variation in characteristics could be due to a change in the quality of NOM in the river or an improvement in extraction techniques or protocols. Since both functional groups are very essential in complexation reactions, the three extracts would interact with metals at different degree. Thus, the inputs of extracts used in the models may not precisely represent the scenario observed in this study.

Component	Species name	% of total concentration
	Cu	
	Cu ²⁺	-
	Cu-HA	99.99
	Cu-DOM	99.99
Cu ²⁺	CuHPO4	-
	Cu-EDTA	99.97
EDTA	Cu-EDTA	0.16
DOM	Cu-DOM	0.22
	Zn	
	Zn ²⁺	7.91
	Zn-HA	97.31
	Zn-DOM	62.93
Zn ²⁺	ZnHPO4	1.56
	Zn-EDTA	99.98
EDTA	Zn-EDTA	1.53
DOM	Zn-DOM	1.24
	Mn	
	Mn ²⁺	9.89
	Mn-HA	73.68
	Mn-DOM	46.66
Mn ²⁺	MnHPO4	18.66
	Mn-EDTA	99.98
EDTA	Mn-EDTA	1.82
DOM	Mn-DOM	0.85

 Table V-6: The percentage distribution among dissolved and adsorbed species of Cu, Zn and Mn in HA, DOM (FA + HA), and

 EDTA calculated using Visual MINTEQ 3.1 model.

6.4.1 UV chromatogram of complexed extracts

UVA₂₅₄ chromatograms generally showed a decrease in absorbance with increase in metal concentration though this decrease was not very significant Figure V-13. A maximum of 3% decrease in absorbance was observed when metals were added to SRHA or SRNOM. The insignificant modification in spectral properties is not surprising considering the very low concentrations of metals added compared to the complexometric titration with the raw water. The low concentrations were however necessary to approach real natural water condition. There was also no change in MW observed upon the addition of metals. Even at the same carbon concentration, the signal response for SRHA was about twice as that of SRNOM, demonstrating the very hydrophobic nature of SRHA and the possible higher complexing capacity of this extract compared to SRNOM. The UV chromatogram of the extracts exhibited fairly broad

peaks and widths of around 2 mins which corresponds to MW of 400 Da to over 20 KDa. The building blocks fraction is thus incorporated under the chromatogram.



Figure V-14: UV chromatograms of Cu and Zn complexes with 5 mgC/L of SHRA and SRNOM measured with HPSEC-UVA254.

6.4.2 SEC-ICPMS chromatogram of complexed extracts

The shape of the chromatograms observed in SEC-ICP-MS for Cu complexation were very different from that of SEC-UV although the retention times were unchanged. Whereas a single peak was observed in SEC-UV, two distinct peaks were observed in SEC-ICP-MS indicating two possible complexing sites for Cu (Figure V- 15). The two complexing sites were made up of similar chromophores since they were not differentiated in SEC-UV measurement. Since this happened only with Cu and not with Zn, it is fair to conclude that, the binding properties of Cu may have influenced its binding to different sites available. Zn however complexed little with the highest molecular weight fraction of both SRHA and SRNOM but essentially with the second eluting fraction that has been highlighted in SRHA-Cu and SRNOM-Cu chromatograms, at retention time of around 7-8 min. Thus Zn, whether or not in the presence

of other elements, prefers to be bonded to the semi-HMW fraction or the building blocks which is in accordance with our previous results, showing that in natural waters, Zn is complexed to the building block fraction (Figure V-7). Mn behaved like Zn and was observed to have complexed only with the second peak.



Figure V- 15: SEC-ICP-MS chromatograms of Cu and Zn complexes with 5 mgC/L of SHRA and SRNOM. Numbers in legend represent concentrations of metals added (μg/L).

