AN INVESTIGATION INTO THE BIODEGRADABILITY OF NATURAL ORGANIC MATTER (NOM) AND THE RESULTING POTENTIAL OF BIODEGRADABLE NOM FRACTIONS TO FORM DISINFECTION BY-PRODUCTS

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by

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I hereby declare that this work has never been previously submitted to this University or in any other University for the award of a **Magister Technologiae degrees: Chemical Engineering**. I declare that this is my work. Quotations in this dissertation from other peoples' work have been correctly cited and referenced.

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DEDICATION

I would like to dedicate this dissertation to my parents

My mother, Ms Treasure Nomcebo Nkosi-Dlamini Sambo and My late father, Mr Antony Maculane, Sambo-May your precious soul, rest in Peace. Thank you for your love and support throughout my life. Thank you both for believing in me, for giving me the strength to chase my dreams. Thank you for always sharing your wisdom, that has shaped me into the person I have become. To the whole Sambo family- you all deserve a thank you. I wholeheartedly appreciate every single one of you- There is no doubt in my mind that without your support and guidance, I could not have completed this research study. This is for you, thank you!!!!

"Until I reach the pinnacles I plan to reach, I am not throwing away my pre-school Speech"- **Sifiso Sambo**

"Uzoy'thola kanjan uhle' ekhoneni"- **Inkalakatha Mandoza**

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Seminar Presentations

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ABSTRACT

Not only does the presence of excessive levels of natural organic matter (NOM) in surface waters affect the raw water quality, but it also impacts the water treatment and supply processes. Other notable challenges caused by NOM is its contribution to bacterial regrowth and the formation of ‘toxic’ disinfection by-products (DBPs). DBPs are nuisance chemicals in water systems as they lead to the production of inferior water quality which may affect human health, the eruption of toxins and various disease-causing microorganisms. Most conventional water treatment plants (WTPs) insufficiently remove NOM, primarily the biodegradable dissolved organic carbon (BDOC) fraction. In the presence of bio-available fractions of NOM, conditions are created for opportunistic pathogens to regrow. While chlorination is crucial for the control of microbial contaminants, the co-existence and interaction of residual chlorine and residual NOM in the WTP lead to the introduction of DBPs such as trihalomethanes (THMs). To maintain the quality of potable water during conveyance, the system must be optimized with adequate control and monitoring, particularly of disinfection and microbial control. A better understanding of the biodegradability of NOM fractions and their potential to form DBPs due to interactions with chlorine residues is required.

This study investigated the character of NOM and its fractions in water treatment plants as well as their biodegradability and influence of these fractions on the THM formation potential (THMFP). The aim was achieved through a combination of conventional and advanced NOM characterization techniques. Raw and treated water from a conventional WTP was characterized through specific ultraviolet absorbance (SUVA) (L/mg.m) to define the NOM composition in terms of aromaticity. The water was further isolated into 3 NOM fractions (i.e. Hydrophilic [Hpi], transphilic [Tpi] and Hydrophobic [Hpo]) through the application of the modified polarity rapid assessment method (m-PRAM). Then, the biodegradability was assessed through the BDOC method, which measures the change in DOC of a NOM sample attached to biologically activate sand over a given period. The THMFP assessment was also conducted on each NOM fraction. Lastly, due to the significant correlation between BDOC and biomass production, the impact of the biodegradability of each fraction on bacterial regrowth potential (BRP) was investigated. This was concurrently together with the BDOC studies by monitoring the concentrations of heterotrophic plate counts (HPC) and Total coliforms (TC) on the first and last day of the experiment. The BRP of each fraction was calculated as the difference between the initial and the final concentration of HPC or TC, and only a $\geq 1x10^3$ increase in the bacterial counts was considered positive for the BRP.

The raw water SUVA ranged between 3.88 L/mg.m and 4.11 L/mg.m, with an even distribution of the Hpi and Hpo NOM obtained through the m-PRAM fractionation. In terms of biodegradability, the Hpi and Tpi fractions were the most biodegradable fractions, with BDOC values of >32% and >29%, respectively. The relatively high BDOC on the Hpi and Tpi fractions substantially contributed to BRP, thereby increasing the HPC to ranges between 121.4 x10^3 cfu/mL to 197.4 x10^3 cfu/mL, respectively, while their impact was less significant to THMFP. The Hpi fraction can be confirmed as the primary cause of bacterial regrowth. The strong correlation (i.e. R^2= >0.9) between BDOC and BRP allows for the prediction of the BRP in a water sample using the BDOC of each of the NOM fractions.

In terms of THMFP, chloroform ($CHCl_3$) was the most abundant, increasing up to 708 µg/L and 611 µg/L for the raw water and treated water, respectively, while bromodichloroform ($CHBrCl_2$) were detected in very low concentrations (<21µg/L) both in raw and treated water. The formation of $CHBrCl_2$ and $CHCl_3$ was mainly ascribed to the Hpo fraction. The high proportion of the NBDOC to the BDOC observed on the HWM Hpo fraction can be attributed to the higher potential of the Hpo fraction to form TTHMs. Significant correlations (R^2) ranging from 0.83 to 0.91 were observed between SUVA and TTHM, confirming that SUVA alone can be successfully used to predict TTHM formation. A relationship between the biodegradability of NOM and DBPFP exists, the less biodegradable the NOM fraction, the more influence they have on the formation potential of DBPs.

The enhanced BDOC method has been successfully optimized for NOM biodegradation studies. The various ways in which systems can be retrofitted to effectively deal with biodegradable NOM can be accomplished through this method. The BDOC is an excellent tool for BOM quantification and is thus crucial in the development of an effective NOM removal strategy. Now that the link between BDOC and TTHM formation has been established, there is a need to conduct an assessment for N-nitrosodimethylamine formation potential (NDMAFP), particularly in the chloraminated distribution network where NDMA is more likely to occur. The study also recommends an investigation into the other NOM fractions such as Hpi-Acids, Hpi-Neutral, Hpo-Base etc., with respect to biodegradability and how they can impact the mechanisms for bacterial regrowth and DBPFP in distribution systems.

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LIST OF ABBREVIATIONS

AC	Activated carbon
AOC	Assimilable organic carbon
AOP	Advanced oxidation processes
BAS	Biological active sand
BDOC	Biodegradable dissolved organic carbon
BOM	Biodegradable organic matter
BRP	Bacterial regrowth potential
DBPFP	Disinfection by-product formation potential
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
ELSD	Evaporative light scattering detector
EPA	Environmental Protection Agency
FEEM	Fluorescence excitation-emission matrices
FTICR-MS	Fourier transform ion cyclotron resonance mass spectrometry
FTIR	Fourier transform infrared
GAC	Granular activated carbon
GC-ECD	Gas chromatography-electron capture detection
HAA	Haloacetic acids
HMW	High molecular weight
HPC	Heterotrophic plate count
Hpi	Hydrophilic
HpiA	Hydrophilic acid
HpiB	Hydrophilic base
Hpo	Hydrophobic
HpoA	Hydrophobic acid
HpoN	Hydrophobic neutral
HPSEC	High-pressure size exclusion chromatography

IARC	International Agency for Research on Cancer
LC	Liquid chromatography
LC-OCD	Liquid chromatography-organic carbon detection
LHWP	Lesotho Highlands Water Project
LMW	Low molecular weight
MALS	Multi-angle light scattering detector
MF	Membrane filter
MIEX	Magnetic ion-exchange
m-PRAM	Modified polarity rapid assessment method
MSD	Molecular size distribution
MWD	Molecular weight distribution
NBDOC	Non-biodegradable organic carbon
NDMA	N-nitrosodimethylamine
NF	Nanofiltration
NMR	Nuclear magnetic resonance
NOM	Natural organic matter
OCD	Organic carbon detector
PAC	Powder activated carbon
Py-GC–MS	Pyrolysis gas chromatography-mass spectrometry
RAC	Resin adsorption chromatography
RI	Refractive index
RO	Reverse osmosis
SA	South Africa
SABS	South African Bureau of Standards
SANS	South African National Standard
SEC	Size exclusion chromatography
SPE	Solid-phase extraction
SUVA	Specific ultraviolet absorbance
THM	Trihalomethane

THMs	Trihalomethanes
TOC	Total organic carbon
TOX	Total organic halides
Tpi	Transphilic
TTHM(s)	Total Trihalomethane(s)
UF	Ultrafiltration
USEPA	The United States Environmental Protection Agency
UV	Ultraviolet light
UV-Vis	Ultraviolet-visible
WDS	Water distribution systems
WHO	World Health Organization
WTP	Water treatment plant

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CHAPTER 1: INTRODUCTION

1.1 Background

Potable water of inferior quality may result in an array of diseases, illnesses, infections and may even cause fatalities. For a life-long safe consumption, the set water quality standards must strictly be adhered to. It is therefore vital that drinking water is of acceptable quality; free of disease-causing micro-organisms and toxins (World Health Organisation [WHO], 2017). Safe drinking water is of utmost concern, particularly in developing and water-stressed countries such as South Africa (SA) (Tshindane *et al*., 2019). Sources of pollution contribute to an array of organic and inorganic pollutants transported to surface water sources (Gwenzi *et al*., 2017). On the other hand, the use of alternative water sources to augment potable water supply has major limitations (e.g. costly) (Marais *et al.*, 2018). Therefore, the reliance on surface water sources is inevitable, and their protection remains a priority for Water Service Providers.

The manifestation of water-related diseases has been linked to the occurrence of various contaminants such as pathogens and natural organic matter (NOM) in drinking water (D'Ugo *et al*., 2016). Even with its considerable persistence in water distribution systems (WDS), NOM is still perceived as an emerging concern in the water industry (Swartz *et al.*, 2018). Its tenacious nature (i.e. stability) not only relies on the natural atomic configuration but also the physicochemical and biological influences drawn from the nearby geographical surroundings (Kallenbach *et al*., 2016). Not only does NOM alter raw water quality, but it also affects the fundamentals of drinking water treatment as it inhibits precipitation precursors, gives rise to colour, regrettable taste and odour within distribution, as well as the introduction of disinfection by-products (DBPs) (Papageorgiou *et al*., 2016). NOM also acts as a fountain of carbon and nutrients for heterotrophic bacteria, thereby promoting bacterial regrowth in distribution systems and ultimately compromising water quality (Arrieta *et al*., 2015; Chowdhury, 2013).

The widespread presence of NOM in water remains a major obstacle in the water treatment and supply industry, which primarily lies within South African municipalities. It is important that the requirements by the South African National Standard (SANS:241 2015) that "potable water coming into the distribution system ought to be microbiologically protected and free of toxins" is met by the Water Service Providers (South African Bureau of Standards [SABS], 2015). Further investigation of NOM is important since the formation of DBPs in natural waters is also well-rooted in its presence. There is a need to further study local water sources with a view to establishing the presence of NOM, its composition, treatability and formation of DBPs thus ensuring better design, optimization and management of the water supply systems based on the localised raw water quality.

1.2 Problem Statement

NOM forms a large part of contaminants present in surface waters and is a serious threat not only to human health but also to the water treatment and supply industry. When present in raw waters, NOM can result in multiple effects in drinking water. These effects can especially arise if the NOM is not sufficiently reduced during the treatment, particularly before the application of chlorine-based disinfectants (Fakour and Lo, 2018). The quality of water is controlled by various physicochemical, hydraulic, and operational parameters (EPA, 2002). The use of disinfectants during treatment is necessary to control microbial contaminants and to reduce bacterial regrowth. However, the simultaneous presence of NOM and residues of disinfectants such as chlorine residues in water systems can induce the formation of unwanted and undesirable DBPs.

Previous studies have reported that bacterial regrowth and loss of disinfectant residuals in contaminated WDS are common challenges due to the presence of residual NOM (Ngwenya *et al.,* 2013). Detectable amounts of bacteria or relatively high bacterial cells in drinking water is not usually an issue, provided they are within the acceptable limits (Hoefel *et al*., 2005; Hammes *et al*., 2008; Vital *et al*., 2012a). However, the excessive growth of bacteria in drinking waters conveyance network can lead to a deterioration in the microbial quality of water and render it unfit for human consumption and industrial usage. As reported by Prest *et al*. (2016), numerous opportunistic pathogens (e.g. *Pseudomonas aeruginosa*) can regrow in WDS even with very low nutrient concentrations available.

The biodegradable fraction of NOM is known as biodegradable dissolved organic carbon (BDOC). Being the labile dissolved organic carbon (DOC) component, BDOC is mineralised by heterotrophic bacteria. Bacterial regrowth within the water supply systems could lead to biofouling, thereby multiplying the counts of coliforms and expedient pathogens such as

Legionella pneumophila, increasing non-compliances in respect to the quality standards and ultimately customer complaints (Hijnen *et al.,* 2017). The reduction of BDOC during water treatment is important as even low levels are enough to support bacterial regrowth in WDS (Prest *et al.*, 2016). WDS that have favourable conditions for bacterial growth, such as the presence of NOM, can result in the loss of disinfectant residuals (Hijnen *et al*., 2017; Prest *et al*., 2016). A loss of disinfectant residual introduces threats to already vulnerable WDS that generally have traces of residual NOM. This is due to the incapability of most WTPs to effectively remove the ever-increasing concentrations of NOM (Krzeminski *et al*., 2019), especially the biodegradable fractions under alkaline conditions (Zanacic *et al*., 2016).

Research has also shown that specific NOM fractions are at the helm for the formation of specific DBPs (Castellon, 2008). Most hydrophobic and acidic fractions give the main reactive precursors for total organic halides (TOX), haloacetic acids (HAAs) and trihalomethanes (THMs) (Croue *et al*., 2000). According to Lu *et al*. (2009), hydrophobic acid (HpoA), hydrophilic acid (HpiA), hydrophilic base (HpiB) and hydrophobic neutral (HpoN) are the main precursors of THMs and HAAs. NOM has been thoroughly researched globally, however, there is currently no modern exploration of the occurrence, distribution and removal strategies of the various NOM fractions in South African source waters (Chaukura *et al*., 2018). As a result, the impacts of NOM in SA are obscured. A typical South African wastewater treatment plant (WTP) relies almost entirely on frequently compromised surface water sources as a result of indirect re-use, and high return flows. Also, taking into account the often-poor capabilities of wastewater treatment plants, and the ever-changing and compromised catchment (Flanders, 2018; Naidoo and Olaniran, 2013), a localised reliable approach for the selection of effective treatment techniques and water quality management must be evaluated for the supply of high-quality water to consumers.

1.3 Justification

The production and proper maintenance of safe water, which is biologically stable in distribution sites is an inherent requirement for water treatment companies, and Rand Water which is the largest bulk water supplier in SA is no exception. The quality of water that is produced at the WTP level (i.e. Rand Water treatment plants as a case study) should be maintained until it reaches consumers. The provision of such quality can be attained by effective control and monitoring of microbial processes during water purification and reticulation. However, to date, Rand Water has not been able to fully identify the NOM fractions responsible for bacterial regrowth or the formation of DBPs in WDS. In light of the observed steady increase in NOM concentrations in many South African raw waters, an informed understanding of its physicochemical and biological impacts is thus strongly sought for. According to the local production standard (i.e. SANS:241 2015), water that is supplied to customers must be fit for purpose and safe for life-long consumption (SABS, 2015). Therefore, it is of vital importance that water service providers meet the local standards for potable water production. However, on-going activities at Rand Water's catchment have the potential to cause an influx of NOM in the Vaal Dam raw water source. This could pose a threat of non-compliance with respect to NOM-related regulatory standards. There has also been an observed proliferation of biofilm formation in Rand Water's distribution network, possibly due to the occurrence of NOM serving as a substrate for microbial growth.

According to WHO (2008), the likely acute health repercussions due to microbial contamination require that its management and protection be given an overriding significance and should never be compromised. Disinfection is, therefore, of irrefutable significance in the provision of guarded drinking water as it is necessary to control microbial contaminants and reduce bacterial regrowth. However, upon exposure to residual disinfectants such as chlorine during the water treatment, the presence of NOM can generate potentially carcinogenic DBPs (Fakour and Lo, 2018). The production of high-quality water relies on highly optimized systems, well equipped to effectively remove contaminants including NOM as well the proper management of distribution systems. In order to be able to select the best treatment technology, optimize water treatment performance, and control biological regrowth, biofilm formation and depositions in the distribution system, it is critical to apprehend the nature and properties of NOM. NOM attributes of having a non-homogenous organic matrix of divergent size, structure and functionality pose difficulties in its characterization, which ultimately impact on its control. To overcome this limitation and successfully remove NOM during water treatment, its composition in the source water must be well understood.

A more acceptable comprehension of the characteristics, properties, as well as the biodegradability potential of NOM and the potential of forming DBPs when it interacts with chlorine residues is required. This approach requires various measurements and characterization techniques for the realization of credible and reliable identification of NOM. The identification of NOM can be achieved through a systematic characterization of the raw water and through the treatment processes that allow the intricate NOM fractions to be singled out for removal and alteration. Furthermore, the characterization of NOM can serve as a fundamental basis for the choice of treatment strategies and monitoring of the performance of process units. This study will endeavour to employ NOM characterization techniques, which can be used to describe NOM characteristics in biodegradation and the formation potential of the associated DBPs (i.e. THMs) in water. Information derived from the dynamics and interactions between NOM and bacterial communities within water treatment processes and distribution systems is crucial not only to delineate the pathways of NOM biodegradation but also to comprehend the evolution and succession of the bacterial community within the water treatment processes. This study is aimed at the quantitative characterization of biodegradable NOM fractions and by extension the non-biodegradable NOM components. Moreover, this study assesses the effect of the biodegradable NOM fractions on microbial regrowth and disinfection by-product formation potential (DBPFP) in a Rand Water WDS, as a case study.

1.4 Aim and objectives of the study

The aim of this study is to investigate the biodegradability of various NOM fractions and their associated trihalomethane formation potentials (THMFPs). In order to address the main aim of the study, the following specific objectives of the study were formulated.

I. To apply comprehensive characterization techniques on raw and filtered water from the Rand Water treatment process. This will be achieved through a combination of conventional bulk (UV, DOC and SUVA) and advanced (the enhanced BDOC method) characterization methods, as well as the modified polarity rapid assessment method (m-PRAM) for the isolation of NOM fractions.

II. To systematically fractionate the NOM on the raw and filtered water samples into hydrophobic (Hpo), transphilic (Tpi) and hydrophilic (Hpi) fractions using the m-PRAM.

III. To assess the biodegradability of the Hpo, Tpi and Hpi NOM fractions by performing the enhanced BDOC method at bench-scale. This will also assist in the assessment of the influence each fraction on the bacterial regrowth in the WDS.

IV. To investigate the NOM precursor (i.e. NOM fraction) responsible for the formation of the individual species of THMs in water. This will assist to establish whether there is a relationship between the biodegradability of NOM and THMFPs.

1.5 Outline of the dissertation

The rest of the 6 (six) chapters discussed in this dissertation are as follows:

Chapter 2: Literature review – Chapter 2 provides a general description of NOM, its impacts on water, treatment processes, characterization and fractionation strategies, biodegradation and its removal from water. The most suitable techniques for quantifying the biodegradable organic matter (BOM), the BDOC technique and application of the m-PRAM for characterization and fractionation, is described in detail.

Chapter 3: Methodology and experimental design - All the experimental methods that were followed to accomplish both the aim and the objectives of this study are described in Chapter 3.

Chapters 4: The characterization of NOM and its removal through the water treatment process – The chapters chronicle the results and discussions on the seasonal characterization of NOM as well as the application of the m-PRAM, and the removal efficiency of the Rand Water conventional water treatment process on each NOM fraction.

Chapters 5: The biodegradability of various NOM fractions and their influence on bacterial regrowth and THMFP- The chapter discusses the findings from the enhanced BDOC technique and the resulting effects of various fractions on the formation potential of THMs and bacterial regrowth.

Chapter 6: Conclusions and recommendations – This dissertation ends by providing a conclusion of this research study. A conclusion that addresses the aims and objectives of the study, lessons learned and ends by providing recommendations for possible future work.
References – All references used in each chapter are listed at the end of that particular chapter.

Appendices – All supplementary information, tables and figures of results are contained in this section.

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CHAPTER 2: LITERATURE REVIEW

2.1 Background

Present in all surface waters, natural organic matter (NOM) which refers to a complex variation of humic and non-humic organic compounds is continually being released by degrading fauna, flora, organic soils and biological activity (Chaukura *et al*., 2018). The chemical composition of NOM varies with source and time, which in turn is attributed to the varying provenances of the precursor material and the degree of transformation at which NOM undergoes (Chaukura *et al*., 2018). NOM attains its overall polarity and reactivity from the integration of other NOM constituents, namely amino acids, oxygen-containing aliphatic and aromatic hydrocarbons, hydroxyl and nitrogen groups etc., (Khare, 2016). The seasonal variations and increasing trends in NOM concentration levels impose threats to the water treatment and supply industry with regards to operation, optimization and effective process management (Baghoth, 2012). NOM is primarily dissolved organic matter (DOM), about 50-75% (Chaukura *et al*., 2018; Garcia, 2011). DOM is the fraction of NOM in the solution that passes through a 0.45 um filter, which only constitutes dissolved organic carbon (DOC). Due to its various weak organic acid functional groups, strong metal complex capacity and lipophilic sorption abilities, DOM plays an important role on surface water chemistry as it affects the chemical and biological processes in the aquatic environments (Artifon *et al*., 2019; Reyes and Crisosto, 2016).

2.1.1 Impacts of NOM during drinking water treatment: Impacts on water quality

Safe drinking water is defined as water with acceptable physical, chemical, and microbiological properties (SANS 241:2015). These properties are mainly dissolved or suspended in the water. Although with no direct health impact, the introduction of taste, odour and colour in water usually indicates the presence of dissolved organic carbon (DOC) (Nkambule, 2012). If DOC is not sufficiently removed through drinking water treatment processes it combines with chlorine residues to form DBPs such as trihalomethanes (THMs which are derivative compounds of methane (CH_4) where one or more of their hydrogen atoms has been replaced by halogen atoms (i.e. Fluorine, chlorine, bromine, or iodine). Examples of THMs chloroform, bromodichloromethane, dibromochloromethane, and bromoform). THMs are implicated as, probable carcinogens, which are detrimental to human life. Epidemiological studies have revealed a link between the ingestion of water with chlorine residues and increased risk to bladder cancer, whereby monitoring was limited to only a few DBPs such as THMs (Li and Mitch, 2018). For THMs and HAAs, the formation of DBPs is not as a result of the respective reaction between methane and chlorine or acetic acid and chlorine, but as a result of complex reactions between NOM, chlorine, and bromine (Br^-) (Castellon, 2008). The generation of DBPs in the course of water treatment can be minimized by limiting the quantity of DBP precursors before disinfection with chlorine (Moncayo-Lasso *et al*., 2008).

When allochthonous DOM is transported from terrestrial to aquatic environments, it impacts the concentrations of macronutrients (i.e. nitrogen and phosphorus) and thereby providing energy and nutrients to microorganisms. Heterotrophic bacteria feed on these nutrients and promote bacterial regrowth in the WDS, thereby altering and compromising the quality of the water (Prest *et al*., 2016). On-going investigations have shown that apart from easily assimilable organic carbon (AOC) in water, there are more complex biodegradable compounds of high importance for the biological stability of the water (Hijnen *et al*., 2014 and Prest *et al*., 2016). Some studies (e.g. Hijnen *et al*., 2017) found no interrelationship between bacterial regrowth and AOC in the drinking water systems where three full-scale plants were assessed. The biodegradability of NOM is habitually evaluated using biodegradable dissolved organic carbon (BDOC) techniques and the potential to increase bacterial regrowth in the distribution system (Kwon *et al.,* 2005). BDOC represents the biodegradable DOC fraction, in both low molecular weight (LMW) and moderately high molecular weight (HMW) organic compounds, which can be assimilated by heterotrophic bacteria. Molecular weight (MW) can be defined as the weight of the molecule to which the ‘average’ atom belongs (Sobantu, 2014).

2.1.2 Impacts on water treatment efficiency, water distribution systems and removal strategies

NOM is typically harmless on their own and in the early stages and its presence of NOM in water was nothing more than an aesthetic challenge due to its contribution to organoleptic parameters. Only in the 1970s did the influence of NOM as a precursor to DBPs became recognized, thus prodding greater interest in its removal (Croft, 2012). Letterman (1999) and Reckhow (2010) have stated that humics serve as precursors of DBPs, whilst specific fractions of NOM act as substrates for biological regrowth in the WDS, especially where oxidation is practised. Other challenges associated with the presence of NOM in wastewater include the increase in coagulant dosages, the transportation of metals and hydrophobic chemicals, corrosion in distribution networks, and the interference in with the adsorption processes during the removal of other contaminants (Croft, 2012). The microbial complexities in a water distribution pipeline relate to how NOM affects pipe material, the hydraulics within a conduit, impact on residual protection, and bacterial regrowth and community dynamics within a pipeline (Prest *et al.*, 2016).

2.2 The characterization of natural organic matter (NOM)

NOM characterization strategies can be loosely categorized as follows: direct activity techniques, fractionation methods and spectrometric (qualitative) techniques (Chaukura *et al.*, 2018). Whereas methods for direct measurement of NOM measure the concentrations of organic matter in the water sample, spectrometric methods quantify the released or the absorbed amount of radiation by chromophores. Fractionation methods, on the other hand, isolate NOM relative to size and polarity. Advanced NOM characterization methods include high-performance size exclusion chromatography (HPSEC), fluorescence spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, PRAM, fluorescence excitation-emission matrices (FEEM), and BDOC (Kwon *et al.*, 2018). Methods that are available for precise determination of the structure of NOM include multidimensional NMR, Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and pyrolysis gas chromatography-mass spectrometry (Py-GC–MS), (Matilainen *et al.*, 2011). Bulk parameters used for the characterization of NOM include UV_{254}, specific ultraviolet absorbance (SUVA) and DOC, and they give details on the aromaticity and amount of humic substances present within the organic matter. UV_{214} detection is a recommended technique for a more detailed analysis of the NOM character with respect to nitrates and nitrites content.

2.3 The fractionation of natural organic matter (NOM)

Often interchangeably used with isolation, the term fractionation refers to physiochemical processes that separate components of the NOM samples, based on physicochemical properties such as acidity, polarity, and molecular size, into more homogenous groupings (Lenheer, 2009). According to Mills and Hunte (1997), owing to the chemical intricacy and diversified nature of NOM, a fractionation helps to minimize the molecular heterogeneity of

NOM and provides more information into its chemical composition. Fractionation isolates the class of composites occurring in the NOM based on physical attributes. Kucukcongar *et al*. (2013) emphasized the importance of also including fractionation when dealing with NOM, as it serves as a useful preparatory method for the examination of the nature of NOM components and their reactions.

2.3.1 High-pressure size exclusion chromatography (HPSEC)

To ascertain the apparent molecular weight (MW) or molar mass (MM) distribution of NOM from various aquatic and terrestrial environments, the SEC/HPSEC technique is usually applied (Świetlik and Sikorska, 2006). The principle of SEC/HPSEC is based on the separation of components due to ionic exclusion and hydrophobic attraction with respect to their size (MW) and shape. The technique requires passing a water sample over a rigid adsorbent substance loaded into a column using a liquid solvent flow, where each analyte in the sample interacts marginally differently with the adsorbent material, thus impeding the flow of the analytes. The elution is dependent on the strength of analyte-adsorbent interaction; if the interaction is weak, the flow of the analyte from the column occurs rapidly and vice-versa (Sobantu, 2014). The method detects MW distribution of small to large fractions of NOM which results from the oxidation process during potable water treatment. NOM studies (Bopape, 2017; Świetlik, *et al*., 2002) have reported HPSEC MW sizes of NOM ranging between 2500 and 300 Da. Other studies have indicated that the MW of NOM can vary from a few hundred to over 100 000 Da; therefore, NOM is highly poly-dispersed in nature (Sobantu, 2014; Debska *et al.*, 2007).

The HPSEC is a quick and reproducible method that separates NOM into six (6) peaks resembling the humic fractions, and the percentages of each, from HMW to LMW fraction (Nissinen *et al*., 2001). The six NOM fractions translate to six peaks, which are generally eluted by HPSEC, with the HMW fraction denoted by Peaks I – II, the LMW fraction denoted by Peaks III-IV, and Peaks V and VI resembling only the LMW fraction. According to Szabó and Tuhkanen (2007), the humic and fulvic compounds that percolate from soils are resembled by the HMW fractions, while the non-humic fraction is represented by the LMW fractions of NOM. The HPSEC can also be used to denote the removal of NOM after each process unit of the water treatment as there will be a visible change in the MW distribution

(Nissinen *et al*., 2001). Also, an understanding of the DBPs and MW distribution of NOM can assist in the optimization of water treatment processes.

Other detectors that can be used together with the HPSEC include the evaporative light scattering detector (ELSD), refractive index (RI), variable wavelength UV-VIS, multi-angle light scattering detector (MALS), and online DOC analysers. The detector is amongst the most commonly used in HPSEC measurements (Sobantu, 2014; Matilainen *et al*., 2011). According to Bopape (2017), the advantage of the HPSEC technique includes the fact that it gives the NOM fractions and the corresponding MW. On the other hand, the disadvantages of HPSEC is that the fractions are grouped into three representative fractions, namely higher, medium and lower molecular weights. In addition, HPSEC cannot be more specific with the detection of specific MW, the statistical averages cannot be calculated and the overall shape of the MW distribution remains unknown (Sobantu, 2014).

2.3.2 Liquid chromatography-organic carbon detection LC-OCD

The liquid chromatography coupled with an organic carbon detector (LC-OCD) is a type of liquid chromatography technique that can quantify both TOC and DOC in water samples at concentrations ranging between 10ppb - 5 ppm. LC-OCD consists of three SEC columns that classify the TOC into fractions of various sizes while taking into account their hydrophobic and ionogenic character (Uyguner and Bekbolet, 2005). It operates by separating the organic mixture by liquid chromatography (LC) followed by their detection with organic carbon detector (OCD), which incorporates acidification and inorganic carbon purging, TOC oxidation to CO_2, and detection by a non-dispersive infra-red detector similar to the traditional TOC analysers, (Humbert *et al.*, 2005).

2.3.3 Resin adsorption chromatography (RAC)- Amberlite XAD 8/4 Resin

One of the most popular techniques for the separation of organic solutes from water is the use of combined XAD 8/4 resins (Thurman, 1985; Leenheer, 1981; Mantoura and Riley, 1975). The resin adsorption chromatography (RAC) technique can be used to isolate and concentrate NOM from water. The procedure (**Figure 2-1**), which uses XAD-type resins, was developed by Leenheer (1981) and is now widely accepted as one of the leading fractionation techniques (Hu *et al*., 2003). In the XAD method, fractionation rests on variations in sorption capacity of dissolved organic carbon (DOC) on the resins under acidic and basic conditions. The three main fractions that can be separated using XAD-8/XAD-4 resins are: (i) the Hpo fraction sorbed and eluted through the XAD-8 resin using 0.1 N sodium hydroxide (NaOH) solution; (ii) the Tpi fraction sorbed and eluted through the XAD-4, also using a 0.1 N NaOH solution; and lastly (iii) the Hpi fraction which adsorbs in neither the XAD-4 or XAD-8 resins (Rho *et al.*, 2019). The procedure requires about 600 mL of samples, which are passed sequentially through the combined resin system starting with the XAD-8 column and followed by the XAD-4 column. At 200 mL intervals, samples of the effluent are taken from the column, and after each run, the Hpo and Tpi fractions of DOM are back-eluted from the column resins using 0.1 M NaOH. To preserve and prevent oxidation of dissolved organic matter (DOM) on the elutes, the samples are immediately neutralized using 0.1 HCL (Malcolm and McCarthy, 1992).

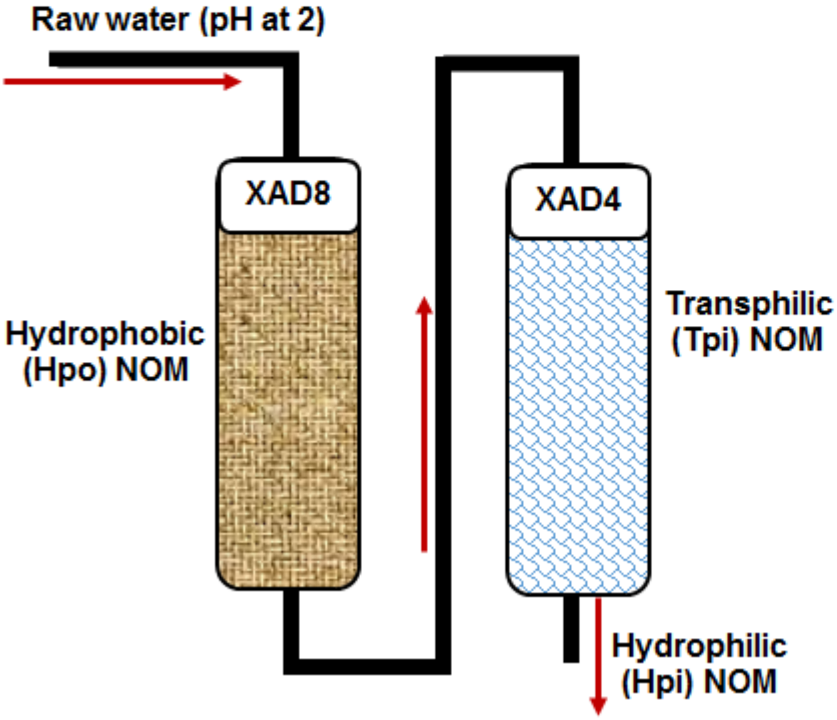

Figure 2-1: Isolation of DOM using XAD 8/4 resins (Rho *et al.*, 2019)

The RAC method is advantageous in that it directly isolate NOM from water, permitting for the processing of large water volumes, and has the potential to separate humic material from inorganic substances, whose reactivity is measured by $SUVA_{254}$ (Kitis *et al.*, 2001). Also, the results obtained are actual DOC measurements of each fraction or as a reciprocal percentage. However, the conventional XAD 8/4 method is extremely time-consuming and labour-intensive when used with general columns, unless coupled to a solid-phase extraction (SPE) technique (Rho *et al.,* 2019).

2.3.4 The modified polarity rapid assessment method (m-PRAM)

The m-PRAM is a derivative of the polarity rapid assessment method) (PRAM) by Rosario-Ortiz *et al*., (2007). The m-PRAM was developed in an attempt to shorten the duration from the original method, yet maintaining the reliability of the information produced in relation to the composition and aromaticity (Nkambule, 2012). NOM was originally categorised into six fractions; however, some studies have successfully classified NOM into three major fractions (i.e. Hpo, Tpi and Hpi fractions) through the use of m-PRAM (Uyak *et al.,* 2014).

The m-PRAM uses three (3) solid-phase extraction (SPE) cartridges (i.e. polar, non-polar and anion-exchange) to map the changes in polarity of NOM in a water sample (**Figure 2-2 and Table 2-1**). The chemical descriptions of these fractions obtained from m-PRAM are summarized as follows, the Hpo fraction is composed of carboxylic acids compounds of 5-9 carbons, 1- and 2-ring aromatic carboxylic acids, aromatic acids, 1 and 2-ring phenols and tannins, proteins with 1- and 1-ring aromatic amines, excluding pyridine and HWM compounds. The intermediate fraction (i.e. the Tpi) is a combination of hydrocarbons and carboxyl compounds, aliphatic amides, alcohols, aldehydes, esters, polysaccharides and ketones with >5 carbons. Lastly, the Hpi fraction comprises of the highly aliphatic acids, also of >5 carbons, hydroxyl acids, sugars, LMW alkyl monocarboxylic acids and dicarboxylic acids, sugars, peptides, proteins etc. The method takes benefit of the various SPE cartridges to isolate the fractions of the NOM present in the water at physical pH and ionic strength, and relative to its polarity and charge. The m-PRAM is a tool which fractionates NOM based on selective adsorption of DOM fractions onto the SPE sorbents.

Table 2-1: m-PRAM SPE cartridges and their respective sorbents (Rosario-Ortiz *et al*. 2007)

Solid-Phase Extraction (SPE) Cartridge	Sorbent type
C18	Hydrophobic (Hpo) compounds
CN	Hydrophilic (Hpi) compounds
NH_2	Weak anion exchange compounds

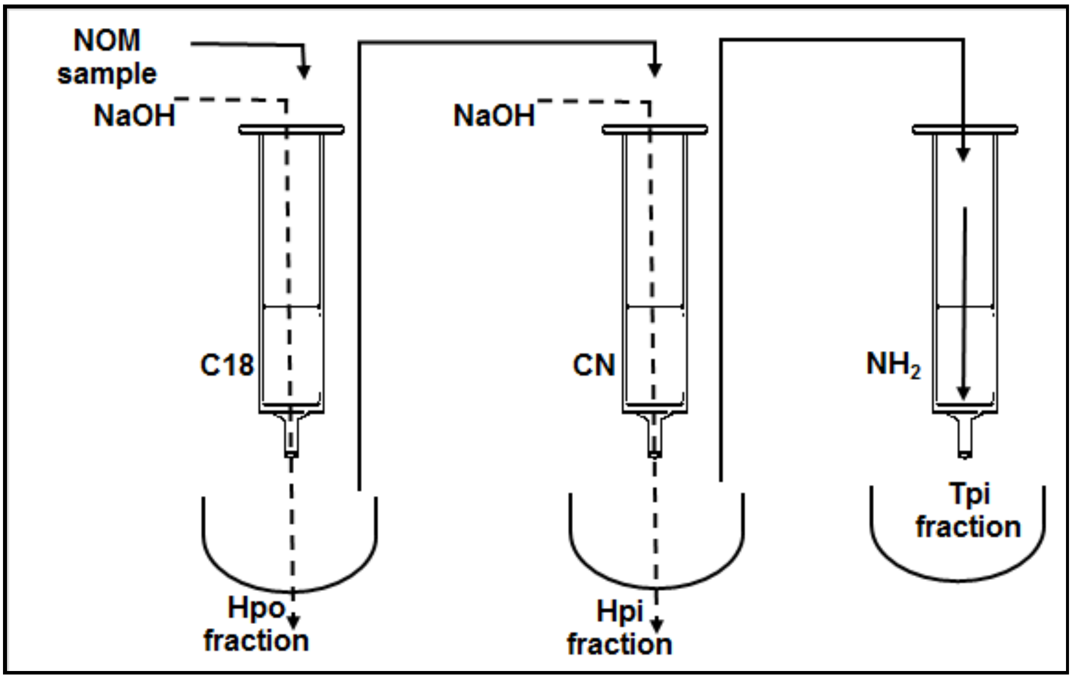

Figure 2-2: The modified polarity assessment method (m-PRAM) setup (Nkambule, 2012)

The m-PRAM has the ability to account for the difference in variation of precursors pre and post the SPE cartridge elution. The parallel and ease of application of the distinctive SPE cartridges permit for simultaneous and multidimensional characterization of the polarity of NOM. Also, the analysis is conducted under atmospheric conditions whereby no pre-treatment is required. This may, in turn, interfere with the chemical properties of the NOM, allowing a precise depiction of its polarity as it exists in the surroundings. The procedure does not require vast amounts of sample and can be carried out regularly thus permitting the evaluation of temporal and spatial changes in the characteristics of NOM to be undertaken. The method also gives reproducible results with variation coefficients of less than 5% (Marais *et al.*, 2018). However, the m-PRAM also has disadvantages, as it only provides information on the polarity of NOM and not on other important aspects of NOM relating to its treatability. In addition, the PRAM does not account for the biodegradable fraction of NOM (i.e. the BDOC) or allow for the collection of sufficient NOM volumes for further structural analysis using other techniques such as NMR and FTIR spectroscopies.

2.4 NOM as a precursor of disinfection by-products (DBPs)

The availability of dissolved organic carbon (DOC) in natural water gives rise to the generation of disinfection by-products (DBPs) (e.g. THMs, NDMA, and HAAs) during the chlorination disinfection step (Awad *et al.*, 2016; Rook, 1977; Reckhow, 2010).

2.4.1 Disinfection practices - chlorination and chloramination

First used as a disinfectant in the late 1800s in the United Kingdom, today chlorine is a globally accepted disinfectant that is used in most parts of the world. It was not until the early 1970s that DBPs were discovered (Richardson *et al.*, 2007). This was after higher levels of chloroform, a THM compound was found in treated potable water than in the source water. To date, at least 600 DBPs have been reported, and the number is still increasing. Although some of the DBPs are regulated, other DBPs are considered as emerging DBPs since they have lower occurrence levels and toxicological effects (Tsitsifli and Kanakoudis, 2018). The production of safe, clean potable water comes with the risk of producing harmful DBPs. The chlorination of residual NOM in water gives rise to a wide range of toxic halogenated organic substances. Carcinogenic nitrosamines (NDMA) are a potential by-product of chloramination, a disinfection process where ammonia is added to chlorinated water to make the residual last longer. While the protection against microbial contamination is of high priority, water distribution systems must also be protected within reasonable measures against the formation of DBPs and other chemical compounds formed unintentionally.

2.4.2 The influence of various NOM fractions on DBP formation and potential

Polarity is one of the most investigated NOM parameters due to its great influence on its reactivity and its ability to influence chemical alterations during treatment processes. In terms of polarity, when using the PRAM technique, NOM can be categorized into three polarity-based groups, namely hydrophobic (polar), transphilic (intermediate) and hydrophilic (non-polar). It is therefore important to look further into each of these fractions, their impacts on DBPs formation as well as their formation potentials. For a very long time, THM formation was equated to the concentration of DOC content and UV absorbance in water (Rook, 1977). This is due to the wide distribution of aromatic humic substances in surface water, which is acknowledged for their strong reactivity with chlorine producing THMs (Dhaouadi *et al.,* 2013).

DOC is regarded as a surrogate measurement for the formation of THM and its presence in potable water may suggest the need for additional water-treatment strategies. Some studies have reported no significant correlation between THMFP and SUVA, and this has proven to be a limitation for the accurate prediction of THMFP in drinking water using UV spectroscopy (Fram *et al.*, 1999). The THMFP has also been found to be not closely related to the aromatic carbon content of DOC isolates or to the partitioning of the DOC by non-ionic macro-porous resins (Fram *et al.,* 1999). This suggests that neither of the measurements is related to the compositional features of the DOC responsible for THM formation (Fram *et al.,* 1999). Awad *et al*. (2016) have reported that the character of DOM (defined by SUVA, MWD and relatively large presence of protein-like or Fulvic-compounds) with DOC and Br concentrations were related to THMFPs of DOM isolates.

Studies such as Awad *et al*. (2016), Chen and Westerhoff (2010) and Kitis *et al*. (2002) have reported that THM formation was more probable to occur from organic Hpo compounds that absorb UV absorbing than from just the overall DOC (i.e. including hydrophilic and non-aromatic compounds). SUVA can be used as an indicator of NOM removal. Owing to the weak regression existing between SUVA and TTHM in the final drinking water, there is a need to incorporate THM formation potential (THMFP) on the individual NOM fractions to determine confidently the likelihood of the specific fractions to form THMs. Other studies have suggested that UV_{254} was a better and reasonable surrogate for THMFP, with a correlation coefficient close to 1 for the raw waters (Golea *et al*., 2017).

Marais *et al*. (2018) observed a strong positive interdependence between the HMW NOM fraction (i.e. the Hydrophobic fraction) and TTHM formation on raw water versus the measured TTHM formation in potable water. This indicates that the aromatic NOM fraction which is abundantly available before treatment was the cause for the formation of THM, especially during summer. The molecular size distribution (MSD) of NOM can also be used to project DBP formation (Matilainen *et al*., 2003; Vuorio *et al*., 1998). A substantial and positive correlation between NOM of larger molecular size was only evident during the summer. This indicates that during summer the formation of TTHM, especially chloroform, was primarily ascribed to HMW NOM. Various studies have shown that the HMW hydrophobic NOM fraction is a precursor of chloroform (Lu *et al*., 2009; Xue *et al*., 2007). However, Dhaouadi *et al*. (2013) reported a higher THMFP due to the presence of transphilic NOM fraction, not the hydrophobic NOM fraction. This is because the transphilic fraction has richer and more active functional groups than the hydrophobic fraction (Dhaouadi *et al*., 2013). This suggests that the removal of hydrophobic and the transphilic compounds during water treatment should be given priority as there is a higher potential for the generation of brominated compounds.

Bromine (Br) has been reported to highly react with hydrophilic compounds as compared to hydrophobic compounds (Hua *et al*., 2015). However, higher concentrations of the bromide ion (Br^-) in the presence of higher UV absorbing and hydrophobic compounds were reported to have minimal influence on the formation of THM (Hua *et al*., 2015). Due to reactions between hypochlorous acid and Hpo NOM fractions, waters without the Br ion mainly form THMs (i.e. CHCl3). On the other hand, waters with the Br ion and the Hpi NOM fractions result to brominated THMs through interactions with hypobromous acid (Awad *et al*., 2016; Chowdhury *et al*., 2009; Liang and Singer 2003). Winter conditions are apparently more favourable towards the formation of THMs from the LMW NOM fraction. It was established that the HMW NOM fraction was the responsible precursor to TTHM and chloroform formation, especially during summer months (Awad *et al*., 2016).

2.4.3 Regulation on DBPs

Categorized as emerging contaminants owing to their perceived effects on human well-being and their frequent formation during WTP, DBPs are regulated differently in different parts of the world. The local regulation standard (SANS 241:2015) requires that the ratio of TTHM should not exceed 1. The THM ratio is calculated from all the THM species as fractions of their acceptable limits (i.e. CHBr2Cl, $CHBr_3$, $CHBrCl_2$ and $CHCl_3$ are ≤100µg/L, ≤100µg/L, ≤60µg/L and ≤300µg/L, respectively) in drinking water. **Table 2-2** illustrates the available normative and rules with respect to control and safeguarding directives of DBPs. The DBPs are categorised according to the International Agency for Research on Cancer (IARC) Health classifications system, where: Group 1 indicates that the agent is a proven carcinogen to humans; Group 2A means the agent is probable carcinogen to humans; whilst Group 2B means the agent is a possible carcinogen; Group 3 means the agent is not classifiable as a carcinogen to humans; and Group 4 indicates that the agent is most likely not carcinogenic (Rosero-Moreano, 2018).