6.5 Metal complexed after SEC separation

The area under the chromatograms after peak integration were compared to the calibration curve developed using EDTA-metal complex to estimate the concentration of metals complexed. Although the actual concentrations of metals complexed to extracts increased, its percentage appeared to decrease with increasing metal concentration. While about 98% of Cu was complexed with SRNOM at 1 μ g/L, only about 42% of the 10 μ g/L Cu added was complexed (Figure V- 16a). Similar trends were observed for Cu and Zn complexed with SRHA although the decrease in complexation was minimal. It has been reported in previous studies that the recovery of metals after column separation were observed to be generally higher at pH

7 than at pH 5 which was attributed to the ability of metals (Cu and Zn) to form more stable dissolved complexes with NOM at high pH Values (Robertson and Leckie, 1999; D. Schmitt et al., 2003; Metreveli et al., 2010).

SRHA was generally better at complexation with all metals than SRNOM except for Cu at low concentrations. This was equally observed in the model where even though there was higher complexation of Zn (97%) and Mn (73%) with HA, their percentages complexed decreased with NOM to 63% and 47% respectively. This high complexing efficiency could be due to the very hydrophobic nature of its composition. Erhayem and Sohn, (2014), observed that at all pHs (4.8 and 7.8) and ionic strengths (10, 50, 100 mmol/L) tested, the adsorption constants of extracts followed the order of SRHA > SRNOM > SRFA. Since the aromatic/aliphatic carbon ratios of SRHA, SRNOM and SRFA are about 1.48, 0.85 and 0.63 respectively (Table V-3), it can be deduced that, the HMW and greater hydrophobicity of SRHA made it to have a higher affinity for metals compared to the other extracts (Yang et al., 2009; Erhayem and Sohn, 2014). Furthermore, the phenolic content of SRHA, SRNOM and SRFA are 3.72, 2.47 and 2.84 mmol/g respectively. Considering the working pH, phenolic groups were the dominant functional groups in complexation, giving another possible reason to why SRHA complexed better than SRNOM. Since aromaticity and functional group content have been linked to adsorption constants, care must be taken when using HA and FA extracts to represent NOM in a natural environment as their interchangeable use might lead to erroneous results. However, since NOM extracts contain a mixture of humic acid, fulvic acid and low molecular weight organic components, it may be the most appropriate extract to mimic the environment (Erhayem and Sohn, 2014). The percentage of metal recovered in this study was comparable to what has been found in literature. At pH 7, the distribution of Cu in humic acids was 29% while about 73% of Zn was complexed with Humic acid (Laborda et al., 2008), while at the same pH, 49 and 2.2% of Cu and Zn were recovered in NOM after SEC separation (Metreveli et al., 2010). One must be careful however when interpreting these results as the NOM source and nature were very different from ours. The concentration of Zn and Mn complexed with SRNOM were very steady indicating that there was likely equilibrium between the dissolved and complexed form of the metals.

Although 100% of recovery was not obtained, this was unlikely due to saturation or absence of complex sites on NOM as have been demonstrated in the model. Moreover, the concentration of metals complexed increased at high metal concentrations. At a given concentration, the amount of Mn complexed was higher than Cu and Zn. For instance, at 10 μ g/L, the molar

concentration (mmol/L) of Mn complexed to SRHA and SRNOM was the highest (Figure V-16b). The order of complexation was Mn > Zn \geq Cu for SRHA and Mn > Cu > Zn for SRNOM. For SRHA, the difference in metals complexed between Cu and Zn was only 2% while that between Mn and Zn for SRNOM was 5%. The stronger affinity of Mn for NOM compared to Zn was evident at both 20 µg/L and 50 µg/L. This order contrasts with what has been reported in literature for the affinity of the metals. The apparent high complexing capacity of Mn observed in this study could be due to interference from the eluent. According to the Visual MINTEQ model, about 19% on Mn²⁺ are expected to be complexed with the phosphate buffer. The complexation properties of Mn need to be further examined especially when no complexed was observed in the natural water samples even though Mn was present (23.6 µg/L).



Figure V- 16: (a) Percentage of metals (Cu, Zn and Mn) complexed after addition of known concentrations of metals to (b) concentration of metal complex of metals complexed (mmol/L) for 5 mgC/L each of SRHA and SRNOM extracts

Additional analyses are still needed to make this method robust. Challenges such as sources of pollution, ageing of the column and its impact on complexation and peak separation need to be assessed. There is also the need to confirm the repeatability of the results and to determine the limits of quantification of this method. The complexation of metals with IHSS extracts under similar conditions could equally be confirmed with electrochemical methods such as voltammetry.