Table 2-2: Rules and regulation to control of DBPs for drinking water (Rosero-Moreano, 2018)

Item	Organization	DBPs	Value mg l^{-1}	IARC health categories*	References
1	World Health Organization (WHO	Chloroform	200	Group 2B	Richardson (2002); WHO, (2017)
		Bromodichloromethane	60	Group 2B	
		Dibromochloromethane	100	Group 3	
		Bromoform	100	Group 3	
		Dichloroacetic acid	50	Group 2B	
		Trichloroacetic acid	100	Group 3	
		Dichloroacetonitrile	90	Group 3	
		Dibromoacetonitrile	100	Group 2B	
		Trichloroacetonitrile	1	Group 3	
2	Environmental Protection Agency (EPA)	Chloroform	30	Group 2B	Richardson (2002)
		TTHMs	80	Group 3	
		Haloacetic acids	60	Group 2B	
3	European Union (EU)	Total trihalomethanes	100	Group 3	Richardson (2002)
4	South Korean Regulation	Total trihalomethanes	100	Group 3	On *et al.* (2017)
		Haloacetic acids	100	Group 2B	
		Dichloroacetonitrile	90	Group 3	
		Dibromoacetonitrile	100	Group 2B	
		Trichloroacetonitrile	4	Group 3	
5	Australian Regulatory Limits	Trihalomethanes	250	Group 3	Alexandrou *et al*. (2017)
6	Colombian Regulation	Trihalomethanes	200	Group 3	Rosero-Moreano *et al*. (2012)

2.5 NOM removal strategies

There are many different water treatment technologies of different potential, which are widely applied for the removal of NOM during the water treatment processes. These technologies include membrane filtration (MF), ion exchange, activated carbon (AC), as well as conventional processes. The effective removal or reduction of NOM is essential in water treatment since there are countless challenges associated with insufficiently removed NOM

(LeChevallier, 2003). These challenges include microbiological regrowth, the formation of DBPs, odour, taste and colour in the final treated water (LeChevallier, 2003). A selected number of these technologies are discussed below.

2.5.1 Activated Carbon (AC)

Activated carbon (AC) is a potent adsorbent used during potable water treatment. AC can be used in a powder or granular form. However, the performance of AC is influenced by the location of the process within the overall treatment train, the pore size distribution and the molecular size distribution of NOM. When used as filter media in the filtration processes, AC if effective in reducing micro-pollutants and organoleptic compounds that cause aesthetic water problems (Hamann *et al.*, 1990). Although granular activated carbon (GAC) is costly when compared with powder activated carbon (PAC), GAC is considered more effective than PAC in the removal of an array of organic compounds (Summers *et al.*, 2011).

2.5.2 Ion Exchange Resins

An anion exchange resin generally comprises of a polymer matrix to which charged functional groups are affixed through molecular or ionic bonding (Dlamini, 2012). The conventional matrix is polyacrylic cross-linkage for structural and configurational solidity. Positively charged ion exchange sites (i.e. ammonium) are attached to the resin matrix. Mobile negatively charged counterions are linked by electrostatic enticement with each positive ion exchange site. The resin exchange capability is determined by the number of fixed charge sites per unit volume or weight of the resin. The strong attraction of NOM for anion resins has been extensively employed as the basis for the reduction of DOC from surface water whereby special highly porous resins which have been magnetized with an iron oxide substance are used. These resins are referred to as magnetic ion exchange resins (MIEX) (Letterman, 1999). The approximate order of selectivity for some common amine-based anion exchangers is: Hydroxyl ≈ Fluoride < Chloride < Bromide < Bicarbonate < Nitrite < Phosphate < Chromate < Sulphate anions. Humic- and Fulvic-acid fractions of NOM are desirable than sulphate ions, but because of their wide range in molecular weights, ranging between 100 Da – 70 000 Da and structures, no precise positioning in their selectivity sequence can be assigned (Boyer and Singer, 2008).

2.5.3 Oxidation processes

In the treatment of potable water, oxidants are generally applied to remove taste, odour and eliminate colour causing compounds from drinking water. According to Zhang *et al*., (2001), oxidants have also proven to enhance the coagulation process, thereby reducing the quantities of coagulants required. The most commonly applied oxidants during potable water treatment are chlorine dioxide, permanganate, chlorine, ozone. Oxidants are often applied as a pre-treatment step, or at the rapid mix basin, however, they can also be applied before filtration after a significant amount of NOM has been removed (Dlamini, 2012). The use of ozonation removes DBP precursors upon subsequent chlorination (Zhang *et al*., 2001). Oxidants are also selective in their effectiveness; for instance, Bose and Reckhow (2007) found that when ozone was applied in raw water, it constantly reacted with the humic fraction of NOM. Other studies have found substantial benefits in combining advanced oxidation processes (AOP) and biological treatment for the removal of DOC (Fahmi and Okada, 2003).

2.5.4 Membrane filtration processes

Over the past decades, membrane filtration strategies have increasingly been employed for potable water treatment. Due to their high permeate flux, membranes have a significant role to play in the removal or reduction of NOM in source water (Zhang *et al*., 2012; Zhang *et al*., 2011). The water treatment industry also uses membrane processes to remove larger residual organic matter that remains after coagulation and in particular dissolved NOM fractions. These membranes are pressure-driven and they include ultrafiltration (UF), reverse osmosis (RO), microfiltration and nanofiltration (NF). Various membranes have varying NOM and particle removal efficiencies (**Table 2-3**). However, membrane fouling remains a major obstacle to maintain efficient operation for extended periods. The hydrophobic fractions of NOM and the high molecular weight compounds cause fouling of the membranes and a decline of flux, which poses major problems to the membrane techniques (Matilainen *et al*., 2006). MF is normally used at advanced treatment steps, in combination with other techniques such as anion exchange resins to prevent the adverse effects associated with the problematic NOM fractions (Kim and Demsey, 2010).

Table 2-3: The various membranes, their respective aperture size and applications (Nakashima *et al.*, 2000)

Membrane #	Type of membrane	Pressure (bar)	Aperture size	Pollutants retained
1	**MF**	0.1–3	0.1–5 µm	Suspended particles including bacteria and dust
2	**UF**	2-10	20–0.1 µm	Various macromolecules including silica, viruses etc.
3	**NF**	5-30	> 1 nm	Salts, synthetic dyes, small solutes (synthetic sugars) etc.
4	**RO**	10-100	0.1–1 nm	Salts

2.5.5 Conventional water treatment processes

Though conventional water treatment processes were not originally intended to remove NOM, the process is capable of reducing NOM concentrations in water. Conventional water treatment processes consist of coagulation, flocculation, sedimentation and filtration, reduces NOM. According to Ødegaard *et al.* (2010), this separation method is used globally and is the most commonly used method for contaminant (including NOM) removal. Its removal principle is centred around the removal of turbidity as this is one of the major surrogates for NOM. According to Matilainen *et al.* (2003), a conventional water treatment process was found to be more successful than others in the removal of high molecular weight (HMW) aromatic organic matter compared to low molecular weight (LMW) and aromatic NOM. There are three (3) primary mechanisms for the removal of NOM through conventional processes. The mechanisms include the; a) direct precipitation of a Me-NOM solid phase through the complexation of NOM with dissolved metal coagulant species (Al or Fe), b) the adsorption of this complexed material onto precipitated $Me(OH)_3$ solids through the complexation of NOM with dissolved coagulant species and c) direct adsorption of NOM onto the surface of precipitated $Me(OH)_3$ solids.

2.6 Biodegradable NOM and measurement techniques

The regrowth of microbial contaminants in water supply networks is a major concern for water utilities. From a microbial perspective, one of the major aims of a potable drinking WTP is to remove all pathogens, particularly bacteria, protozoa, and viruses, and to minimize the likelihood and potential for heterotrophic bacteria regrowth in the water distribution systems (Nkambule, 2012). The extent of bacterial regrowth relies on the presence of a substrate and the lack of residual protection. To effectively minimize the formation of undesirable DBPs and bacterial regrowth, while simultaneously providing for adequate disinfection, modifications to treatment philosophies may be required. This may require the removal of the DBP precursor material. Techniques used for the biodegradability of NOM either measure the assimilable organic carbon (AOC), the biodegradable dissolved organic carbon (BDOC) or the bacterial growth potential (BRP) of the water under investigation.

According to Grünheid *et al.* (2005) and Shuang *et al.* (2007), DOC is the carbon which remains in a sample after passing the sample, typically through a 0.45 - 0.7 µm filter. These authors are also of the view that DOC exists in two fractions, viz; BDOC and non-biodegradable dissolved organic carbon (NBDOC) fractions. The biodegradable fraction (BDOC) represents the DOC mineralized by the heterotrophic bacteria (Laurent *et al*., 2005), and the portion of DOC consumed by these heterotrophs has been largely used as an indicator for quantifying and differentiating NBDOC from BDOC in water systems. DOC content in water includes both LMW and HMW organic compounds. The biodegradable organic matter content in treated water is amongst the key constituents influencing bacterial regrowth in distribution systems (Laurent *et al*., 2005). **Figure 2-3** shows the relative distribution of carbon in water.

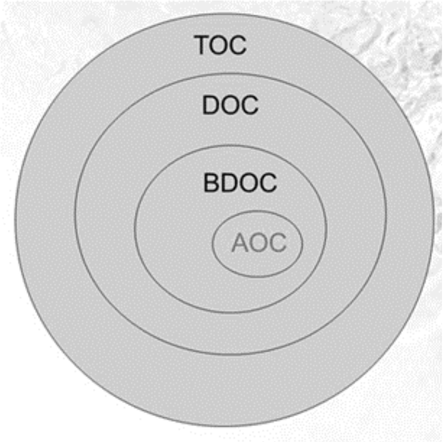

Figure 2-3: Relative distribution of carbon in drinking water (Check light, 2019)

The biodegradability of NOM is often assessed with regards to BDOC and its inhibition of bacterial regrowth (Kwon *et al.,* 2005). The BDOC technique is used largely as a parameter for quantifying and distinguishing between non-biodegradable dissolved organic carbon (NBDOC) and BDOC in water systems. This technique envisages the regrowth of bacteria in distribution systems by quantifying the amount of biodegradable organic matter in the water after water treatment (Laurent *et al*., 2005).

It represents the biodegradable DOC fraction, in both LMW and moderately HMW organic compounds, which can be assimilated by heterotrophic bacteria (**Figure 2-4**). Compared to the AOC and BRP methods, the BDOC technique is principally aimed at determining the amounts of organic carbon that can be removed from the water. Hence, it is the decrease in DOC that is measured as opposed to quantifying the biomass produced (**Figure 2-4**).

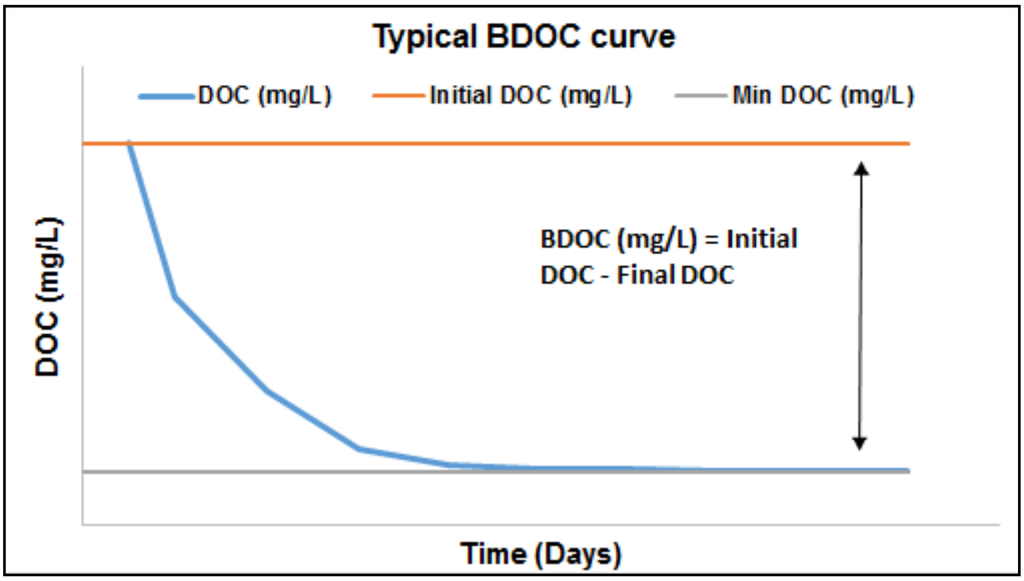

Figure 2-4: A typical DOC/Biodegradation curve (WHO, 2001)

Successful reduction of BDOC during water treatment is important as even low levels of DOC can be enough to support bacterial regrowth in water distribution and pipeline systems. Amongst other factors that create conducive conditions for the bacterial regrowth, BDOC remains the main substrate (Momba *et al.,* 2002). The absence of BDOC after water treatment limits bacterial regrowth in the distribution pipeline (Yavich *et al.,* 2004). BDOC can also be linked to chlorine demand and DBP formation potential (Escobar and Randall, 2001). The removal of HMW NOM, total organic carbon (TOC) and colour agents in a WTP does not necessarily reduce the biofilm formation potential, and the assimilable organic carbon relates more to low molecular weight (LMW) NOM (Hem and Efraimsen, 2001). It can, therefore, be concluded that the removal of HMW NOM does not always reduce the organic carbon available to bacteria and could therefore still influence bacterial regrowth in the pipe distribution system.

To address the problems associated with biodegradable organic matter (BOM), which for purposes of this research study, BOM has been equated to BDOC. This relates to the fact that organic matter present in water is primarily in dissolved form; therefore, the particulate component of NOM is negligible. According to Page *et al.* (2002), all techniques for BOM quantification in water include the utilization of bacteria. Certain measuring techniques or methods measure BDOC by focusing on bacterial regrowth capabilities. Other methods quantify the microbial activities by assessing the changes in chemical properties in test water, while others, measure the actual DOC concentrations in water (Page *et al.*, 2002). Methods available for BOM are discussed in below.

2.6.1 Batch cultures of known bacterial species

A widely used method, the assimilable organic carbon (AOC) method, which measures the mass of organisms that can multiply or grow in a water sample is considered proportional to the quantities of biodegradable organic carbon. In this method, a low amount of bioassay organisms is injected into culture vessels carrying pure test water. For the next several days to a week, the cultured bioassay organisms are secured and incubated at 15°C. Thereafter, the cell densities are enumerated with an adequate frequency to characterize the growth curve in the population and to obtain the highest cell densities (Page *et al.*, 2002). The density or mass of the cells is proportionated and converted to acetate (a good source of carbon) concentration using yield factors derived through empirical studies. The *Pseudomonas fluorescens* and *Spirillum strain NOX* isolated from drinking water are the two bacterial species or bioassay organisms primarily used in the AOC method. The AOC method is very sensitive to the determination of organic carbon due to the potential of the cultured organisms to easily grow even at minimal concentrations. The AOC method is time-consuming and only gives an indication of the regrowth potential of bacteria, not a definite measure of carbon concentration. However, the AOC method has advantages due to its sensitivity and precision.

2.6.2 Cultures of autochthonous bacterial species

This is the second major group of BOM measurement and it involves analyzing the regrowth of indigenous or autochthonous bacteria in water samples. This method emanates from varying techniques for measuring growth such as the BRP method based on turbidity quantification (Page *et al*., 2002b). To ensure BOM is the only limiting factor, the method uses sterilized water samples which are topped up with a mineral salt solution placed in a cuvette under guarded temperatures. The inoculum (i.e. biologically active sand) is simply prepared by cleansing off the bacteria from the membrane used to sterilize the sample. To produce an initial density of 5×10^4 cells/ml, the cell numbers and densities are first modified and adjusted to achieve the target. While turbidity measurements are being made, the cuvette is continuously stirred and the system is operated until the batch cultures accomplish an immobile or stationary phase. The bacterial community is denoted by growth curves over time and the two parameters depicted on the curve are the growth rate, signified by the slope during the exponential increase and the growth factor (i.e. a proportion of the final turbidity to the initial turbidity). Due to the bacteria being autochthonous, the results are particularly fitting to the water sampled, although it does measure the growth of suspended bacteria instead of attached bacteria (Page *et al*., 2006; McDowell *et al*., 2006).

2.6.3 Cultures rooted in the quantification of variations in DOC concentrations

Techniques based on the analysis of DOC are together known as BDOC assays. BDOC is the change between initial and lowest DOC analyses over a specified period. This is a direct method of measuring DOC and the DOC measurement values are expressed in units of carbon. This method has the advantage of accounting for the DOC that is non-biodegradable (NBDOC). **Table 2-4** shows how the BDOC method has evolved over the years with modifications made on the original BDOC method.

Table 2-4: Transformations made to the indigenous BDOC method

No:	Enhancement pursued	Alteration	Reference
	The indigenous BDOC	Formalized BDOC technique based on DOC analyses	Servais *et al*. (1987)
1.	**Increase the size of the BDOC pool measured**	Use of bacteria attached on the sand- biologically active sand	Joret and Levi (1986) Park *et al*. (2004)
2.		Increased incubation time – to grow more bacteria	McDowell *et al*. (2006)
3.	**Increased speed**	Use of attached bacteria instead of suspended bacteria	Trulleyová and Rulik (2004) Park *et al*. (2004)
4.		Use of a recirculation batch reactor	Gimbel and Maizer (1987) Lucena *et al*. (1990)
5.		Using a plug flow bioreactor	Volk *et al*. (1997)
6.	**Reduction of potential contamination**	Removal of particulate matter by filtering of the test water	Kaplan *et al*. (1994)

2.6.3.1 BDOC method with batch cultures

This strategy involves the use of solid whereby batch cultures of microorganisms are either in suspended or attached to it. The steps involved in this approach include 1) re-inoculating the filtered water with bacteria, 2) a preliminary determination of the DOC quantities, 3) incubation of the BOM, and lastly, 4) a continuous analysis of the DOC from the incubation vessel at specified time intervals until constant DOC levels are achieved. The incubation period can last from 10 days to a month. The mechanisms of BDOC metabolism through suspended inoculum differ for distinctive water with various traits (Volk *et al.,* 1997). Also, according to McDowell *et al*. (2006), DOC generally contains more of the refractory, slowly or non-biodegradable compounds and a 28-day incubation could understate BDOC concentrations by up to 25% in comparison to extended periods (e.g ±100 days). It was based on the findings of this method that a 'state-of-the-art' technique involving the speedy determination of relatively labile DOC was required. According to McDowell *et al*. (2006), the technique needed to include a ±42-day incubation period, allow for continuous and reproducible analysis of CO_2 production. According to Kaplan *et al*. (1994), the technique was relatively rapid and easy to conduct and is both sensitive and accurate. A lower detectable concentration in the BDOC test is 0.1 mg C/L or much less and its precision was envisioned at a wider range between 11 and 160 µg C/L for 109 distinct water sources in the United States of America (Kaplan *et al*., 1994). The indigenous BDOC method was marginally altered by Kaplan *et al*. (1994) in a venture to lessen the defilement of organic carbon and enhance the detection limit.

2.6.3.2 BDOC with batch cultures and biologically active sand

The BDOC with batch cultures and sand was developed particularly for potable water by Joret and Levi (1986). The method uses bacteria fixed on the sand as the inoculum. The inoculum (sand), which is colonized by bacteria is taken from an operational sand filter at a WTP and cleaned with test water until no increase of or constant DOC is detected in the wash water. About 100g of the inoculum is put in an Erlenmeyer flask (approximately 500ml) and is topped up with 300ml of test water (i.e. the ratio is 1:3 for the sand and water respectively). To initiate an aerobic process, the batch cultures or the contents of the flasks are oxygenated at 1.1 ml/s with air compressed by two water washes. The DOC concentration is continuously monitored until minimum concentrations are detected, usually between 3 and 4 days. This BDOC technique involving bacteria attached on sand has advantages similar to those of the traditional BDOC technique described earlier, in that it is a rapid approach, easy to conduct and is both sensitive and precise. In comparison to the BDOC determination using suspended bacteria, this method was found to give a superior performance as the latter underestimated BDOC by 5-25% (Trulleyová and Rulík, 2004; Park *et al*., 2004). Amongst other factors, the BDOC underestimation was attributed to lower microbial diversity, the lower metabolic activity of suspended bacteria etc. In this regard, the attached bacteria technique holds the most advantages for BOM (i.e. BDOC) measurement. However, one challenge of this method is the difficulty in the cleaning of the sand, which when not done properly interferes with the DOC detection levels.

2.6.3.3 BDOC with bioreactors

The BDOC with bioreactors is designed to operate in a continuous flow as opposed to recirculation. The BDOC in this method is measured as the difference between the inflow and outflow DOC. According to Kaplan *et al*. (1995), the original design consisted of a sand glass column filled with test waters in an upflow mode at 240 mL/hour. Later on, this design was reconfigured to include beads as support for bacteria. Factors that impact on the BDOC bioreactor method include the colonization, exposure period, temperature and DOC levels in the influent. The continuous flow bioreactor has the advantage of being a rapid method and the results generated are nearly real-time. However, the continuous flow bioreactor method requires a considerable amount of time, approximately 100 days for colonization. The method may also potentially underestimate the BDOC content as the bioreactor effluent takes into account the DOC released by biofilm microorganisms. Similarly, with other BDOC techniques, the bioreactor needs carbon limitation; therefore, the addition of inorganic nutrients and oxygen are also essential to create aerobic conditions.

2.6.4 BOM/BDOC quantification and estimation of bacterial regrowth potential

To conclude and cement the decision of choosing the BDOC for the experimental phase of the research study, the two widely used methods (i.e. AOC and BDOC) are summarized. There currently is no definite technique for BOM quantification that can be employed as a standard for all other assays. The various available techniques all offer different minimum detection limits and applicability. Escobar and Randall (2001) have suggested that measuring only one of these parameters (i.e. BDOC or AOC) can under- or overestimate the regrowth potential of bacteria in the water. Adoption of both approaches can provide information that can be used to optimise WTPs for the removal of NOM and management of BDOC in water supply systems. The merits of the method involving an attachment of BDOC to sand over AOC and other BDOC techniques (including BDOC with suspended bacteria method) serves as a justification for adopting this method for this research study. These merits are:

a) In contrast with BDOC measurements, the AOC is less accurate as it underestimates the BOM content in water. The observation that the estimation value of BOM using AOC is generally lesser than that of BDOC is common. This is attributable to the variability in the metabolic potentials of the bacteria.

b) AOC is derived from the metabolic abilities of two (2) types of bacteria (i.e. *Pseudomonas fluorescens* and *Spirillum strain NOX),* while BDOC is derived from the metabolic processes of an unknown but an array of species, which can be considered a real representation of actual scenarios in a distribution system.

c) AOC assumes the yield coefficient for the bioassay organisms and these may differ conditional to the character and properties of the BOM assessed.

d) Lastly, the microorganisms for the BDOC assay hails from the test water, which suggests the selection for specific metabolic pathways has already begun.

2.7 Conclusions

NOM is, without doubt, a global concern in the water sector, as it affects raw water sources, water treatment processes, and distribution systems. Though a lot has been done in addressing the challenges with NOM, its continuous influx in water bodies, its spatial and seasonal variations make NOM one of the most challenging contaminants to deal with in the water treatment and supply industry. Due to these complexities associated with NOM, there is a need to continuously characterize NOM and its fractions, specific to the raw water source, the intake to the water treatment processes, as well as the various treatment steps.

Due to the inherent heterogeneous nature of NOM, a number of approaches are often sought to ensure all of its properties are evaluated. The seasonality in the character and structure of NOM is apparent from the literature and as such the characterization requires a different approach which will give more insights on the NOM. NOM characterization techniques such as TOC, UV_{254}, SUVA and more advanced methods such as HPSEC, FEEM, NMR allow the quantitative analysis of NOM, however, these techniques cannot simultaneously separate and characterize the different fractions of NOM. Therefore, there is a need for the separation (i.e. fractionation) and characterization of the different fractions of NOM; hence the adoption of the m-PRAM for isolation of bulk NOM which can isolate NOM relative to size and polarity.