7. Conclusion

It has been demonstrated in our published review paper presented as chapter I, that the interaction between NOM and metals could have an impact on the efficiency of water treatment techniques. Whereas some techniques may positively benefit, others may be impacted negatively. It is thus important to develop robust and sensitive techniques that are capable of characterization NOM-metal interactions at trace level in natural environment. This study has developed a novel quantitative method for the characterization of NOM-metal using HPSEC-ICP-MS. This method made it possible to quantify the concentration of metals bounded to each NOM fractions and to monitor the evolution of metal complex during water treatment processes. Copper for instance has been demonstrated through samples from different sources to be complexed to almost all fractions of NOM while Zn and Pb preferentially complexed with the building block fraction. Toxic metals like As and Cr which complexed more with the LMW fractions should be a matter of concern. This is because the weaker functional groups present in this fraction can easily cause complexed metals to be bioavailable with a little change in environmental conditions. As NOM were removed, the proportion of metals complexed also generally reduced. It has further been established that metals are capable of modifying the spectral properties of NOM especially at higher metal concentrations. The LMW fractions are the most vulnerable because upon metal addition, weakly bounded cations are displaced to be associated with weaker sorption sites that are mostly abundant in the LMW fractions (Wu et al., 2004). The findings in this study are very novel, because existing studies about the interaction of NOM fractions with metals based on their molecular weights are mainly focused on MW > 1.5 KDa. Thus, a significant portion of NOM fractions were not considered in these studies. Since the very HMW fractions are easily eliminated by coagulation processes, there is the need to focus on the characteristics of the other fractions that would be subsequently removed by secondary treatment techniques. There is the need however to apply this quantitative method to different water sources to test its robustness. Once validated, it could be applied to assess directly the effect of NOM-metal complex on treatment techniques, such as quantifying the concentration of metals in a complex that can result in significant aggregation of NOM and consequent clogging of membrane pores. An analytical protocol is also needed in ascertaining if indeed NOM-metal complex was intact during column separation (especially for natural samples with mixed ligands) and the inertness of the column over usage. The issue of vial contamination equally presented a great setback for this method.

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General conclusions and perspectives
The different aspects of this work were all aimed at responding to the primary objective of this study, which was to characterize Natural Organic Matter (NOM) and its complexes in drinking water treatment processes. In order to have an overview of previous studies that have been done on the characterization of NOM, this thesis started out with a comprehensive review to gather information on NOM-cations complexation and its impact on water treatment. This oriented the focus of the study to two main angles:

- To combine the physical and spectroscopic properties of NOM fractions to enhance its characterization and the monitoring of disinfection by product precursors.
- To develop an innovative quantitative and qualitative method for the distribution of trace metals in NOM fractions under natural environmental conditions.

The study was carried out primarily with a steric exclusion chromatography (SEC) coupled with two spectral detectors (UV and Fluorescence) and an elemental detector (ICP-MS). With the help of a calibrated column, NOM was divided into 5 different classes; biopolymers, humic substances, building blocks, low molecular weight acids and neutrals. Thus, once NOM was separated into different fractions based on their molecular sizes, the spectral and complexing properties of the fractions were studied independently. This approach provided supplementary information to what was obtained from classical UV and fluorescence spectrophotometers.