Conventional processes are still widely used and remain the most preferred treatment option in relation to capital investments and operating costs. However, conventional processes cannot sufficiently remove NOM, particularly the BDOC component. The co-existence of NOM and chlorine in water gives rise to the formation of DBPs. Literature has also highlighted the need to address NOM and its individual fractions as precursors to DBP formation in potable water. The disinfection practices are unlikely to change; chlorination still takes precedence over other disinfectants, particularly in the developing world. More insight on the precursors is sought and this can be achieved by characterizing and isolating the various NOM fractions, to assess them individually for their influence on DBP formation.

There is a need to augment water treatment processes’ capability to efficiently reduce NOM which is a great contributor to DBP formation.

Various characterization and fractionation techniques have been applied in an attempt to gain more understating on NOM, however, there are still facets of NOM (e.g. the biodegradable fraction of NOM) that require further exploration. Quantification of the biodegradable fraction of NOM is still a challenge, as only two techniques (i.e. AOC and BDOC) are available for its measurement. Sufficiently reduced BDOC during water treatment is important as even minute BDOC concentrations are reportedly adequate to supplement bacterial growth in the water distribution system. The literature suggests that the AOC method has too many limitations such that it cannot be relied upon, therefore the BDOC remains the only viable option and needs to be explored further to ensure optimum quantification of biodegradable NOM. There is a need to design an affordable, rapid and a simple technique to quantitatively assess the BDOC potential by each fraction of NOM.

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CHAPTER 3: MATERIALS AND METHODS

3.1 Chemicals and reagents

The chemicals and reagents used throughout the research study were purchased by the Rand Water Scientific Services Accredited Laboratories and they were all of analytical grade. All standard solutions were prepared using deionized water (Milli-Q system). Their applications are further discussed in their respective sections below.

3.1.1 Sodium hydroxide

The available grade of sodium hydroxide (NaOH) is 40 g/mol. To prepare a primary stock solution of 1 *N* of NaOH, a 4 g (NaOH) was weighed and dissolved in a 1000 mL deionised milli-Q water in a volumetric flask. The solution was then diluted 10 times to create a secondary stock solution of 0.1 *N* (NaOH) and was stored in sealable glass containers with stoppers.

3.1.2 Methanol

Methanol (CH_3OH) was supplied as with a concentration of 99% as CH_3OH. To prepare a 5 % primary stock solution, about 50.5 mL of the 99% as CH_3OH was pipetted and diluted in a 1000 mL of deionised milli-Q water in a volumetric flask.

3.1.3 Monopotassium phosphate

To prepare a 0.1 % monopotassium phosphate (KH_2PO_4) solution, approximately 1.3 g of the 136,086 g/mol (KH_2PO_4) was weighed and dissolved in a 1000 mL of deionised milli-Q water in a volumetric flask

3.1.4 Ammonium chloride

To prepare a 0.1 % ammonium chloride (NH_4Cl), approximately 4 g of the 53,491 g/mol (NH_4Cl) and dissolved in a 1000 mL of deionised milli-Q water in a volumetric flask

3.1.5 Sodium acetate

Two primary stock solutions of sodium acetate ($NaCH_3$) were prepared, a 5 mg/L and 10 mg/L. Approximately 0.028g and 0.057g ($NaCH_3$) were weighed and dissolved in 1000 mL of water, to form 5 mg/L and 10 mg/L ($NaCH_3$), respectively.

3.1.6 Sodium thiosulphate

To prepare a 0.1 N sodium thiosulphate ($Na_2S_2O_3$) solution, approximately 49.66 g of the 158,11 g/mol ($Na_2S_2O_3$) was weighed and dissolved in a 2000 mL of deionised milli-Q water in a volumetric flask.

3.2 Bulk water sampling

3.2.1 Description of the study area

The Vereeniging WTP is one of the two (2) major WTPs in the Rand Water Treatment chain The Vereeniging WTP receives water supply from the Lesotho Highlands Water Project (LHWP), via the Vaal Dam impoundment located in the Free State Province of South Africa (SA). The Vaal Dam is the fourth largest dam in SA and has a surface area of approximately 321 km^2 and an average depth of 22,5m. The dam water is abstracted via the Lethabo weir, which is situated at Viljoensdrift next to Lethabo Power station. The raw water is then pumped to the Vereeniging WTP which has been in operation since 1923 and was Rand Water’s first river intake water treatment and pumping site. The plant has a total capacity of approximately 1200 ML/day of water. After treatment, the water is pumped at a head of approximately 200m to the main booster pumping station, Zwartkopjes and its three satellite booster pumping stations, Palmiet, Eikenhof and Mapleton. The area of supply covers the entire Gauteng Province and extends as far as Rustenburg and Carletonville in the North West Province, Heilbron in the Free State and Bethal in Mpumalanga.

3.2.2 Sample collection and preservation

Raw and treated water samples, before the application of the chlorine disinfectant, were collected from the Rand Water conventional water treatment plant (WTP) (**Figure 3-1**). The treatment steps of the Rand Water WTP consist of a screening process, coagulation (lime-poly or lime-silica), spiral flocculation channels, sedimentation, re-carbonation, rapid sand filtration, primary chlorination and secondary chloramination (through the addition of ammonia on water containing residual chlorine at a preferred ration of 4:1). The samples were collected on a weekly basis over a period of 15 months, between August 2018 and November 2019. The selected time period was to ensure that all weather seasons in a year are covered. The South African seasons are as follows; Winter (June – August); Spring (September – November); Summer (December – February); and Autumn (March-May).

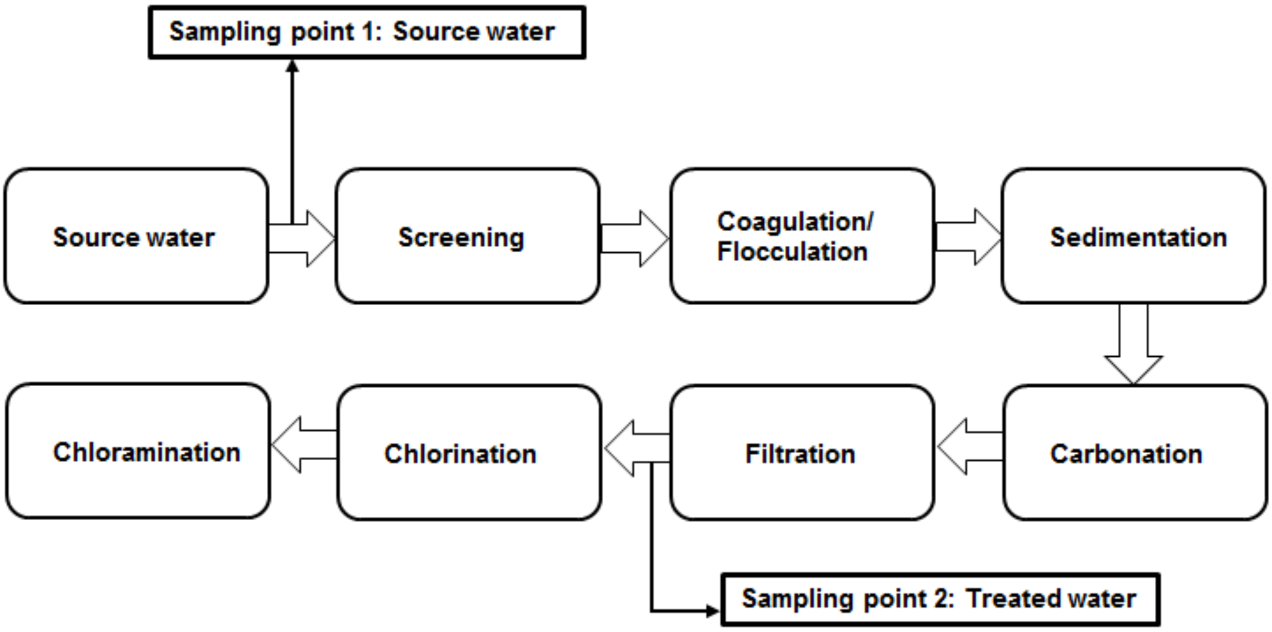

Figure 3-1: Raw (1) and treated (2) water sampling points within the Rand Water WTP

Sampling and preservation were done in accordance with recommendations by Daoudi (2000) and Radtke (2002). Samples were kept at 4ºC during transportation and refrigerated in the laboratory. Sample preservation done in a proper way minimizes the potential to reduce or lose target analytes (Baghoth, 2012). All other analyses such as UV, DOC were conducted at Rand Water SANAS accredited Laboratories. Raw water samples were used to collect NOM characterization data from the raw water source in the catchment area of Rand Water. The samples were used to investigate the character of the NOM, the biodegradation of various NOM fractions, and the DBP formation potential through bench-scale chlorination experiments. The procedures followed are explained further in the forthcoming sections.

3.3 The characterization and fractionation of the natural organic matter (NOM)

The modified polarity rapid assessment method (m-PRAM), which employs solid-phase extraction (SPE) cartridges with different polarities was used for NOM fractionation and characterization was conducted using the bulk NOM parameters. For the m-PRAM technique, which is a derivative of the PRAM technique, the method was performed in conformity with the procedural steps narrated by Nkambule (2012). Instead of the original 6 fractions (Rosario-Ortiz *et al*., 2007), the m-PRAM gives 3 fractions (excluding the acids and neutrals.) and is thus enough for the characterization of NOM with respect to aromaticity and composition. The 3 fractions were deemed adequate for addressing the aim and objectives of this research study. The m-PRAM method is a rapid technique; thus considered to be less time-consuming. Other NOM characterization techniques, which were employed for further characterization of the Bulk NOM and the various NOM fractions, are UV_{254}, DOC, and SUVA.

3.3.1 m-PRAM experimental procedure

NOM was separated into 3 major NOM fractions: namely, hydrophobic (Hpo), hydrophilic (Hpi) and transphilic (Tpi). The following three solid-phase extraction (SPE) cartridges were chosen for m-PRAM: C18 (non-polar), CN (polar) and the NH_2 (weak-anion exchange). These cartridges were chosen to obtain the respective Hpo, Hpi and Tpi fractions of NOM. The following sections give further descriptions into the m-PRAM procedure and characterization (**Section 3.3.1.1 - 3.3.1.2**)

3.3.1.1 Preparation and cleaning of SPE cartridges

The three SPE cartridges (i.e. C18, CN and NH_2) were washed sequentially with a 5% methanol solution and deionised water to ensure that any UV absorbing material was completely removed from the cartridges before the m-PRAM was conducted (**Figure 3-2**). The use of methanol provides for the conditioning, which is necessary for SPE cartridges as it wets and settles the bed, activates the packing materials, and removes any residual process materials. The deionised water was filtered through all the SPE cartridges for ≥ 20 minutes to ensure that cartridges are free of UV absorbing particulates (**Figure 3-2**). The effluent from the cartridges was analysed for UV_{254} at 4 minutes' intervals or until constant UV_{254} values were obtained. The constant or low UV_{254} values were regarded as an indication that the SPE cartridges are clean, free of DOC and are ready for the extraction of the three NOM fractions.

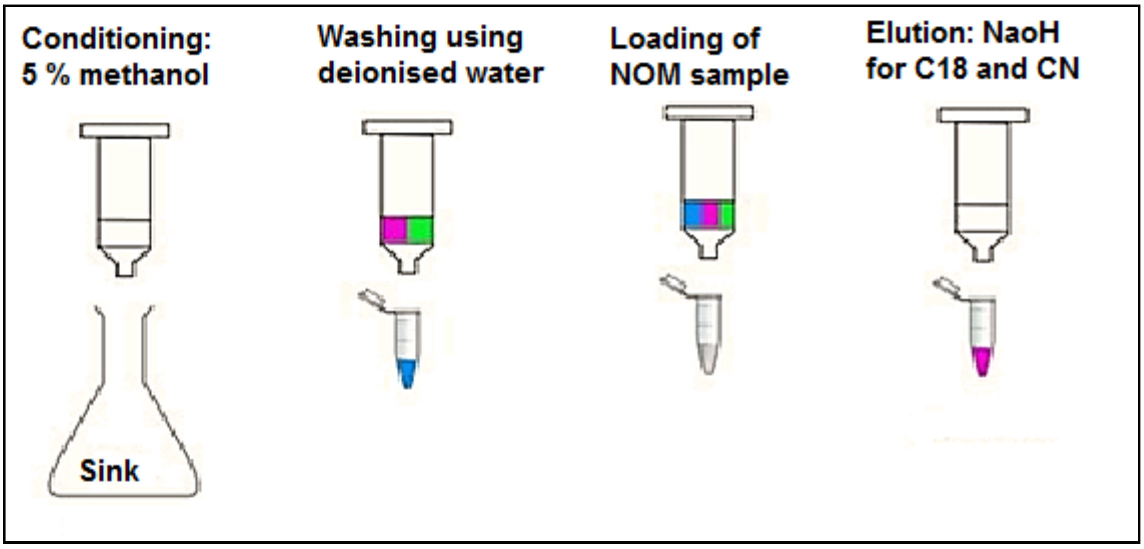

Figure 3-2: Conditioning and washing of the SPE cartridges for the m-PRAM (Khanyile *et al.*, 2019)

3.3.1.2 m-PRAM extraction of the NOM fractions

To remove any particulate matter from the samples, they were first filtered with a Whatman 0.45 µm S-PAK membrane filters before fractionating through the 3 SPE cartridges at a flow rate of 1.2 mL/min (5 inches Hg, 0.1 bar) (**Figure 3-3**). The method uses Phenomenex® solid-phase extraction (SPE) cartridges to fractionate NOM in terms of polarity and uses the C18 (non-polar), CN (polar) and the NH_2 (weak anion exchange) cartridges to obtain the Hpo, Hpi and Tpi NOM fractions, respectively. NOM was fractionated into 3 major NOM fractions that represents the aromatic structure or composition of the NOM. For the Hpo and Hpi fractions, samples were filtered directly through the C18 and CN cartridges, respectively, thus resulting in the extraction of the fractions. To obtain the Tpi fraction, the water samples were filtered through the respective Hpo and Hpi cartridges prior to elution through the NH_2 cartridge (**Figure 3-3**). For every 6 mL of the sample, 10 mL infusion of 0.1 M sodium hydroxide (NaOH) stock was filtered through the C18 and CN cartridges to elute the Hpo and Hpi fractions respectively. Thereafter, the filtrate from each SPE cartridge (i.e. Hpo, Hpi and Tpi) was analysed for UV_{254} and DOC. The DOC and UV were used to compute the SUVA amount of each fraction as described in **Equation 2**.

$$SUVA(L/mg.m)_{254} = \frac{UV_{254}\left(cm^{-1}\right)\times 100}{DOC\left(\frac{mg}{\ell}\right)} \qquad [2]$$

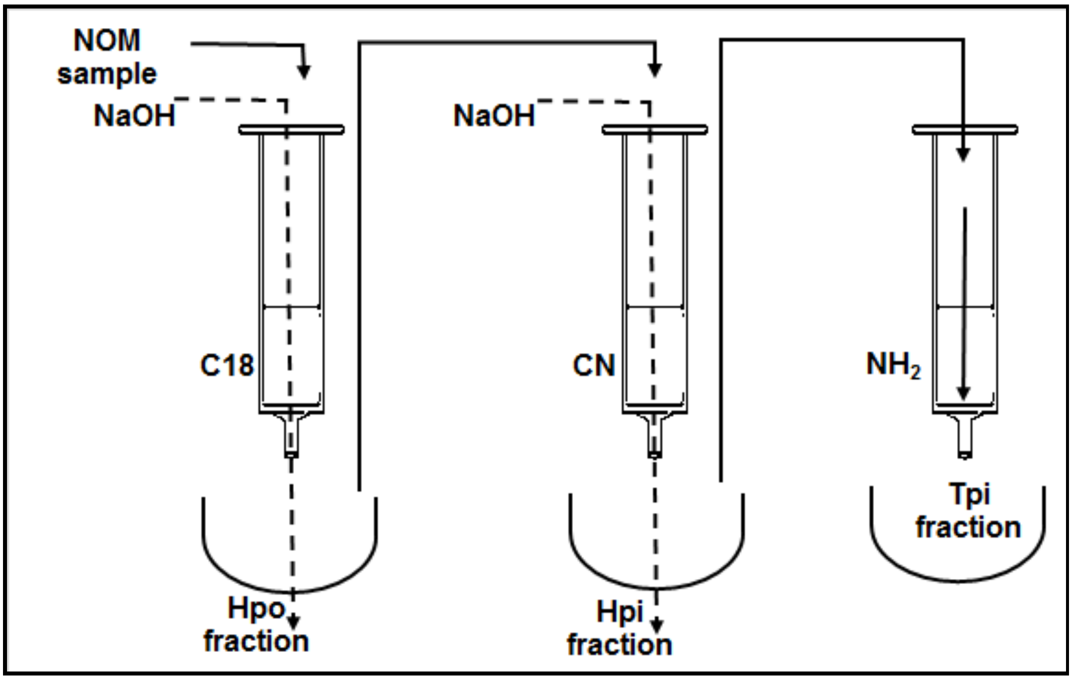

Figure 3-3: Experimental set-up for the SPE and m-PRAM (Nkambule *et al*, 2012)

3.3.2 Bulk NOM characterization techniques

3.3.2.1 Dissolved Organic Carbon (DOC)

Each of the 3 NOM fractions obtained from raw and treated water was analysed for DOC using a Shimadzu TOC-L analyser, a method specified under EPA 415.3 approved method (Potter and Wimsatt, 2005). The instrument oxidizes organic carbon from a sample to produce carbon dioxide (CO_2), which is then measured as TOC or DOC. For DOC analyses, water samples were filtered using a Whatman 0.45 µm filter paper to remove any particulate organic matter. Thereafter, the CO_2 was measured by a combination of conductivity and non-dispersive infrared detector and reported as DOC in mg/L. To ensure the instrument was functioning optimally, standard carbon solutions were used to calibrate the instrument making use of hydrogen phthalate (KHP) of concentrations ranging from 1 to 30 mg/L.

3.3.2.2 Ultraviolet absorbance at 254 nm (UV_{254})

To ascertain the aromaticity of the NOM fractions, the filtered water samples (raw and filtered water from the WTP) and the NOM fractions obtained through the m-PRAM was analysed for UV_{254} through a spectrophotometer- Cary 60 UV-Vis developed by Agilent Technologies. Due to the organic matter structure consisting of double bonds between the carbon atoms that absorb ultraviolet light at this wavelength (254 nm), the UV_{254} measurement was used as an indication of the aromaticity of NOM (Edzwald and Tobiason, 2011). The UV_{254} is easy to use, less time-consuming technique and is easily accessible to WTP personnel (Lobanga *et al.,* 2014).

3.3.2.3 Specific ultraviolet absorbance (SUVA)

The SUVA of a water sample is defined by its UV absorbance at a specified wavelength specific for its concentration of DOC. The SUVA is strongly correlated to the percentage aromaticity of the dissolved fraction of organic matter in the water sample (Hansen *et al*., 2016). The SUVA was computed as a fraction of the UV_{254} and DOC, as previously described in **Equation 2**. The relationship between SUVA and humic matter removal and TTHM formation makes the SUVA value of a water sample beneficial when predicting the treatability of the water (Fakour and Lo, 2018).

3.4 Biodegradability of NOM assessment

3.4.1 Experimental procedures or set-up

3.4.1.1 The sampling of the inoculum and inducing proliferation of the bacterial cells within the inoculum

Biologically active sand (BAS) was sampled from currently used filtration units at the WTP. The BAS was used to trace the BDOC over a 4-day period. The BAS was covered with raw water and incubated in a fish tank for 7 days to encourage the growth of heterotrophic bacteria **(Figure 3-4).** The use of BAS instead of single-strained bacteria avoided the mineralisation and degradation of specific NOM fractions (Axmanová *et al*., 2006). Approximately 5 kg of BAS was transferred into a fish tank (L= 91cm, b= 32 cm, and h= 38 cm), which was thereafter covered with raw water for 7 days.

Figure 3-4: A 7-day inoculation of biological activated sand (BAS) set-up (picture taken by S Sambo, 2019)

This type of inoculation is meant to encourage the growth of heterotrophic bacteria. Sufficient amounts of monopotassium phosphate and sodium acetate ($NaCH_3$) were added to the BAS to provide nutrients for the bacteria. After the 7-day proliferation period, the BAS was rinsed sequentially with 10 aliquots of 0.1 M sodium thiosulphate ($Na_2S_2O_3$) solution (i.e. 10 ml of the 0.1 M $Na_2S_2O_3$ and 490 mL deionised water), inverting the sand each time until no DOC was released from the sand. This constitutes the initial DOC in the samples in the sand sample.

3.4.1.2 BDOC setup and monitoring

A 100 g of the rinsed sand was put in 500 mL Schott bottles and 300 mL of each of the NOM fractions was thereafter added to the Schott bottles. Sodium acetate ($NaCH_3$) solutions of 5 or 8 mg/L (a source of carbon in biodegradation) was used as a control with a view to ascertaining the biological activity in the water with the BAS inoculum (Yang *et al.,* 2012). Each of the NOM fractions was dosed with an inorganic nutrient solution by adding N and P (0.1% KH_2PO4 and 0.1% NH_4Cl) for providing nutrients to the existing bacteria. This was to warranty that carbon was the only restriction in the DOC degradation study. The addition of inorganic nutrients to the setup has no interferences on the BDOC set-up and results obtained (Mann *et al*., 2015; Abbott *et al*., 2014). The prepared samples were shielded with foil (to prevent photodegradation) and kept at a temperature range of 22ºC. Moreover, the samples were aerated to maintain high oxygen transfer efficiency through a connected air compressor that bubbles air inside the samples at intervals of a maximum of 2 bubbles per second **(Figures 3-5).**

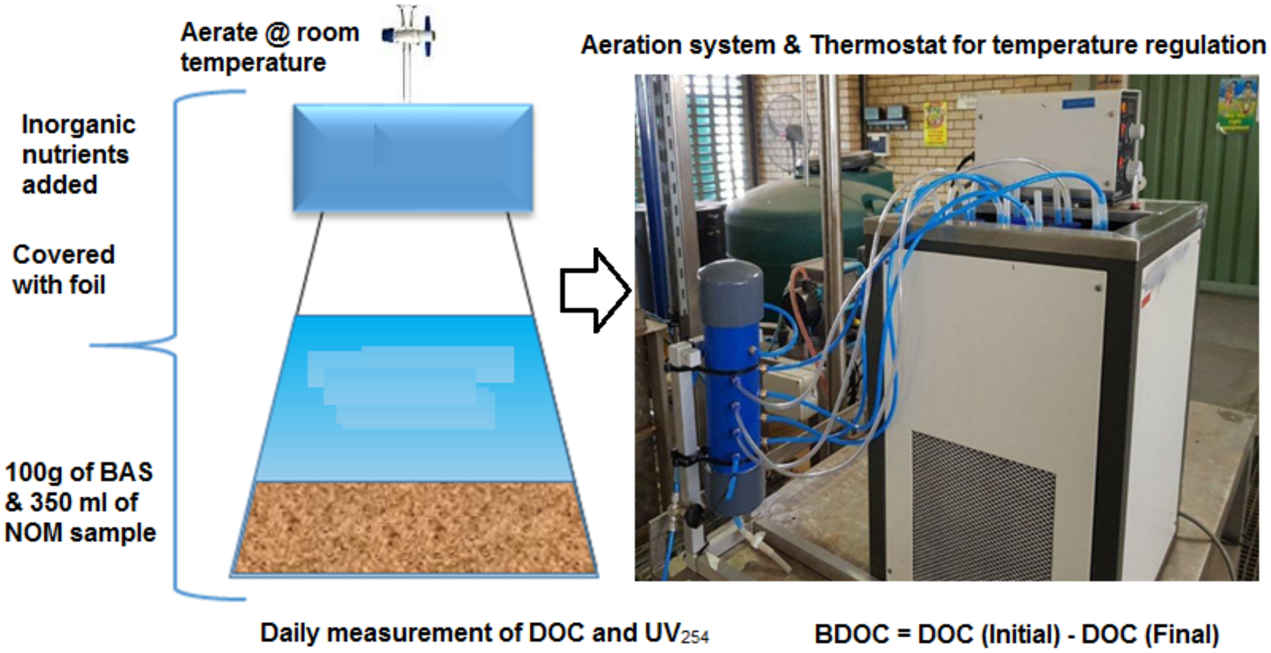

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Figure 3-5: The enhanced biodegradable dissolved organic carbon (BDOC) setup (Picture taken by S Sambo, 2019)

Samples were filled to capacity and thus prevent any escape of the DOC. The samples were collected over a 4-days period in 50mL glass containers and thereafter analysed for DOC. The BDOC was then determined as the difference between initial DOC concentration and lowest DOC concentration (**Equation 3)** (Escobar and Randall, 2001).

$$BDOC = DOC\ (Initial) - DOC\ (lowest\ within\ the\ 4\ days) \qquad [3]$$

3.5 Disinfection by-product formation potential (DBPFP) analyses

The formation potential of DBPs was specifically studied for total trihalomethanes (TTHMs), TTMs are a combination of dibromochloromethane (CHBr2Cl), bromoform (CHBr3), bromodichloromethane (CHBrCl2), and chloroform (CHCl3) which are formed when chlorine interacts with NOM in the presence of the bromide ion. A combination of SPE and gas chromatography-electron capture detection (GC/ECD) techniques were used for the analysis of THMs.