The first aspect of this work focused on natural water samples from both pilot and full-scale treatment plants of DOC2Cs project partners. Since the composition of each fraction was known, the chromatographic signals were used to monitor the effects of treatment techniques on NOM fractions. For instance, it was observed from the chromatograms of samples obtained from the full-scale treatment plant of PWN and DWG that, coagulation step removed almost all biopolymers, followed by the high molecular weight humic substances (38-80% according to UVA₂₅₄) and a very small part of the low molecular weight compounds (neutrals, building blocks and acids). These observations were confirmed using a SEC-OCD. Other processes like ultrafiltration and nitrification did not significantly remove NOM apart from the residual fraction of biopolymers. The biopolymer fraction was observed to be composed of protein-like fluorophores and hence its fluorescence and absorbance of UV light was less likely due to the presence of colloids but rather the presence of tryptophan-like components. A technique to determine the quantum yield of this fraction after chromatographic separation has been proposed which needs to be validated with other techniques in subsequent studies. SEC-UV/FLD have also demonstrated that they can be used as an effective surrogate for OCD

detector, especially for the characterization of humic substances and building blocks. Even though the PARAFAC components did not correlate well with THMFP, the good correlation observed between both HL, UV₂₅₄ signals and THMFP suggests that HL and UV₂₅₄ can be potentially used for the monitoring and prediction of DBPFP. There is already enough evidence in literature on the use of UVA254 as a surrogate to DOC. This study thus proposes the HL signal which also reflects the chemical composition and predicts the reactivity of organic matter as an alternative to UVA₂₅₄. The advantage of the FLD over UVA is its high sensitivity. These spectral detectors also have an economic advantage over OCDs. By comparing two treatment lanes of DWG, it was observed that the raw waters contained a mixture of hydrophobic and hydrophilic components which contributed to their DBPFP at varying extent. The humic substance fraction of NOM, which is known to be more hydrophobic, contributed most to the formation of DBPs, while the LMW and hydrophilic fractions contributed least. It was further observed that, the reduction in THMFP during treatment processes was mainly due to the preferential removal of highly reactive organic THM precursors and the gradual transformation of NOM to less reactive fractions. Regarding the 4 different resins tested in this study, their efficiencies in removing NOM were in the order of IRA410 > PPA860S > TAN-1> IRA958 according to both SEC-UV and SEC-OCD, while their efficiencies in reducing THMFP were in the order of IRA410 > PPA860S \approx TAN1 > IRA 958. The use of resins may however be a challenge for waters with high anionic compounds like sulphates. There is also the need to verify the possible release of organic compounds by IEX resins which serve as a precursor for the formation of N-nitrosodimethylamine (NDMA).

The second aspect of the study characterized both natural water samples and standard extracts. The evolution of the spectral properties of NOM in natural water samples were monitored by doping samples with increasing amount of Aluminium and Zinc (0 to 150 μ M). A decrease in the UVA₂₅₄ signal was observed for most fractions. The decrease was greater for the HMW fractions and more intense with Al than with Zn. The observed decreases were related to a decrease in the concentrations of free ligands in solution following the complexation of organic matter with these metals. The LMW fractions of NOM behaved differently while a shift in the retention time was generally observed, indicating a change in the size of the fraction. When the interaction of the naturally present metals with NOM were analysed after chromatographic separation, it was observed that copper for instance was complexed with all organic matter fractions, unlike zinc and lead which complexed preferentially with the building blocks. The high sensitivity of this method allowed for the speciation of metals complexed to organic matter

at very low concentrations (μ g/L), closer to natural environment conditions. An innovative method has been developed to calibrate the instrument to quantify the concentration of complexed metals. A column washing procedure has also been incorporated into the method to maintain the cleanliness of the column during analysis.

Notwithstanding the success and ingenuity of this novel method, there is the need for more samples from different sources to make the method robust and to determine among other things, if the preference of metals for NOM fractions is dependent on the concentration of the metals and/or the properties of NOM. An investigation needs to be conducted on the causes of elevated background noise of SEC-ICP-MS especially at low DOC concentration. Once the method has been fully developed, it could be used to validate the effect of NOM-metal complex on treatment techniques as have been outlined in chapter I. The initial plan for this phase of the study, which was to validate the effect of NOM-metal interactions (at trace metal concentrations) on membrane filtration could be revisited. Below is the proposed experimental plan for this phase.