3.5.1 Sampling and sample preparation

As previously described, the m-PRAM was applied to both the raw and treated water acquired from the Rand Water WTP. Thereafter, the NOM fractions were used for assessment of the trihalomethanes formation potential (THMFP). All glassware was cleaned using deionised water. To confirm that the water was indeed free of organic material, the water sample was run through a TOC analyser before use. All used glassware was soaked overnight in a 3 mg/L chlorine solution to ensure that it was chlorine demand free. The same sample containing the chlorine used for soaking was analysed for free chlorine, and the chlorine residual was always above 1 mg/L thus confirming that all the chlorine demand was met. The glassware was rinsed a couple of times with deionised water to eliminate all residual chlorine. All samples used for the analysis of THMs were collected in duplicate making sure that the samples overflow from the container. This eliminates the headspace and ensures the elimination of air bubbles which may accumulate while collecting and sealing the container

3.5.2 Sample chlorination and incubation

In accordance with Standards Methods (2017) for THMs and other DBPs, 40 mL of the water samples were collected in 45 mL glass amber vials (**Figure 3-6**). Temperature and pH were kept constant for all samples. A 1000 mg/L chlorine solution (primary stock solution) was made from a 12.5% sodium hypochlorite (NaOCl) solution. Each NOM fraction was dosed with 14 mg/L of chlorine (**Equation 4**) and covered with the screw caps and inverted for a thorough mix.

$$\begin{aligned}
&C_1V_1 = C_2V_2 \\
&1000\left(\frac{mg}{\ell}\right)\times V_1 = 14\left(\frac{mg}{\ell}\right)\times 40m\ell \\
&V_1 = \frac{14\left(\frac{mg}{\ell}\right)\times 45m\ell}{1000\left(\frac{mg}{\ell}\right)} \\
&\quad = 0.56m\ell
\end{aligned} \qquad [4]$$

Where C_1 and C_2 are the prepared chlorine stock solution (i.e. 1000 mg/L) and the sought after concentration (14 mg/L)), respectively, and V_1 and V_2 are the unknown required volume from the stock solution (mL) and the volume in which the sought after concentration was prepared on (i.e. 40 mL, or the final volume) respectively.

The high dosages of chlorine were deliberately administered to ensure detectable residual chlorine at the end of the experiment and to promote easy quantification of the THMFPs. Once the samples were thoroughly mixed with chlorine, they were filled to overflow to ensure that there was no headspace between the sample and the cap. The bottles were shielded with aluminium foil paper to avoid photodegradation of chlorine (**Figure 3-6**). After 30 min, the residual chlorine was analysed using a HACH Colorimeter. After the standard 7-days incubation at pH 8.3 and 25 °C, chlorine residual of between 3 and 5 mg/L was detectable. To prevent further formation of THMs after the 7-day incubation period, 1 g of ascorbic acid was added to the samples to eliminate the residual chlorine prior to analysis of the THMs.

Figure 3-6: Sample bottles covered with foil used for the THMFP analysis (Picture taken by S Sambo, 2019)

3.5.3 Trihalomethanes (THMs) - Solid-phase extraction (SPE) and GC/ECD method

An SPE and a headspace sampler coupled to gas chromatography (GC) were used to analyse for the individual forms of THMs, namely: bromoform, chloroform, dibromochloromethane and bromodichloromethane. The detection limits on the instrument are as follows: chloroform 0.21 µg/L, bromoform 0.36 µg/L, dibromochloromethane 0.33 µg/L and bromodichloromethane 0.27 µg/L. A capillary GC column (HP-5) was used to separate the THMs, which were detected through an electron capture detector (ECD). The THMFPs for each NOM fraction was calculated according to **Equations 5**.

$$THMFP = THM\ (final, day\ 7) - THM\ (initial, after\ 30\ min\ of\ incubation) \qquad [5]$$

3.6 Assessing the influence of BDOC on bacterial quality of water

Studies such as Jin *et al.* (2018) found a significant correlation between slowly biodegradable organic compounds (NBDOC) and biomass production potential. A positive correlation between NBDOC and regrowth in the water reticulation systems exists (Hijnen *et al.,* 2018). The regrowth was assessed with Aeromonas bacteria, heterotrophic plate counts (HPC) and Total coliforms (TC) (Hijnen *et al.,* 2018). To assess the impact of biodegradable NOM of each fraction on bacterial regrowth, samples from each fraction of both the raw and treated water were collected on the first and last days of the BDOC experiment. Each of the three (3) NOM fractions were subjected to the BDOC test as explained in earlier **Section 3.4**. Thereafter, the samples were analysed for bacterial surrogate parameters. The commonly used surrogates for the bacterial pathogen, include Coliforms and Enterococci. For this study, TC and HPC were analysed following the approved standard methods (**Section 3.6.1-3.6.2**).

3.6.1 Heterotrophic plate count (HPC) analyses

The heterotrophic plate count (HPC) is a method that can be utilized to approximate the counts of live, culturable heterotrophic bacteria in water. According to standard methods 19th Edition (9215A), the method also measures the variation of conditions during water treatment and distribution. The solid plate count is re-liquefied in boiling water and thermo-regulated to temperatures between 44 °C and 46 °C. Using a pipette, aliquots of each sample (0.1 mL and 1.0 mL) were transferred into the centre of a 100x15mm sterile petri dish whereby about 12 mL of the thermo-regulated liquid agar was also added to the petri dish and mixed by swirling the plate. Prior to being inverted and incubated at ±35° C for approximately 48 hours, the plates were allowed to solidify for about 10 minutes. All samples were run in duplicate. After the 10 minutes, the colonies that had formed in or on the plate count media were counted and the results were reported as CFU/mL using **Equation 6**. For diluted samples, the dilution factor was multiplied with the counts to obtain the final CFU/mL.

$$CFU/mL) = \frac{Colonies\ counted}{Actual\ volume\ of\ sample\ plated\ (mL)} \qquad [6]$$

3.6.2 Total coliforms

The standard method (9222) (2017) recommends the membrane filter (MF) technique for the monitoring of the total coliforms (TC) group monitoring in drinking and various other natural waters. This group is defined as facultative anaerobic, non-spore-forming, rod-shaped bacteria that form colonies with unique properties on distinct media., Negative (-) cytochrome oxidase and positive β-galactosidase test reactions are fabricated when sterilized cultures of coliform bacteria are tested. The TC method is a rapid technique that can be performed on very large quantities of samples and the results are reproducible (Standard Methods, 2017). Method 9222c uses the *Fluorogen MI transmitter, a* membrane filter (MF) medium, which concurrently identifies and distinguishes both *Escherichia coli* and TC and in water samples within 24-hours depending on their particular enzymatic activities. When subjected to ultraviolet-light at wavelengths between 365 and 366 nm and at 35°C, the bacteria that produce fluorescent colonies within 24 hours are referred to as *Coliform bacteria*, while in the medium the emergence of a blue colour signifies the presence of *Escherichia coli* colonies. Highly turbid waters interfere with the precision of the method. The TC method requires a standard 100 mL to be filtered if the analyses are conducted for potable water purposes.

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CHAPTER 4: THE CHARACTERIZATION OF NOM AND ITS REMOVAL THROUGH THE WATER TREATMENT PROCESS

4.1 General information on NOM

The composition of NOM changes with source and time, and this is attributed to the varying origins of the precursor material and the degree of transformation at which NOM undergoes (Chaukura *et al*., 2018). These seasonal variations and increasing trends in NOM concentration levels impose threats to surface waters and to the water treatment and supply industry (Baghoth, 2012). The Rand Water conventional process is not immune to the challenges imposed by NOM pertaining operation, optimization and effective process management. To adequately enhance and improve these processes, the characterization and quantification of NOM at the various treatment stages is critical. It is also essential to understand and be well-positioned to predict the reactivity of NOM or its fractions within the treatment chain. Therefore, the chapter aimed at characterizing NOM through bulk NOM parameters (i.e. DOC, UV_{254} and SUVA) and isolating the A18 raw and treated water (i.e. before chlorination) by means of polarity into 3 fractions, the Hpi, Tpi and Hpo fractions. Furthermore, apart from presenting the variability in the NOM character and polarity, the chapter also presents the NOM removal efficiencies throughout the Rand Water conventional processes.

4.2 Bulk NOM characterization of the raw and treated waters using SUVA

Specific ultraviolet absorption (SUVA), a variable that provides information on the complexion of NOM and its fractions, was used for the bulk characterization of NOM (Hansen *et al*., 2016). SUVA is also an indicator or measure of the treatability of raw water by coagulation and flocculation (Fakour and Lo, 2018). It is strongly correlated to the hydrophobic organic acid fraction, the percentage aromaticity and molecular weight of DOM in the water sample (Hansen *et al*., 2016). SUVA gives a quantitative measure of aromatic quota per unit concentration of the DOC, and most researchers have used the SUVA variable for the evaluation of NOM reactivity and the prediction of NOM reduction during potable water treatment (Edzwald and Tobiason 2010; Parsons *et al*., 2004).

4.2.1 SUVA characterization of raw water

SUVA values were obtained from two different and independent monitoring programmes, namely the bulk characterization of the raw water (A18) - Internal and external (**Figure 4-1** and **Figure 4-2**) respectively. The characterization of the raw water was undertaken during the period of September (2018) to September (2019) (**Figure 4-2**). The mean SUVA obtained from the two programmes were 3.88 L/mg.m and 4.11 L/mg.m for the internal and external programmes, respectively, with the majority of the data skewed towards the high SUVA value range. As indicated by Edzwald and Tobiason (2010), water dominated by hydrophobic NOM is characterized by SUVA values that are greater than 4 L/mg.m. According to Kitis *et al*. (2002), SUVA > 4 L/mg.m is indicative of a dominant high fraction of aquatic humic matter, highly aromatic, higher molecular weight (HMW) Hpo compounds. High SUVA values also indicate the ease with which water can be treated, particularly by conventional clarification. The high content of aromatic organic compounds translates to a greater tendency of the NOM to react with disinfectants to create DBPs. The mean DOC and UV_{254} obtained for the bulk NOM characterization were 4.22 mg/L and 16.11 m^{-1}, and 4.81 mg/L and 19.79 m^{-1}, for the respective external and internal monitoring programmes (**Figures 4-1** and **4-2**). Historically, the UV_{254} has reached highs of 99.3 m^{-1} (~~Marais~~ *et al*., 2018). therefore, the UV_{254} results obtained in this study can be classified as low UV_{254} for the raw water, with no major changes in its seasonal distribution (i.e. ranges between 10.52 m^{-1} and 22.85 m^{-1}) (**Figures 4-1** and **4-2**) throughout the 2018/2019 period. Considering that only DOC concentration >7 mg/L in water sources is considered high (Drever, 2005), the raw water source was relatively characterized by low DOC (<5.5 mg/L). Despite the low UV_{254} and DOC concentrations on the raw water, the SUVA, a function of both parameters (UV_{254} and DOC) remains high indicating the presence of a large portion of humic matter in the water and that NOM will influence the coagulant dosage and a greater likelihood to react with disinfectants (i.e. chlorine) to create DBPs.

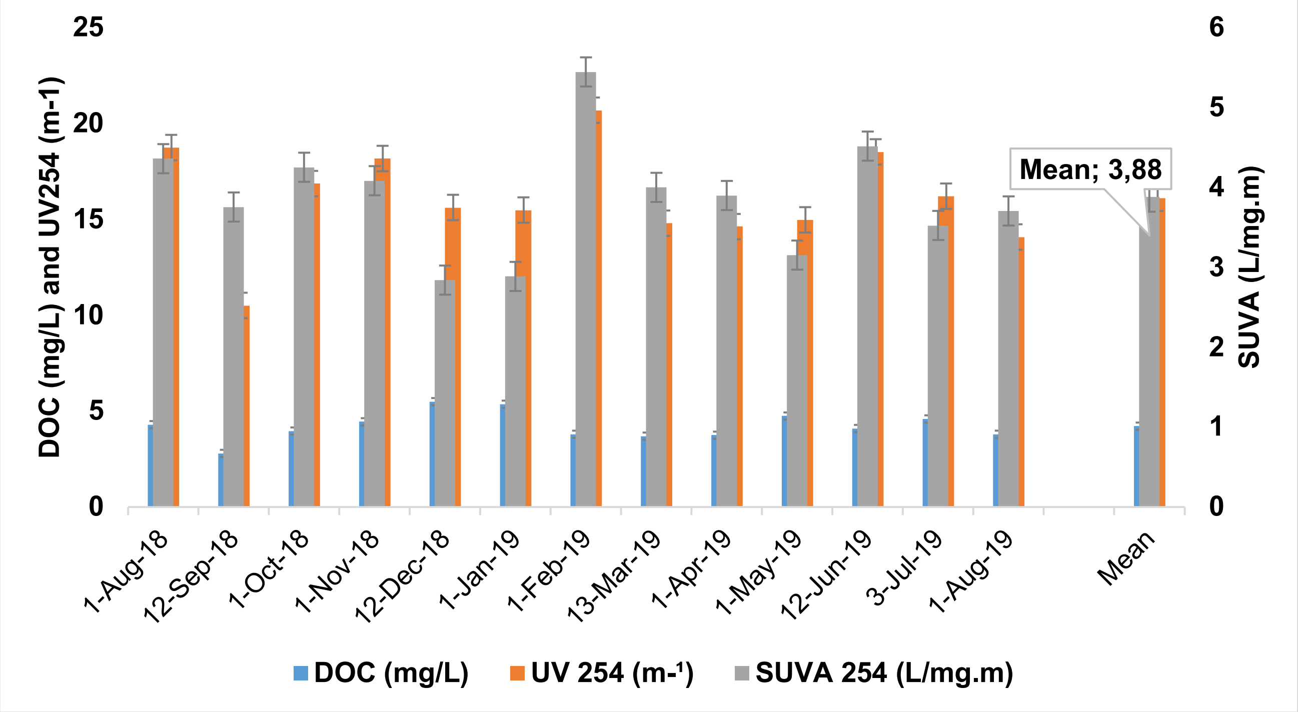

Figure 4-1: Bulk NOM and SUVA characterization of the raw water source (A18)- External monitoring programme

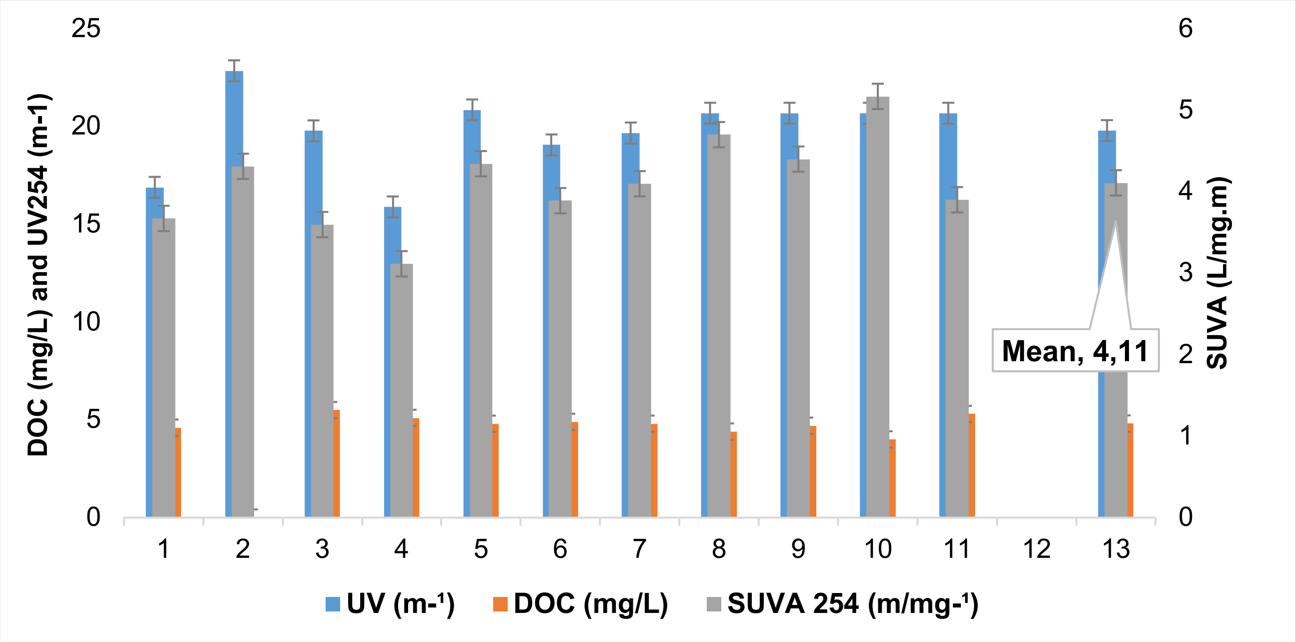

Figure 4-2: Bulk NOM and SUVA characterization of the raw water (A18)- Internal monitoring programme

Figures 4-3 to **4-5** and **Table 4-1** shows the interdependency of the bulk NOM characterization parameters and the Spearman correlation coefficient (ρ). The Spearman correlation coefficient does not assume any distribution about the data (non-parametric), and the correlation coefficient ranges between -1 and 1 (i.e. $-1 \leq \rho \leq 1$). For cases where ρ

=1 or ρ =-1, a perfect linear relationship exists (either positive or negative). Whereas ρ = ±5 implies a moderate relationship, ρ = ±7 is indicative of a strong relationship. A weak or no correlation is characterized by ρ value that is close to 0. The p-value (different from ρ) is used to test the significance of the relationship or correlation between the two parameters. The p-value can then be used to test a hypothesis, where a p-value closer to 0 means there is an insignificant relationship between the parameters and as the p-value approaches 1, it signifies an increase in the significance of the correlation. **Figures 4-3** to **4-5** scatter plots with 95% prediction eclipses, presenting selected variables from the bulk NOM monitoring programme. This also intends to highlight the interdependency of the bulk NOM parameters and how they relate. The prediction eclipse accounts for the random variation of the individual data sets and the uncertainty in estimating the mean. The rest of the relationships amongst the bulk NOM parameters are presented in **Table 4-1**.

The results (**Figure 4-3** and **Table 4-1**) shows a positive relationship between UV_{300} and UV_{254}, as evidenced by a positive correlation coefficient of 0.93. UV_{300}, which is often used as an operational parameter (Lobanga *et al*., 2014), and has also been used as an indirect measure of DOC (Nkambule, 2012). Furthermore, UV_{300} can be correlated to colour, nitrates, and aromatics hydrocarbons (Tethys, 2020; Ecotech, 2018)

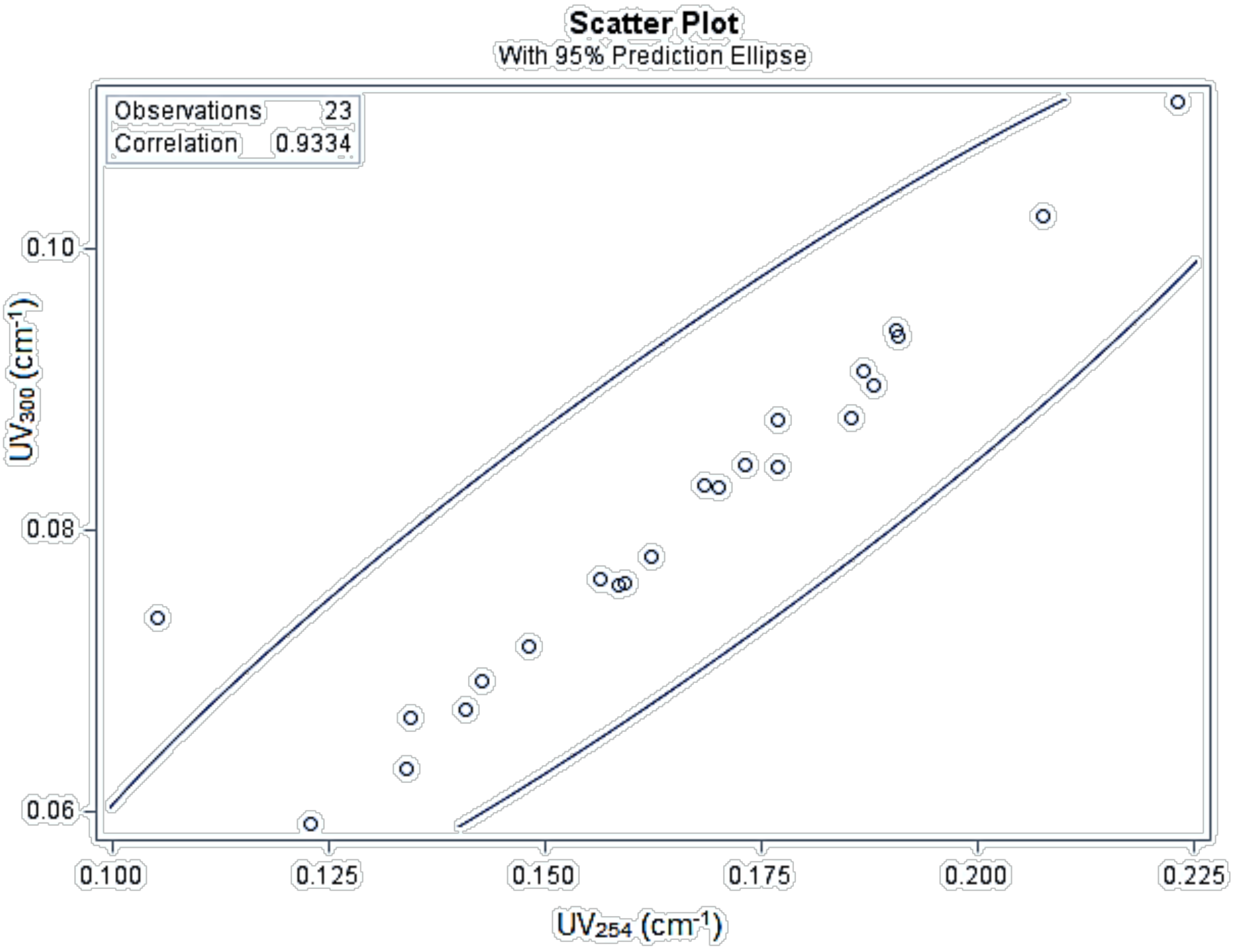

Figure 4-3: The correlation between UV_{254} and UV_{300}

Although dependent on the variations in the chromophores found in the NOM, the most reliable wavelengths in terms of NOM measurements are in the range 220 to 280 nm (Korshin *et al*. 2009). Constant results were obtained in a study conducted by Lobanga *et al*., (2013), where NOM removal by GAC adsorption was evaluated by measuring the UV-absorbance at three (3) different wavelengths (nm) (i.e. UV_{254}, UV_{272} and UV_{300}) and obtained consistent results. Due to this, it is not surprising that a good correlation (ρ =0.71) was observed between $SUVA_{254}$ and UV_{300} **(Figure 4-4)**.