Experimental plan

1. Mono elements

		DOC pH		μg/L		mg/L	
		(mg/L)		Al^{3+}	Fe ³⁺	Ca^{2+}	Na^+
NOM extracts				0	-	-	-
		6	7	20	20	50	20
				100	100	100	100
				250	250	250	200
Natural sample	water			250	250	250	200

2. Multi elements

	DOC	pН	$Al^{3+}(\mu g/L)$	$Al^{3+}(\mu g/L)$	$Fe^{3+}(\mu g/L)$	$Fe^{3+}(\mu g/L)$
	(mg/L)		+	+	+	+
			Ca ²⁺ (mMol)	Na ⁺ (mMol)	Ca ²⁺ (mMol)	Na ⁺ (mMol)
NOM extracts	6	7	100 + 5	100 + 5	100 + 5	100 + 5
Natural water			250 + 5	250 + 5	250 + 5	250 + 5
sample						

Experimental Procedures

1. Clean water flux measurement -10 mins

- 2. Sample filtration **30 mins**
- 3. Mechanical cleaning **30 sec**
- 4. Clean water flux measurement Repeat step 2 & 3 for 4 times 120 mins
- 5. Chemical cleaning 10 mins
- 6. Clean water flux measurement Repeat step 1 10 mins

Total experiment duration/ analysis - 180 mins

Backwash experiments

- 1. Backwash with MQ
- 2. Backwash with filtrate
- 3. Backwash with $MQ + Fe^{3+}$

There may equally be the need to compare this method with other existing techniques such as voltammetry to verify if NOM-metal complex is intact during column separation.

The contamination of vials has also observed highlighted. The new glass vials were contaminated with metals especially with Zn (> 177 μ g/L), Mn, and Ni. Washing them with either MQ or acid before was not effective, thus there is the need to develop a more inert vial.

Supplementary data



SI-1: Extraction procedure for solid-phase source extracts



S III-1: Contribution of NOM fractions to the (a-b) HL fluorescence signals for both DWG and SWW drinking water samples. C 1, C2 and C3 represent campaigns 1, 2 and 3 respectively.



SIII-2: Correlation between (a-b) HL fluorescence signal and (c-d) UVA₂₅₄ with DOC for samples from SWW and DWG during three campaigns each, (e-f) Correlation between UVA₂₅₄ and HL signals of the three campaigns of DWG and SWW



SIII-3: humic-like (HL) signal removal efficiency at each fraction. (1-biopolymers, 2-humic substance, 3-building blocks, 4-LMW acids, 5- LMW neutrals)



S III-418: (a) Concentration of PL fluorophores derived from the calibration curve and represented as TU and (b) Change in TU concentration during treatment processes. Nitri= Nitrification, Coag= Coagulation, Chlori & SF= chlorination and sand filtration, GAC=Granular activated carbon, Clean=final chlorination. C 1, C 2 and C 3 represent campaigns 1, 2 and 3 respectively.



SIII-5: Correlation between the organic carbon and organic nitrogen content of the biopolymer fraction. Dataset represents 28 water samples from project partners (DWG, PWN and SWW)



SIV-1: Correlation between THMFP and PL signal for lane B.