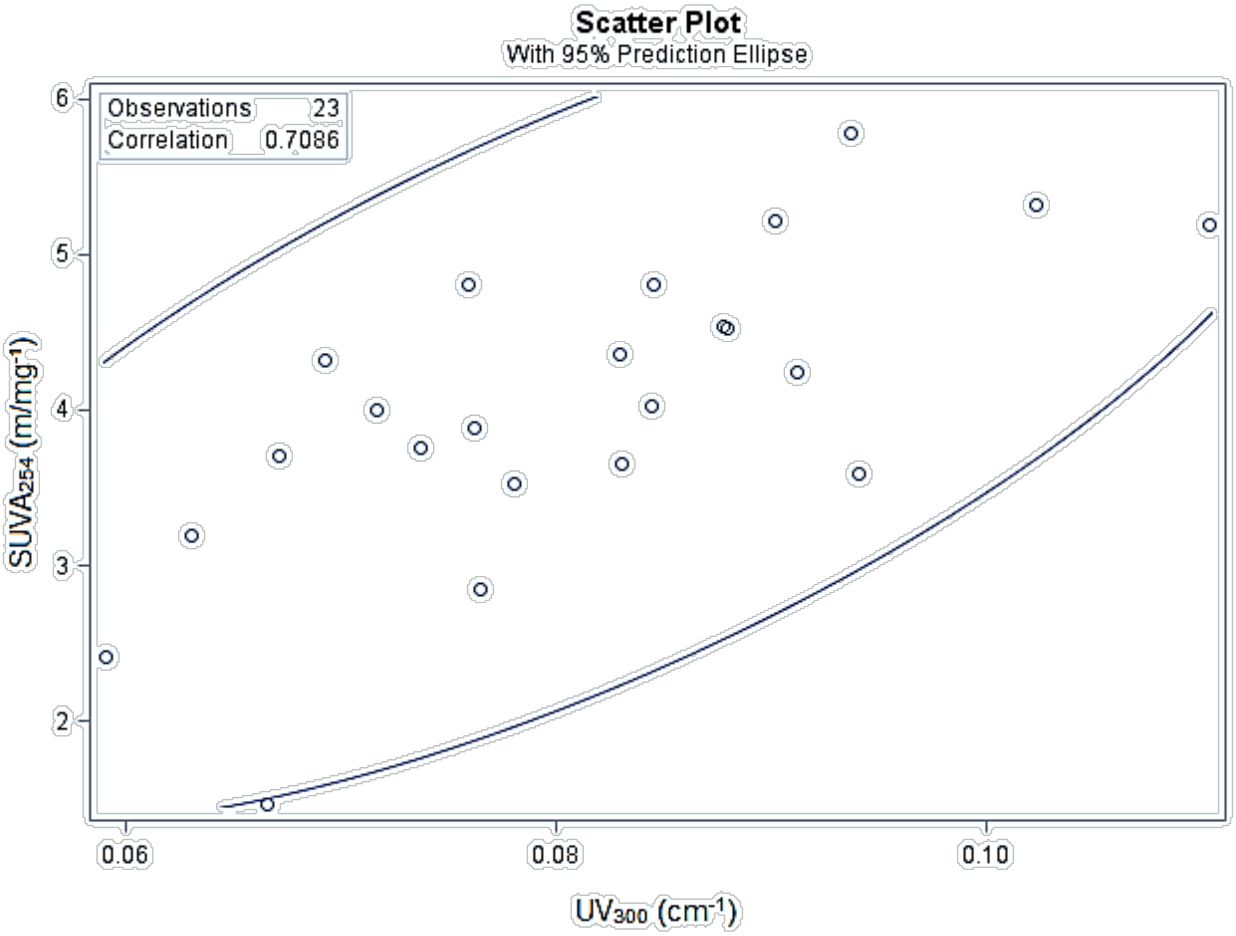

Figure 4-4: The correlation between SUVA$_{254}$ and UV$_{300}$

At ρ = 0.14, being the highest correlation coefficient recorded between turbidity and SUVA (**Figure 4-5**) or any other bulk NOM parameter in this study, turbidity is not a good indicator for NOM quantification. Turbidity has always been used as a surrogate parameter for total suspended solids, while UV and TOC or DOC are majorly used as NOM surrogates (Wattanachira *et al*., 2004). The poor correlation between turbidity and SUVA can be confirmed by the following removal efficiencies for NOM surrogates obtained from Wattanachira *et al*., (2004): 32% for DOC, 48 % for TOC, 47% for UV$_{254}$ and 98% for turbidity removal. A 98% removal for turbidity vs. a range of 32%-48% for all the other surrogates omits turbidity as a reliable surrogate for NOM quantification in water. More of the correlations on the bulk NOM parameters are presented in **Table 4-1** and **Appendix A**.

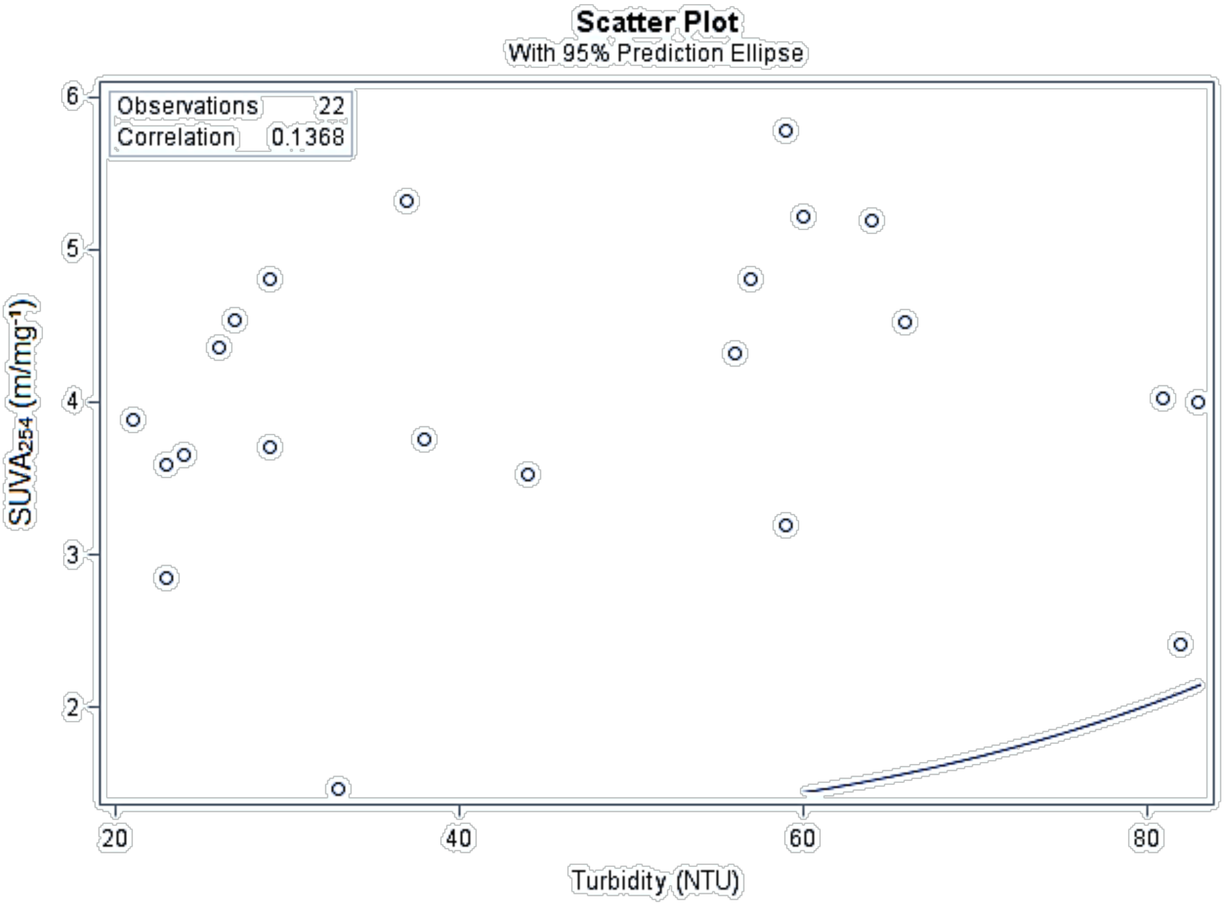

Figure 4-5: The correlation between $SUVA_{254}$ and turbidity

Table 4-1: Spearman correlation coefficients-Bulk NOM characterization parameters

BULK NOM PARAMETERS MONITORED		SUVA$_{254}$ (L/mg.m)	DOC	UV$_{254}$ (m^{-1})	Humic acids (mg/L)	UV$_{300}$ (m^{-1})	Turbidity (NTU)
SUVA$_{254}$ (L/mg.m)	Correlation coefficient (ρ)	1.00	-0.66	0.70	0.41	0.71	0.14
	p-value		0.00	0.00	0.06	0.00	0.33
	Number of observations	23.00	23.00	23.00	22.00	23.00	22.00
DOC	Correlation coefficient (ρ)	-0.66	1.00	0.00	-0.29	-0.01	-0.20
	p-value	0.00		1.00	0.18	0.95	0.37
	Number of observations	23.00	23.00	23.00	22.00	23.00	22.00
UV$_{254}$ (m^{-1})	Correlation coefficient (ρ)	0.70	0.00	1.00	0.35	0.97	-0.01
	p-value	0.00	1.00		0.11	<.0001	0.96
	Number of observations	23.00	23.00	23.00	22.00	23.00	22.00
Humic acids (mg/L)	Correlation coefficient (ρ)	0.41	-0.29	0.35	1.00	0.35	0.00
	p-value	0.06	0.18	0.11		0.11	0.99
	Number of observations	22.00	22.00	22.00	22.00	22.00	21.00
UV$_{300}$ (m^{-1})	Correlation coefficient (ρ)	0.71	-0.01	0.93	0.35	1.00	-0.08
	p-value	0.00	0.95	<.0001	0.11		0.73
	Number of observations	23.00	23.00	23.00	22.00	23.00	22.00
Turbidity (NTU)	Correlation coefficient (ρ)	0.14	-0.20	-0.01	0.00	-0.08	1.00
	p-value	0.33	0.37	0.96	0.99	0.73	
	Number of observations	22.00	22.00	22.00	21.00	22.00	22.00

4.2.2 SUVA characterization of treated water

The characterization of the treated water was undertaken during the period of November (2018) to September (2019) **(Figure 4-6)**. The average values of 3.90 mg/L and 7.53 m^{-1}

were recorded for DOC and UV_{254}, respectively. The calculated mean SUVA value of 2.04 L/mg.m indicates a large reduction in the NOM, particularly the Hpo fraction following treatment. Water samples with SUVA value ≤2 L/mg.m contain mainly non-humic matter, which is generally more Hpi than the other NOM fractions (Sillanpää *et al.*, 2015a). Hidayah *et al.* (2018) also reported that the coagulation process is effective in the removal of NOM, particularly the Hpo fraction that mainly constitutes humic substances. Coagulation in WTP is primarily used for turbidity removal, however, it is a very crucial process for the reduction of NOM from surface waters (Nottle, 2013; EPA, 2010). Generally, coagulation is considered as the most cost-effective process for the removal of NOM (Hidayah *et al.*, 2018). According to Kitis *et al*. (2001), SUVA values of treated waters are not easily reduced to levels below 1.5 L/mg.m when using conventional water treatment (coagulation). The low SUVA values obtained were as a result of the low UV_{254} and DOC concentrations found in the treated water (**Figure 4-6**). The effectiveness and efficiency of the WTP is directly linked to the levels of DBPs formed such as THMs. When operated optimally, the conventional process can reduce the DOC content by 30–60%, with preferential removal of the Hpo fraction (Ghernaout, 2014). According to EPA (2010), rapid sand filtration on its own is unable to fully remove NOM (THM precursor) and for complete removal, the process may be supplemented with other advanced processes.

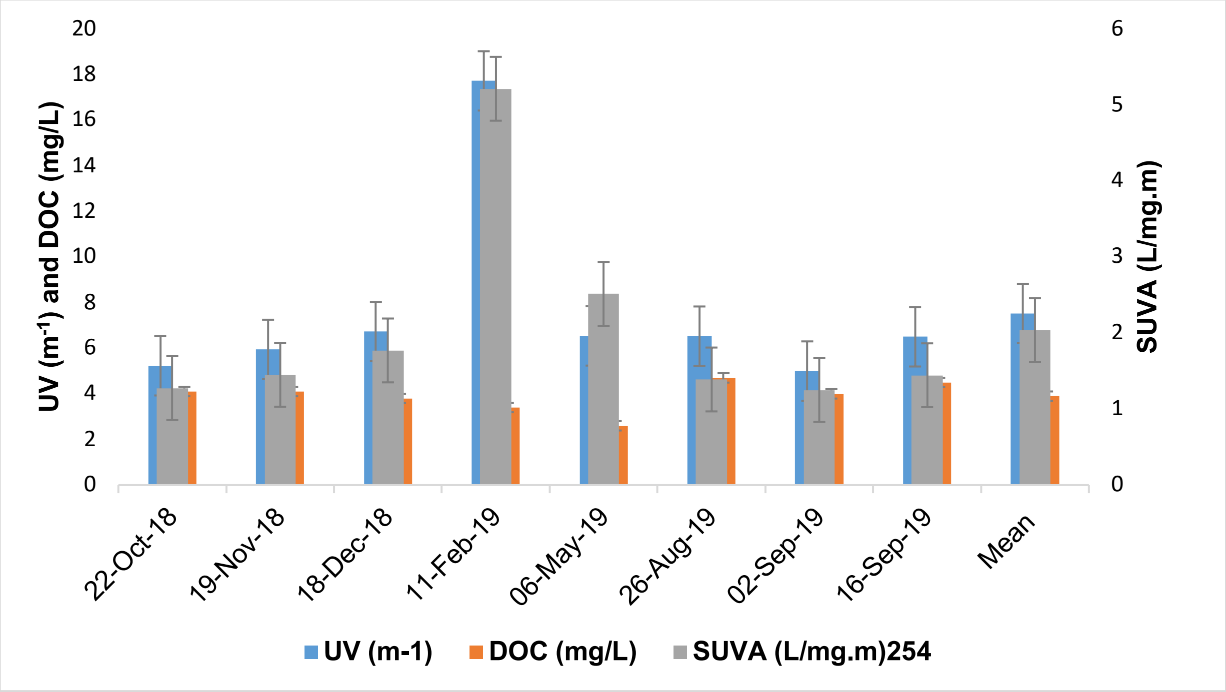

Figure 4-6: Character of the treated water between November 2018 and September 2019

4.2.3 The removal of NOM through the conventional water treatment processes

This section presents the results for bulk NOM removal, estimated using the two (2) surrogate parameters, the UV_{254} and DOC. Both the DOC and UV_{254} can give a measure of the aromatic content of NOM in water (Özdemir and Üngör, 2017). This was conducted to measure the efficiency of the conventional system towards the removal of NOM and to map the differences in the humic content between the raw and treated water. The removal is presented as the ratio of the UV_{254} or DOC on the NOM sample before and after treatment. Supplementary information on the removal of NOM and its fractions is presented in **Appendix B**.

4.2.3.1 Removal of bulk NOM- Ultraviolet absorbance at 254 nm (UV_{254})

The mean UV_{254} absorbance of the raw and treated waters was 20.04 m^{-1} and 7.67 m^{-1}, respectively. The UV_{254} removal was found to be a mean of 61.72%, indicating consistently and sufficiently removed light-absorbing substances (**Figure 4-7**). These results validate findings reported by Marais *et al*., (2018)., on the same conventional water treatment process where 61% UV_{254} removal was reported. According to Sharp *et al*., (2006), a typical removal between 10–60% is often attainable, solely using the coagulation-flocculation process, however, it can rise up to 90% dependent on the water treatment process, the quality of raw water and coagulation conditions. The reported NOM removal rate also compares fairly well with the previously reported rates of up to 75% for UV_{254} removal in a conventional water treatment process (Nkambule, 2012).

Water with high SUVA (i.e. 3.88 - 4.11 m/mg-¹), is indicative of a dominant high fraction of aquatic humic matter and composed mainly of the Hpo compounds. This is highly aromatic water and can be treated with ease (Teixeira and Nunes, 2016). The huge reduction in UV_{254} can also be explained by the reduction in treated water SUVA (i.e. 2.04 L/mg.m). There is a correlation between SUVA values and UV_{254} removal rates; high SUVA values of the raw water often implies high removal rates of UV_{254} (Teixeira and Nunes, 2016). The high rates (i.e. ≥50%) of UV removal indicate that the large aromatic portion of NOM in the water is preferentially removed by the conventional process. The lowest removal of UV_{254} (14.31%), which could also be considered an outlier for UV removal, was observed during winter (the month of August) (**Figure 4-7**). The UV_{254} removal rate was at its peak (75.82%) during spring (the month of September) (**Figure 4-7**). The low UV_{254} indicates a reduction in the content of the aromatic substance in the water, thereby potentially reducing the THMFP of the water (Szerzyna *et al.*, 2017).

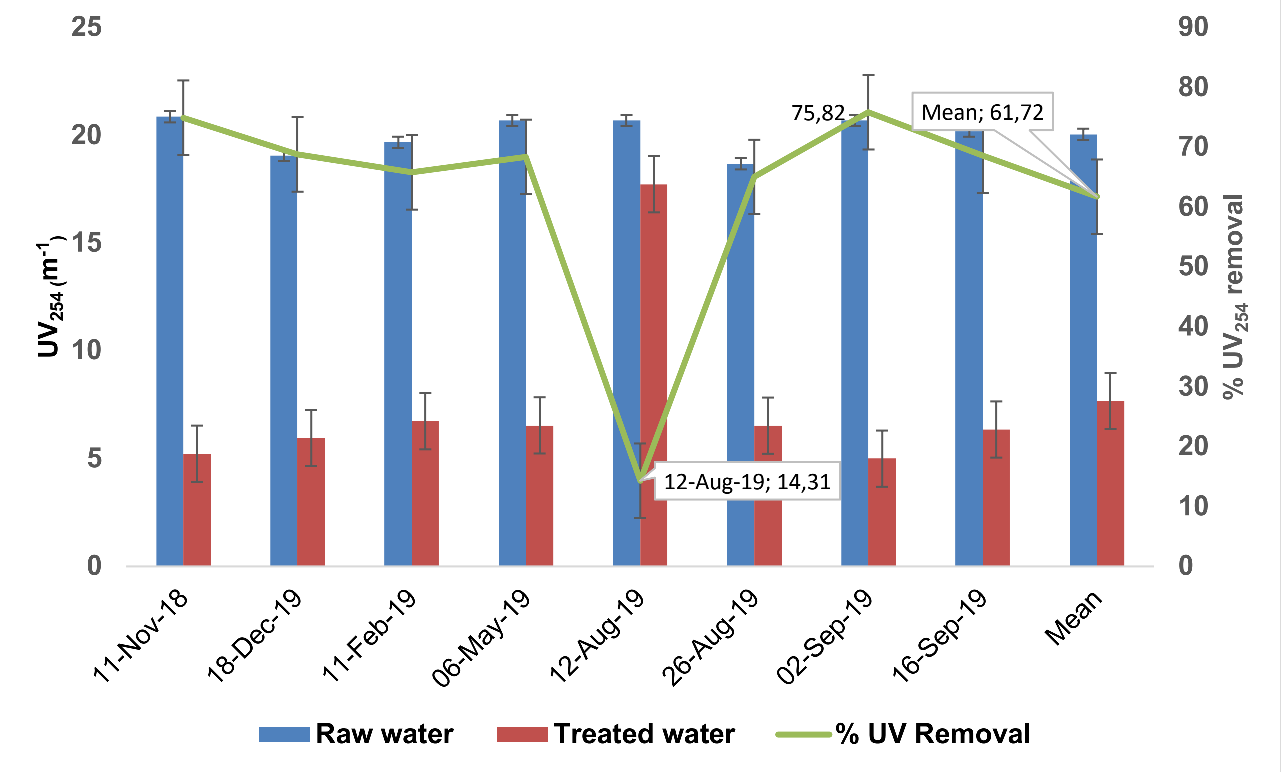

Figure 4-7: Efficiency of the WTP for the removal of UV_{254} from raw water to treated water

4.2.3.2 Removal of bulk NOM- dissolved organic carbon (DOC)

Figure 4-8 shows the reduction of DOC between the period November (2018) and September (2019), and respective mean DOC removal values of 4.7 and 3.9 mg/L were achieved for raw and treated water. In terms of DOC, the calculated NOM removal was 16.78% (**Figure 4-8**). Similar DOC reductions (up to 15% by conventional coagulation were reported by Nkambule (2012). Typically, the highest levels of DOC in raw water result in the highest percentage of DOC removal (US. Geological Survey, 2013). On the contrary, the opposite was observed for the finished water (i.e. high DOC levels gave low DOC removal percentages). Some studies have found a good correlation between the DOC treatability and SUVA of water (Weishaar *et al*., 2003), while others have found results contrary to this notion (Shutova *et al*., 2014). With a mean of 16.78 % in the reduction of DOC, the results are in agreement with studies opposing the existence of a high correlation between SUVA (i.e. 3.88 L/mg.m-4.11 L/mg.m) and DOC treatability. However, with a mean DOC of 4.7 mg/L (**Figure 4-8**) in the treated water, the concentration is still acceptable, as the World Health Organization (WHO) standards prescribe 5 mg/L as the maximum allowable DOC levels in drinking water. On the other hand, the maximum allowable DOC level set by the South African National Standards (SABS, 2015) is 10 mg/L. The results indicate that even with the low removal efficiency of the conventional water treatment plant in DOC removal, the DOC levels were still within the permissible limits for drinking water purposes throughout the study period.

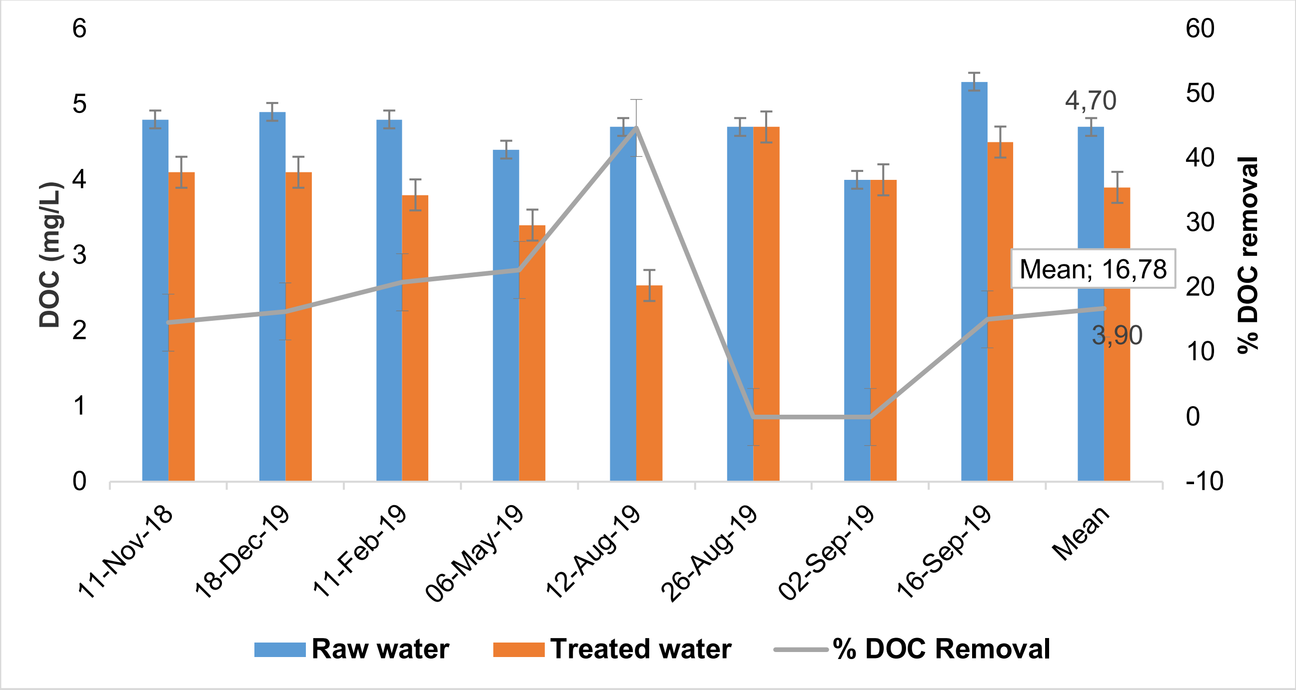

Figure 4-8: Efficiency of the WTP for the removal of DOC from raw water to treated water

4.3 m-PRAM classification of the various NOM fractions in raw and treated water

4.3.1 m-PRAM classification and UV_{254} characterization of raw water

The m-PRAM fractionation of the raw and treated waters resulted in the isolation of three (3) polarity fractions of NOM, the hydrophilic (Hpi), transphilic (Tpi), and hydrophobic (Hpo) fractions as shown in **figures 4-9 and 4-10** respectively. The distribution of the NOM fractions in the source water was as follows: Hpo > Hpi > Tpi fractions, with mean UV_{254} values of; 17.74 m^{-1}, 15.65 m^{-1} and 5.26 m^{-1}, respectively **(Figure 4-9)**. Even though the Hpo fraction is the most dominant fraction, the m-PRAM showed an almost equal distribution of the Hpo and Hpi fractions on the Vaal Dam, A18 raw water source (**Figure 4-9**). Historically, the UV_{254} values in the A18 raw water source ranged between 12 m^{-1} and 99.3 m^{-1}, between 2010 and 2018, with an average of 35.4 m^{-1} on the year 2017/2018 (Marais *et al*., 2018). The results obtained in this study show that there were no major changes between 2017 and 2019 in the NOM concentrations as the UV_{254} was constantly below 21 m-^{1}. Other studies have reported UV_{254} as low as 9.5 m^{-1} to a maximum of 19 m^{-1} (Özdemir and Üngör, 2017) and others a wider range between 16 m^{-1} and 78 m^{-1} (De Oliveira *et al*., 2018).

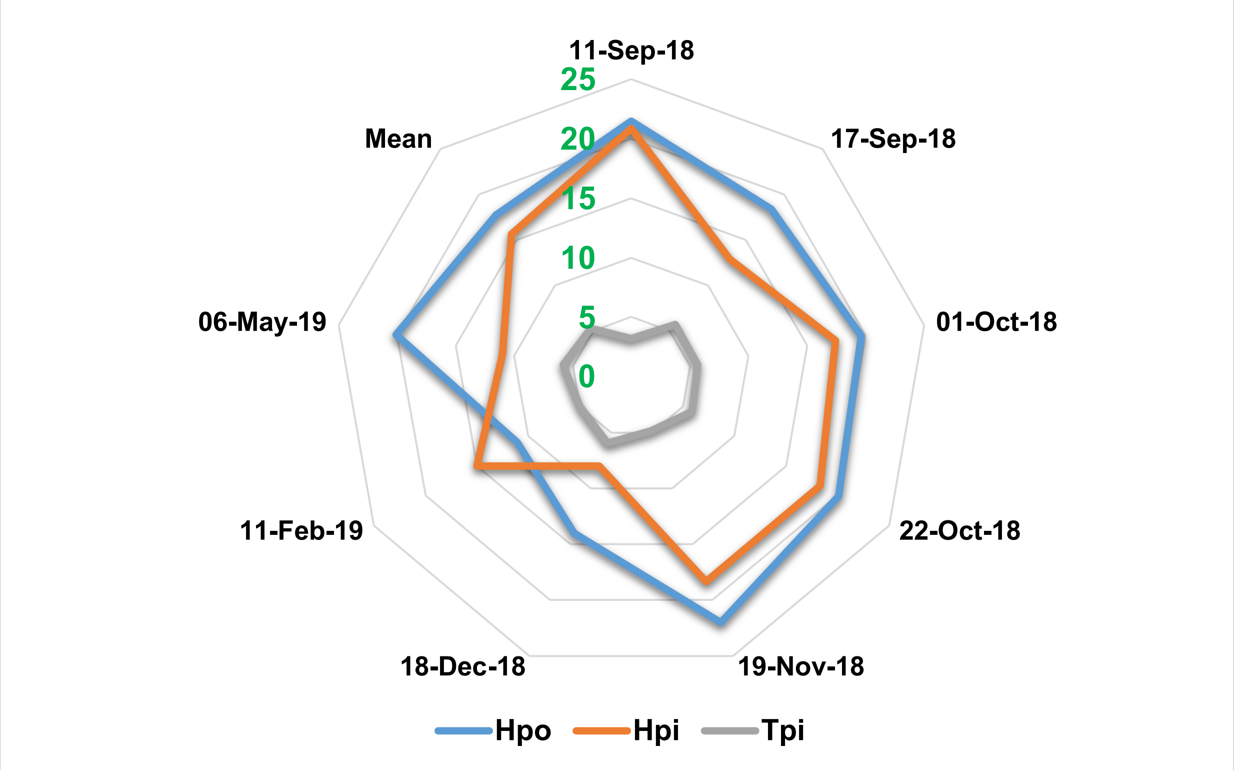

Figure 4-9: m-PRAM fractionation and UV_{254} characterization of the fractions in raw water

4.3.2 m-PRAM classification and UV_{254} characterization of treated water

The UV_{254} (m^{-1}) measurements in the treated water for the Hpo, Hpi and Tpi fractions were 7.39 m^{-1}, 6.46 m^{-1} and 4.58 m^{-1} (**Figure 4-10**), respectively, indicating a substantial removal (>58%) of aromatic NOM within the source water (Sillanpää *et al.*, 2015a). This is also evident in **figure 4-10**, as the most dominant fraction in terms of UV_{254} in the treated water was now the Hpi fraction, confirming that greater removal by conventional clarification was indeed on the Hpo fraction. The reduction in the UV_{254} measurements indicates a relationship between the refractive substance content and the concentrations of organic substances responsible for the colour of the water. The water samples generally exhibited low UV_{254} values, thus suggesting that the samples may be of low humic substances content. There is a direct and positive correlation between the UV absorbance in the raw water and the removal efficiency. The higher the UV on the NOM fractions on raw water, the higher the removal percentage observed on the treated effluent (**Figure 4-9** vs **4-10**). A positive correlation between SUVA and UV_{254} removal rates was confirmed by Teixeira and Nunes (2016); higher SUVA values of the raw water result in high UV_{254} removal as also observed in this study. The removal of the individual fractions is further discussed in the following **section 4.3.3.**

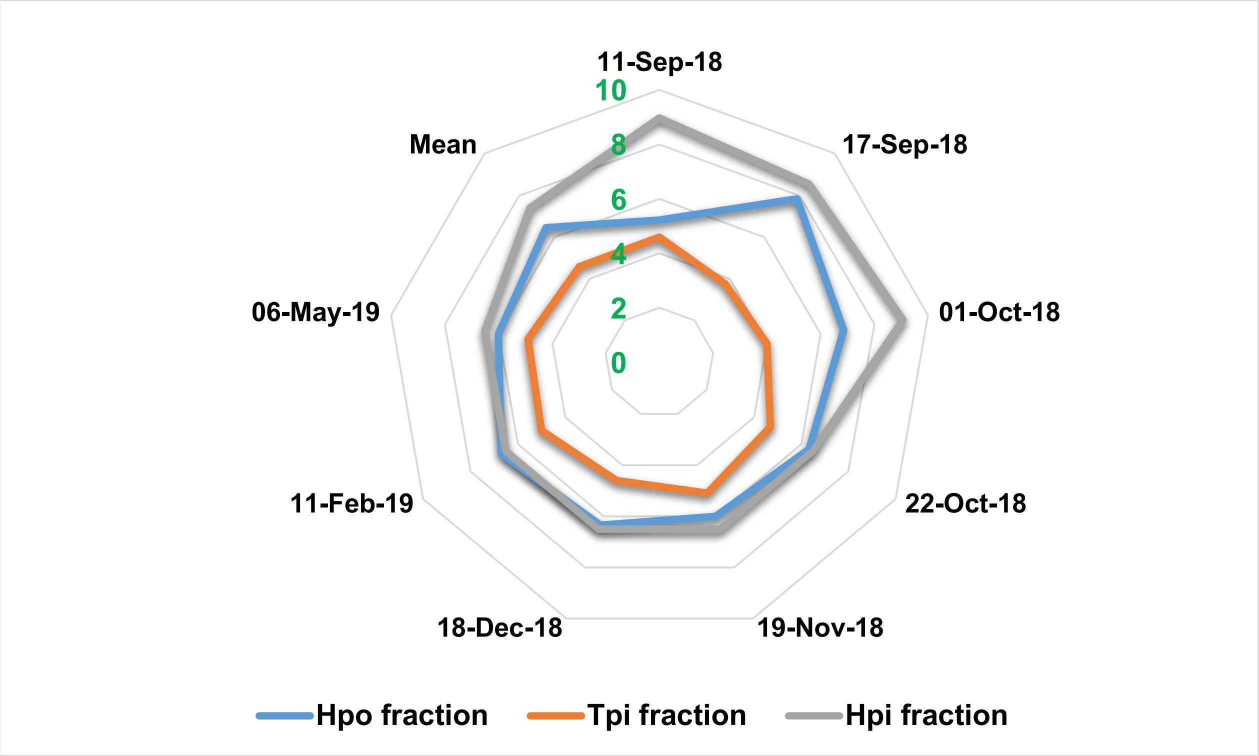

Figure 4-10: m-PRAM fractionation and UV_{254} characterization of the fractions in treated water.

4.3.3 Removal of the individual NOM fractions using conventional water treatment processes

4.3.3.1 Removal of NOM polarity fractions- Ultraviolet absorbance at 254 nm (UV_{254})

The UV_{254} removal for each of the fractions (i.e. the Hpi, Tpi and Hpo) was calculated as the ratio of the UV_{254} on each fraction before and after treatment (**Figure 4-11**). The highest removal efficiency of the WTP amongst the fractions was observed on the Hpo fraction, with a 58.72% reduction, while the Tpi and Hpi NOM fractions were reduced by 13.06 % and 58.33% respectively (**Figure 4-11**). The Hpo fraction mainly consists of highly insoluble humic and Fulvic acids which are difficult to dissolve in water, thereby allowing for relatively easy removal by conventional clarification processes. The concentration of NOM was found to decrease by between 48 % and 76 % at the different conventional WTPs, where the NOM was predominantly characterised of HMW NOM (Krzeminski *et al.,* 2019). The higher content of acidic functional groups on the Hpi fraction is difficult to destabilise by the coagulation process, it is expected that the Hpi fraction will have the least removal by normal coagulation. Also, the Hpi fraction has a high affinity for water and primarily comprises of low molecular weight (LMW) carbohydrates, proteins and amino acids. However, the results emanating from this study suggest that although not equal to the removal of the Hpo fraction, almost similar UV_{254} removal was also observed for the Hpi fraction. Limited amounts of the Tpi fraction removal was achieved by the conventional treatment processes (**Figure 4-11**). An almost similar trend was observed by Nkambule (2012), where nanomaterials were used for the removal of NOM fractions.

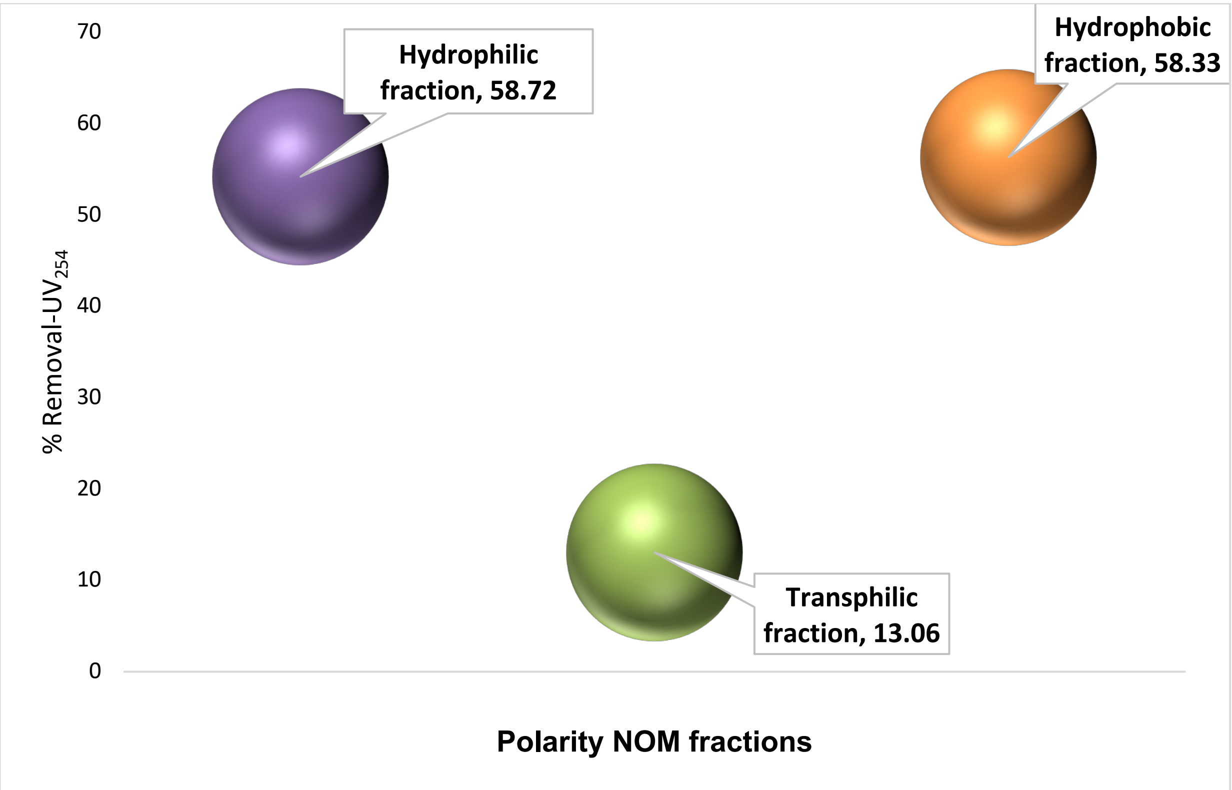

Figure 4-11: % Removal of the various NOM fractions by conventional processes- UV absorbance

4.3.3.2 Removal of NOM polarity fractions- dissolved organic carbon (DOC)

As previously reported, the mean removal of the bulk DOC from the raw water was 16.78%. Aligned to reports by Matilainen *et al*., (2010) and Hidayah *et al*., (2018) etc., which reported higher removals for the Hpo fraction of NOM, the highest removal in DOC content amongst the various NOM fractions was observed on the Hpo NOM fraction, with a mean DOC removal of 19.95% (**Figure 4-12**). These studies reported that HMW NOM fractions are easily removed by coagulation and clarification process. However, it must be noted that these studies were only considering an overall removal, not specifically looking at removals of each fraction by DOC. Furthermore, even though there may be exceptions, DOC measurements in isolation cannot give detailed insight into the evolution of NOM within the treatment process, hence the use of other surrogate parameters such as UV_{254} (Haarhoff *et al*., 2010; Chow *et al*, 2004a etc.).

The second highest DOC removal by fraction was observed on the Hpi fraction with a mean DOC removal of 6.17% (**Figure 4-12**). The removal of the Hpi fraction was expected to be poor by conventional coagulation processes, and as a result, this fraction dominates in treated water (Sharp *et al*., 2006). A number of studies (Zhan *et al*., 2010; Ghernaout *et al*., 2009; Uyak and Toroz, 2006) attribute the difficulty to remove this fraction to its transformation from a soluble state to an insoluble state during treatment. The removal of the intermediate molecular weight Tpi fraction was found to be less than both the Hpo and Hpi fractions, as denoted by a mean of 3.57% (**Figure 4-12**). According to Sarpola (2013), the removal of the Tpi and Hpi fractions is lower than the Hpo fraction, owing to their high sensitivity to the coagulation pH. Dhaouadi *et al*. (2013) reported a higher THMFP due to the presence of Tpi NOM fraction, not the Hpo NOM fraction. This is because the Tpi fraction has richer and more active functional groups than the Hpo fraction (Dhaouadi *et al*., 2013). This suggests that the removal of Hpo and the Tpi compounds during water treatment should be given priority as there is a higher potential for the generation of brominated compounds.

Parsons *et al.* (2004) stated that high levels of DOC in the raw water result in high DOC removal rates. However, in this study, a clear relationship could not be established between the DOC in the raw water and the rate of DOC removal for all the NOM fractions. Nkambule (2012) reported DOC reductions of up to 15% using the coagulation process, whereas other studies reported DOC removal rates of between 30% and 60% using the same process (Ghernaout, 2014; Hansen *et al.,* 2018). The overall interpretation is that the DOC removal is low, regardless of which fraction. As previously reported in **Section 4.2.1 (SUVA characterization of raw water),** the mean SUVA values obtained from two different and independent monitoring programs ranged between 3.88 L/mg.m and 4.11 L/mg.m. This can be attributed to moderately higher SUVA values achieved. This is supported by numerous studies where due to SUVA values ≤4 L/mg.m, the removal of DOC by conventional clarification was low (Ghernaout, 2014; Edzwald and Tobiason, 1999; Edzwald, 1993).

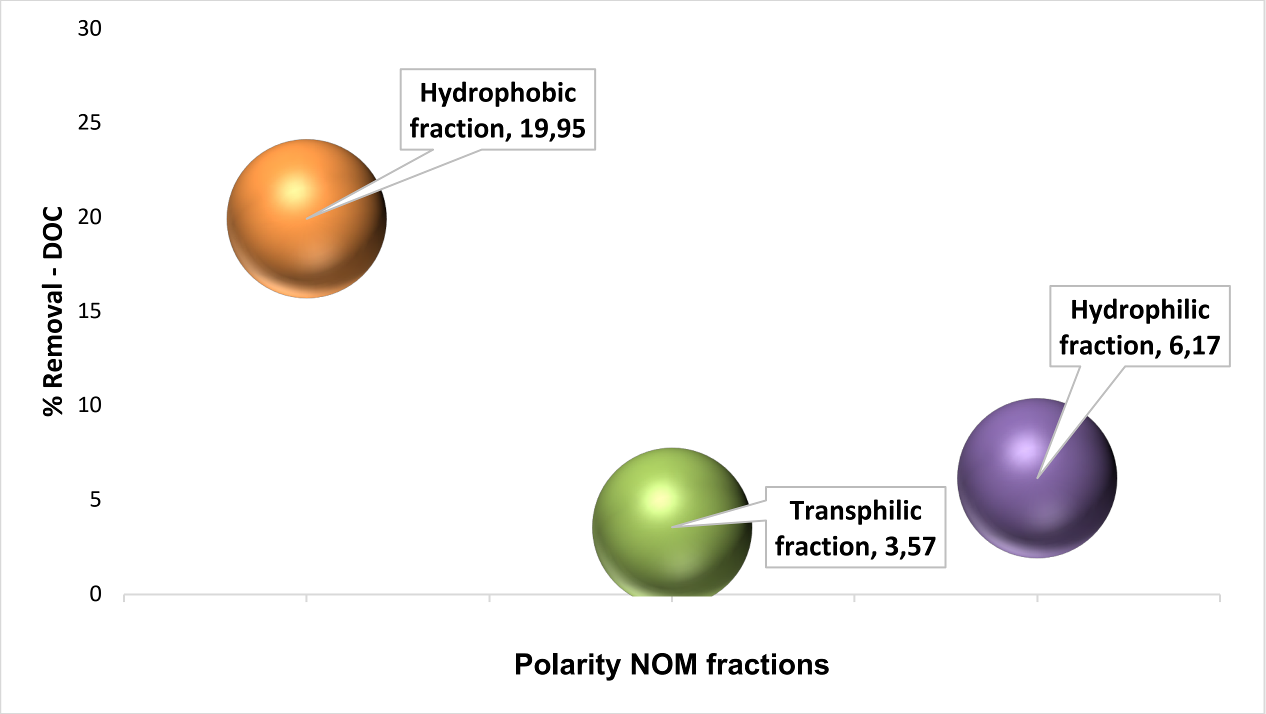

Figure 4-12: Removal of the various NOM fractions by conventional processes- dissolved organic carbon (DOC)

4.4 Conclusions

In many ways, NOM can affect the efficiency of a water treatment plant and the quality of final drinking water with issues such as bacterial regrowth and the formation of DBPs. Sufficiently reduced NOM during treatment is therefore essential, and this requires a better understanding of the character of the NOM in question. This chapter focused on the characterization of NOM within the Rand Water conventional treatment process using bulk NOM parameters (i.e. DOC and UV_{254}). The isolation of the NOM into various fractions and then assessing their removals through the conventional water treatment plant also formed a pivotal component of this chapter.

The characterization of NOM using the conventional SUVA method indicates the aromaticity of the NOM and helps in predicting NOM removal. However, the aromaticity is only an attribute to define the NOM composition and not the actual concentrations of the humic and non-humic substances. Applying advanced NOM isolation techniques, such as the m-PRAM helps in by providing a further discernment of the NOM, thereby allowing for further characterization of the different NOM fractions. With SUVA values ranging between 3.88 L/mg.m and 4.11 L/mg.m, and the even dispensation in UV_{254} between the Hpo and Hpi fraction obtained through the m-PRAM, the characterization revealed that the raw water is a combination of both the Hpi and Hpo NOM, a mixture of non-humic and aquatic humic compounds. Continuous and effective characterization of the NOM will lead to the improvement of its removal efficiency. The use of advanced NOM isolation and characterization tools also lead to an informed outlook on the contribution and role by each of the various NOM fractions, particularly with regards to removal efficiencies of the system, coagulant dose and demand, disinfectant requirements etc. In terms of removal, the conventional treatment process was fairly effective in the reduction on NOM, particularly the HMW Hpo fraction of NOM. An efficient reduction of the Hpo fraction will minimize the DBP formation potential in the treated water and throughout the distribution network. Determining the exact removal rates for each of the fractions at different stages of the treatment processes in a WTP helps in focusing more on NOM control and removal.