SV-12: 3D EEM contour view of natural organic matter in raw water sample

Résumé

Les matières organiques naturelles (MON) présentes dans les ressources en eau est une problématique majeure dans les filières de production d'eau potable. Cette problématique liée à la présence et à l'augmentation continue des teneurs en carbone organique, en particulier dans les eaux de surface, et son impact sur les filières de potabilisation, a donné naissance au projet DOC2C's dans le but d'étudier et de proposer des moyens durables pour réduire la MON. L'objectif de cette étude était de fournir une technique innovante pour la caractérisation de la MON et de ses complexes avec les métaux traces. Cette étude a été réalisée en utilisant plusieurs techniques comme la chromatographie d'exclusion stérique (HPSEC) couplée à des détecteurs spectraux (UV et Fluorescence) et à un détecteur élémentaire (ICP-MS). Les méthodes développées ont été appliquée sur des pilotes de traitement et à grande échelle, dans les usines de potabilisation, pour suivre l'évolution de la MON pendant le traitement et la formation potentielle de sous-produit de désinfection (SPD). La substance humique de la MON, dont on connait hydrophobicité, contribué plus à la formation de SPD, tandis que les fractions légères et les plus hydrophiles y contribuent moins. Nous avons montré l'efficacité d'élimination de la MON sur des filières de traitement à l'échelle pilote, ainsi le processus de coagulation permet d'éliminer presque tous les biopolymères, 38 % des substances humiques et 17 % des substances à faible poids moléculaire. Les résines échangeuses d'ions en suspension a éliminent 75 % des substances humiques, 70 % des building blocks, 78 -81 % des faible poids moléculaire, tandis que l'ultrafiltration sur membranes céramiques semble n'avoir eu qu'un faible impact sur les biopolymères. Les résultats obtenus à l'aide des détecteurs spectraux ont été comparés à ceux d'un détecteur de carbone (SEC-OCD). Il a été démontré que la composition de la fraction de biopolymère ressemblait à des protéines, responsables de sa fluorescence semblable à celle du tryptophane. Les propriétés de complexation des différentes fractions de la MON ont également été étudiées après séparation par taille. Le détecteur élémentaire (ICP-MS) a permis de mettre en évidence les fractions préférentielles pour les métaux. Par exemple, alors que le Cu se complexe avec toutes les fractions de la MON, le Zn et le Pb préféraient se complexer avec le building blocks. La compétition entre des métaux traces et différentes fractions de la MON a également été observées. Une méthode quantitative innovante a également été mise au point avec un étalonnant la SEC-ICP-MS en utilisant l'EDTA comme ligand et validée à l'aide des étalons IHSS de matière organique (SRHA, SRFA) pour les ligands et les solutions étalons de Cu, Mn et Zn.

Mots-clés : Matières organiques naturelles, Complexation, UV absorbance, Fluorescence, métaux traces

Abstract

Natural organic matter (NOM), present in waters poses severe challenges to all stages of drinking water treatment. A disturbing phenomenon is the rapid changes in the quantity and quality of NOM observed recently, which necessitates the adaptation of drinking water treatment processes. This worrying trend gave birth to the Interreg DOC2C's Project which sought to investigate the possibility of improved NOM removal from surface waters through innovative, knowledge and research-based methods. The goal of this study was to provide an innovative technique for the characterization of NOM and its complexes with trace metals. This study was performed with the help of a size exclusion chromatography (HPSEC) coupled with spectral detectors (UV and Fluorescence) and an elementary detector (ICP-MS). The developed methodology was applied to both pilot and full-scale drinking water treatment plants to monitor the evolution of NOM during treatment and the potential formation of disinfection by products (DBPs). The humic substance fraction of NOM which is known to be more hydrophobic contributed most to DBP formation while the LMW and hydrophilic fractions contributed least. After humic substance removal, the decrease in DBP formation was related to the removal of building blocks. In quantifying NOM removal efficiency of the pilot water treatment plant, coagulation process removes almost all biopolymers, 38% of humic substances and 17% of low molecular weight (LMW) substances, with little impact on building blocks. Suspended ion exchange resin eliminates 75% of humic substances, 70% of building blocks, 78% of LMW acids and 81% of LMW neutrals, while ultrafiltration on ceramic membranes seems to have had only a small impact on biopolymers. The results obtained from the spectral detectors were compared with that of a carbon detector (SEC-OCD). The composition of the biopolymer fraction was shown to be made up for protein-like components responsible for its tryptophanlike fluorescence. The complexing properties of the different fractions of NOM was also studied after separation by size. The elemental detector helped in demonstrating the preferred fraction(s) for metal complexation. For instance, whereas Cu complexed with all NOM fractions, Zn and Pb preferred to be complexed with the building blocks. Complexation and competition of the trace metals at different NOM fractions were also observed. An innovative quantitative method was also developed by calibrating the SEC-ICP-MS with EDTA and the method confirmed using organic matter standards (SRHA, SRFA) for the ligands and Cu, Mn and Zn standard solutions.

Keywords: Natural organic matter, Complexation, UV absorbance, Fluorescence, trace metals