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CHAPTER 5: THE BIODEGRADABILITY OF VARIOUS NOM FRACTIONS AND THEIR INFLUENCE ON BACTERIAL REGROWTH AND THMFP

5.1 The relationship between NOM and microbe populations in water

An elemental attribute that governs the growth of all microorganisms is the presence of carbon or nutrients. To strive, heterotrophic bacteria relies on the availability of nutrients and energy; they, therefore, utilize organic carbon as a source (Prest *et al*., 2016). The intrusion of allochthonous DOM in aquatic environments impacts the concentrations of macronutrients and thereby providing energy and nutrients to microorganisms. Apart from the presence of AOC, which is a subgroup of biodegradable NOM, there are more complex biodegradable compounds of critical importance for the biological stability of the water (Hijnen *et al*., 2014; Kwon *et al*., 2005). These can be easily quantified by the BDOC, which can be applied to various fractions of NOM. Not only does the BDOC indicate which fractions have the potential to induce bacterial regrowth in the distribution system, but also the fractions which are responsible for DBPs formation. If DOC is not sufficiently removed through drinking water treatment processes it combines with chlorine to form DBPs such as THMs, a probable carcinogen (Li and Mitch, 2018). To sufficiently improve and optimise these processes, the isolation and quantification of BDOC by the various NOM fractions is essential. Therefore, the chapter aimed at evaluating the biodegradability of the various NOM fractions and their resulting potential to form DBPs (i.e. THMs).

5.2 The biodegradability of NOM

To ensure consistency, the temperature was kept constant at ±20˚C for both the inoculation of BAS and the BDOC experiment, thereby eliminating thermal degradation of the DOC. The results presented in **Table 5-1** serves as confirmation that the sand (inoculum) was indeed biologically active sand (BAS). The sodium acetate solutions of 5 mg/L or 10 mg/L were used interchangeably as controls for the NOM fractions throughout the experimentation. The mean % BDOC achieved for the $NaCH_3$ solutions was 42.23 and 50.87 for the $NaCH_3$ solutions of 5 mg/L and 10 mg/L, respectively. The BDOC ranged from 28.57% to 56.36% and 11.82% to 92.93% for the $NaCH_3$ solutions of 5 mg/L and 10 mg/L, respectively. The huge standard deviations as shown in **Table 5-1** are acceptable as they confirm the

Part of the work presented in this chapter has been submitted for publication to the Journal of Water Process Engineering, 21 February 2020 variability of the BAS from one experiment to the other. Studies such as Simon *et al*. (2013) and Yang (2012) have reported higher biodegradation rates for sodium acetate under aerobic conditions. The high biodegradability of sodium acetate ascertains its use as a control in biodegradation studies. Supplementary information on the biodegradability of NOM and the various fractions is presented in **Appendix C**.

Table 5-1: Biodegradability potential of the sodium acetate control samples

Biodegradation test no	**% biodegradable dissolved organic carbon (BDOC)- NACH$_3$ control samples**	
	NaCH$_3$ (5 mg/L)	**NaCH$_3$ (10 mg/L)**
1.	56.36	11.82
2.	42.86	86.73
3.	50.00	41.67
4.	42.86	92.93
5.	28.57	77.50
6.	33.33	32.50
7.	-	12.94
Min	**28.57**	**11.82**
Mean	**42.33**	**50.87**
Max	**56.36**	**92.93**
Ơ	**10.26**	**34.52**
N	**6**	**7**

5.2.1 Raw water- BDOC on the various NOM fractions

Table 5-2 show the biodegradation on the raw water NOM fractions evaluated over a period of 4 days. **Figure 5-1** is a graphical presentation of the tabulated results including the biodegradation of sodium acetate The biodegradation of the various NOM fraction is of the order Hpi >Tpi > Hpo **(Table 5-2; Figure 5-1)**. The BDOC minimum, mean and maximum percentages for the Hpi fraction were 5.56%, 38.07% mg/L and 78.26% respectively (**Table 5-2**). The non-humic fraction, LMW Hpi fraction has been reported to be highly biodegradable thus making a substantial contribution to bacterial regrowth (Karnik *et al*., 2005; Andersson, 2001). A comparative analysis of the biodegradation rates of the Hpi fraction (**Table 5-2 and Figure 5-1**) and the Tpi and Hpo fractions has revealed a superior biodegradation rate associated with the Hpi fraction. The Tpi NOM fraction demonstrated a biodegradation rate that is in between those of the Hpi and Hpo fractions (**Table 5-2** and **Figure 5-1**). The following data were obtained for the Tpi fraction: a minimum BDOC percentage of 7.07%, a mean of 29.22% and a maximum of 64.67% (**Table 5-2**). The Tpi NOM fraction consists mainly of humics and LMW acids. The Tpi fraction has been reported to be more biodegradable than the Hpo and Hpi fractions (Andersson, 2001).

Furthermore, the results reported herein indicate that the Hpo fraction is not easily biodegradable (**Table 5-2** and **Figure 5-1)**. Due to its low affinity for water, the Hpo fraction is less soluble and easily removed from the water. This makes the Hpo fraction less available for biological activity. According to Karnik *et al.* (2005), the humic fraction of the NOM is less biodegradable but exhibits a high potential for the formation of THMs. This is in agreement with the results as shown in **Figure 5-1** in terms of biodegradability.

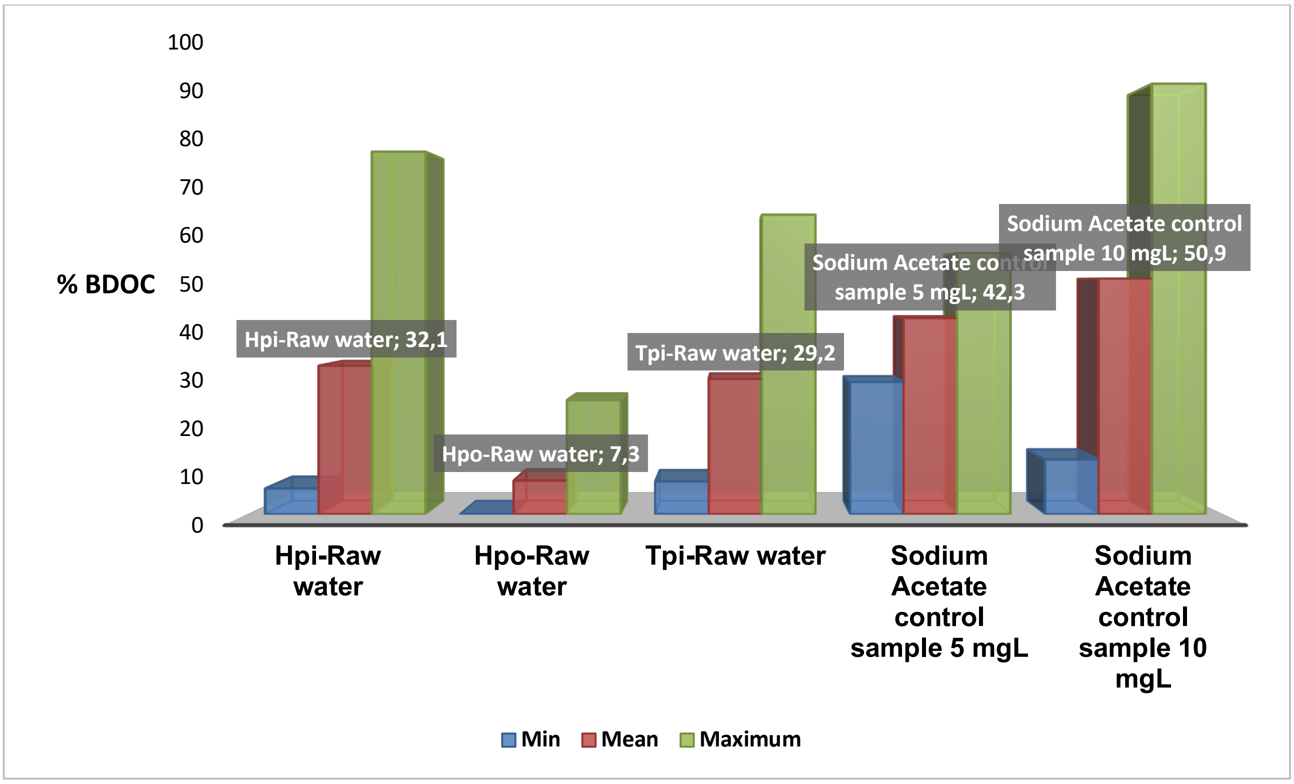

Figure 5-1: Mean percentage BDOC of the individual NOM fractions and sodium acetate control samples

Table 5-2: The biodegradability of raw water NOM fractions

Biodegradation test no	**% Biodegradable dissolved organic carbon (BDOC) by fractions**		
	Hpi-fraction	**Tpi-fraction**	**Hpo-fraction**
1.	32.90	61.02	0.00
2.	17.74	23.75	8.06
3.	19.23	64.67	0.00
4.	78.26	19.17	0.00
5.	67.69	14.29	0.00
6.	53.13	14.29	3.57
7.	20.00	38.46	24.64
8.	25.00	35.38	23.53
9.	9.76	7.07	20.00
10.	23.53	22.50	0.00
11.	5.56	20.78	0.00
Min	**5.56**	**7.07**	**0.00**
Mean	**32.07**	**29.22**	**7.25**
Max	**78.26**	**64.67**	**24.64**
Ơ	**23.85**	**18.91**	**10.29**
N	**11**	**11**	**11**

5.2.2 Treated water- BDOC on the various NOM fractions

Table 5-3 show biodegradation on the treated water NOM fractions obtained through the m-PRAM. The biodegradation of the various NOM fraction occurring in the treated water was of the order Hpi>Tpi>Hpo **(Table 3; Figure 5-2).**

The BDOC minimum, mean and maximum percentages for the Hpi fraction were 11.27%, 35.22% and 88.65% respectively (**Table 5-3**). As previously reported, the non-humic fraction (i.e. Hpi) of NOM is typically highly biodegradable than the humic and Fulvic acid fraction (i.e. Hpo); thus promoting bacterial regrowth in the water supply systems (Karnik *et al*., 2005). The Hpi fraction is easily dissolved in water and is composed mainly of low LMW carbohydrates, amino acids etc. Its high solubility in water renders it difficult to remove by simple coagulation. It, therefore, forms a huge component of the bio-available fraction of NOM which can easily be degraded by heterotrophic bacteria. As demonstrated by

Andersson (2001), the Tpi NOM fraction occurring in the treated water, which is mainly composed of humics and LMW acids, have higher biodegradation rates relative to the Hpi and Hpo fractions (**Figure 5-2**). The minimum, mean and maximum BDOC percentage values obtained for the Tpi fraction was found to be 3.00%, 35.75%, and 81.67%, respectively (**Table 5-3**).

Throughout the course of the experimentation, the Hpo fraction was continuously characterised by low BDOC rates (**Table 5-3** and **Figure 5-2)**. The low BDOC rates result from the low affinity of the Hpo to water, thus rendering this fraction not easily or slowly biodegradable (Karnik *et al*., 2005). The low biodegradability potential of Hpo suggests that this fraction exhibits a high potential for the formation of THMs. The BDOC provides an estimate of the biodegradable fraction of any sample to the non-biodegradable dissolved organic carbon (NBDOC) content of the same sample. BDOC is also indicative of the potential of bacterial regrowth, the biostability of the water and disinfection requirements in distribution systems (Vital *et al.*, 2010).

Table 5-3: The biodegradability of treated water NOM fractions

Biodegradation test no	**% Biodegradable dissolved organic carbon (BDOC) by the fractions**		
	Hpi-fraction	**Tpi-fraction**	**Hpo-fraction**
1.	50.65	13.33	0.00
2.	40.00	3.00	0.00
3.	15.09	21.43	24.14
4.	19.05	33.64	0.00
5.	41.94	81.67	60.00
6.	11.27	53.33	5.11
7.	88.65	67.50	21.82
8.	15.15	12.09	0.00
Min	**11.27**	**3.00**	**0.00**
Mean	**35.22**	**35.75**	**13.88**
Max	**88.65**	**81.67**	**60.00**
Ơ	**26.20**	**28.71**	**21.19**
N	**8**	**8**	**8**

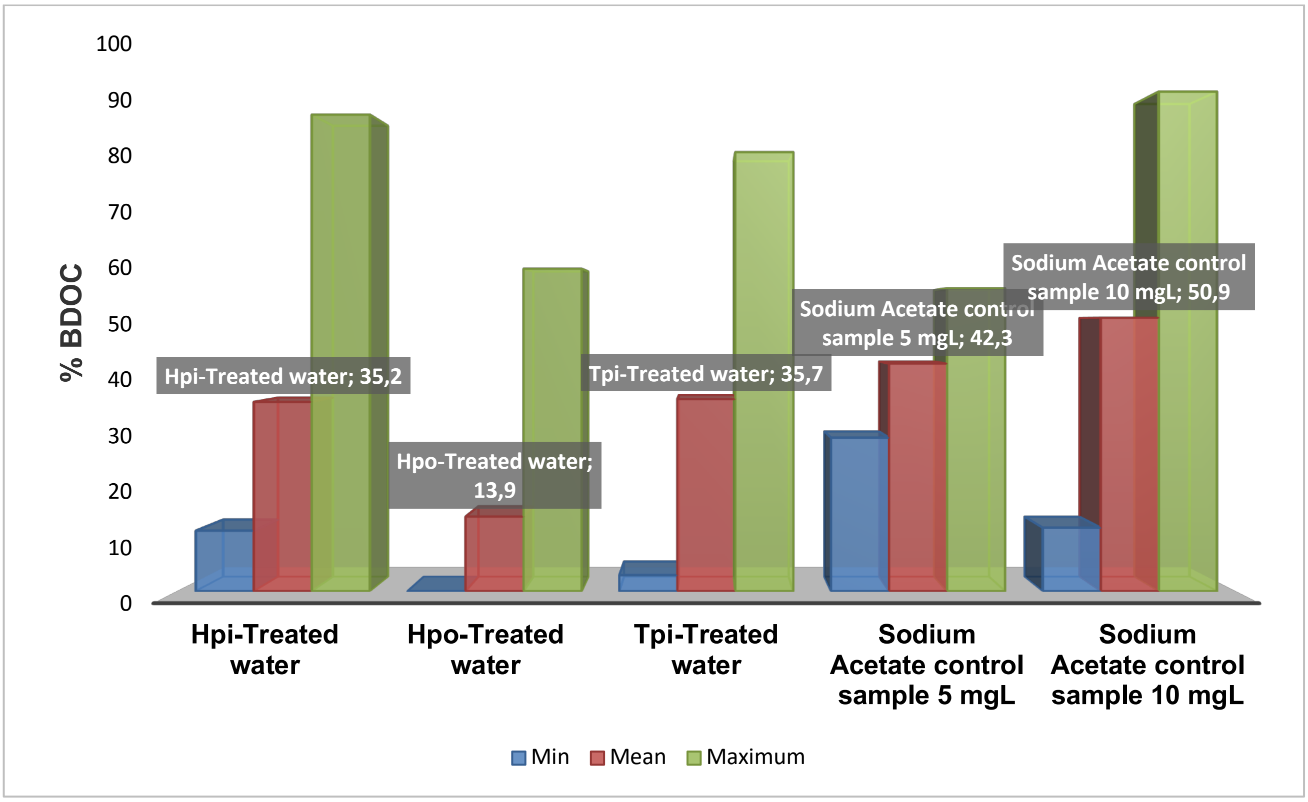

Figure 5-2: Mean BDOC percentage of the individual NOM fractions and sodium acetate control samples

The following relationships can respectively depict the biodegradability of the raw and treated water fractions; Hpi>Tpi> Hpo and Tpi ≥ Hpi> Hpo (**Figure 5-3**). Only a fraction of the DOC is biodegradable, as evidenced by the obtained BDOC ranging between 7.25% and 35.75% across all the NOM fractions. The least biodegradable fraction is the Hpo fraction in both the raw and filtered waters; the Hpi and Tpi showed similar BDOC traits. The observed BDOC percentages for all the fractions investigated is in agreement with those of other studies (e.g. Vital *et al*., 2010; Van der Kooij, 2002), whereby up to 44% of the DOC was reported to be readily available for bacterial growth. Results generated herein are accepted as a true reflection of the biodegradability potential for the A18 raw water source and the treated effluent from the Rand Water Treatment Plant, due to the following reasons:

(i) Auto decomposition and photodegradation were successfully eliminated as potential contributors to the degradation of DOC. Photochemical processes can lead to rapid DOC losses of up to 30% within a period of 2 weeks (Mann *et al*., 2012).

(ii) The temperature was well regulated and kept constant throughout the experimentation phase; therefore, impacts associated with varying temperatures were eliminated.

(iii) In addition, all other interferences such as the availability of DOC in the BAS and nutrients availability were accounted for and correctly regulated.

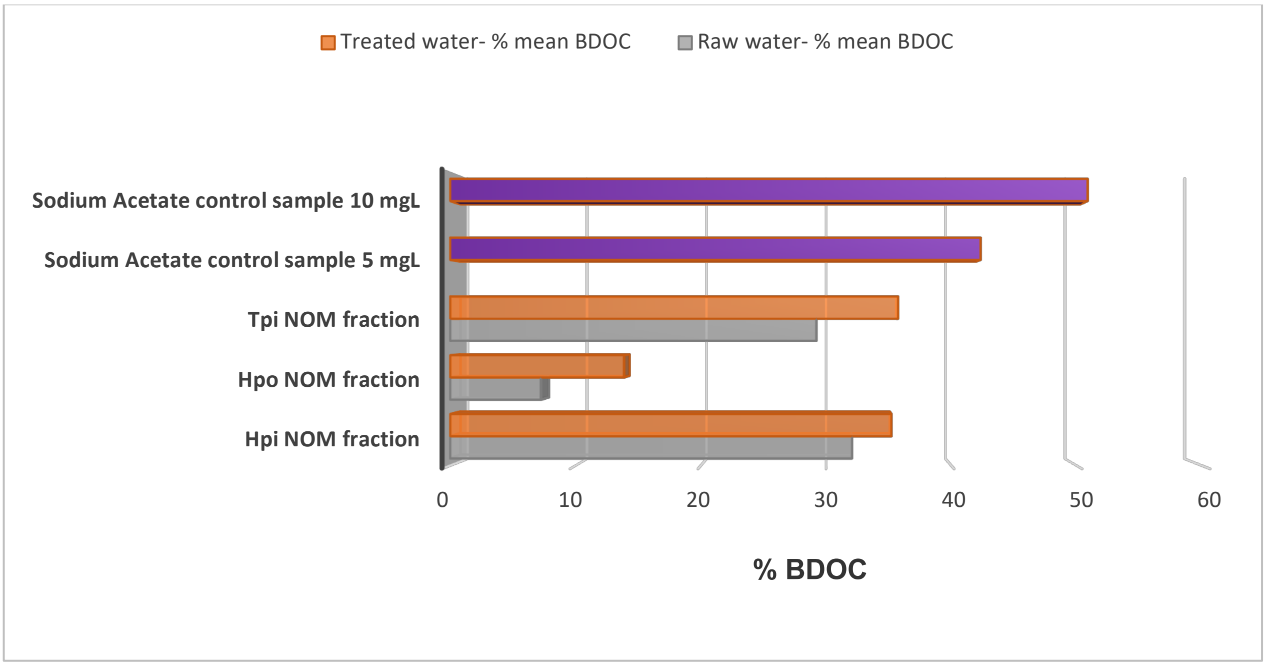

Figure 5-3: Overall BDOC trends on the various fractions in both raw and treated water

5.3 The effect of biodegradable NOM on bacterial regrowth

The BRP specific to the individual fractions was calculated as the difference between the initial and the final concentration of HPC after the 4-day BDOC measurements. For bacterial growth to be considered as positive growth, a minimum increase of 1 x 10^3 cells mL^{-1} was required, otherwise, the growth was considered insignificant; hence the elimination of the treated water results as they were constantly characterised of low HPC, before and after the BDOC. Total coliforms results are also not presented in this section as they also showed very little or negative growth in both the raw and treated water. The detection of relatively low amounts of bacterial cells in treated water samples does not constitute a health hazard since such low levels of bacteria fall within acceptable limits and standards (Hammes *et al.*, 2008; Hoefel *et al.*, 2005).

Results reported herein are expressed as net growth in heterotrophic plate count (HPC) (BRP) and % growth (**Table 5-4** and **Figure 5-4**). DOC and HPC were monitored as two complementary parameters in order to gain more information into the relationship between the bacteria and their utilization of NOM or the various fractions of NOM (i.e. Hpo, Hpi and Tpi) in water. As previously reported, the most biodegradable NOM fraction in raw water is the Hpi fraction followed by the Tpi fraction; then the Hpo fraction is the least biodegradable fraction. A similar trend was observed for the BRP in raw water, whereby the order of contribution to bacterial regrowth by the NOM fractions in raw water was as follows: Hpi > Tpi > Hpo fraction **(Figure 5-4)**. The raw water contained 14.5 x 10^3 cfu/mL and 55.0 x 10^3 cfu/mL of HPC before and after the BDOC, respectively, indicating a BRP of 40.5 x 10^3 cfu/mL (**Figure 5-4** and **Table 5-4**). Amongst the NOM fractions, the Hpi had the highest BRP of 197.4 x 10^3 cfu/mL. The intermediate MW NOM fraction, the Tpi showed an intermediate BRP to that of the other two fractions (i.e. Hpi and Hpo) and was characterized of a BRP of 121.4 x 10^3 cfu/mL (**Table 5-4)**. The lowest BRP of 2 x 10^3 cfu/mL was observed on the Hpo fraction of NOM (**Table 5-4**). A good correlation denoted by an R^2= 0.96, between BRP and BDOC was established (**Table 5-4**). Falkinham *et al. (*2001), found a correlation coefficient (R^2) of 0.64 between BRP and BDOC, however, this was on overall BDOC in a NOM sample and not specific to certain NOM fractions. The higher correlation further serves as a validation for the existence of a close link between BDOC and bacterial regrowth in water (Prest *et al*., 2016).

Potable WDS are generally infiltrated by heterotrophs which utilise BDOC as a source of carbon and nutrients (Servais *et al*., 1992). Conducive environments with ample BDOC allow other opportunistic potential pathogens (e.g., *Legionella*) and other micro-organisms to proliferate. This bacterial biomass production becomes the start of a complex food chain which may alter the biostability of potable water. According to Church *et al*, (2000), bacterial regrowth was found to favourably replicate when subjected to both glucose and amino acids, indicating that bacterial growth may be closely correlated with the Hpi fraction of NOM. This was confirmed by the findings in this study, as the results indicate that bacterial regrowth responds well to the organic enrichment of the Hpi and partly the Tpi fractions of NOM. If DOC is greatly reduced during treatment, particularly the Hpi and Tpi fractions of NOM, the BRP can be lessened in water supply systems. The positive correlation allows for the prediction of the BRP in a water sample using the BDOC of each of the NOM fractions.

Table 5-4: The influence of BDOC on BRP of the various NOM fractions

NOM fractions	HPC (cfu/mL)		BRP	% increase (HPC)	Relationship between BRP and % BDOC	
	Before BDOC	After BDOC				
Hpi fraction	10600	208000	197400	1862	197400	32.07
Tpi fraction	19600	141000	121400	619	121400	29.22
Hpo fraction	40000	42000	2000	4.5	2000	7.22
The correlation coefficient (R^2) between BDOC and BRP (n=9)					0.96	

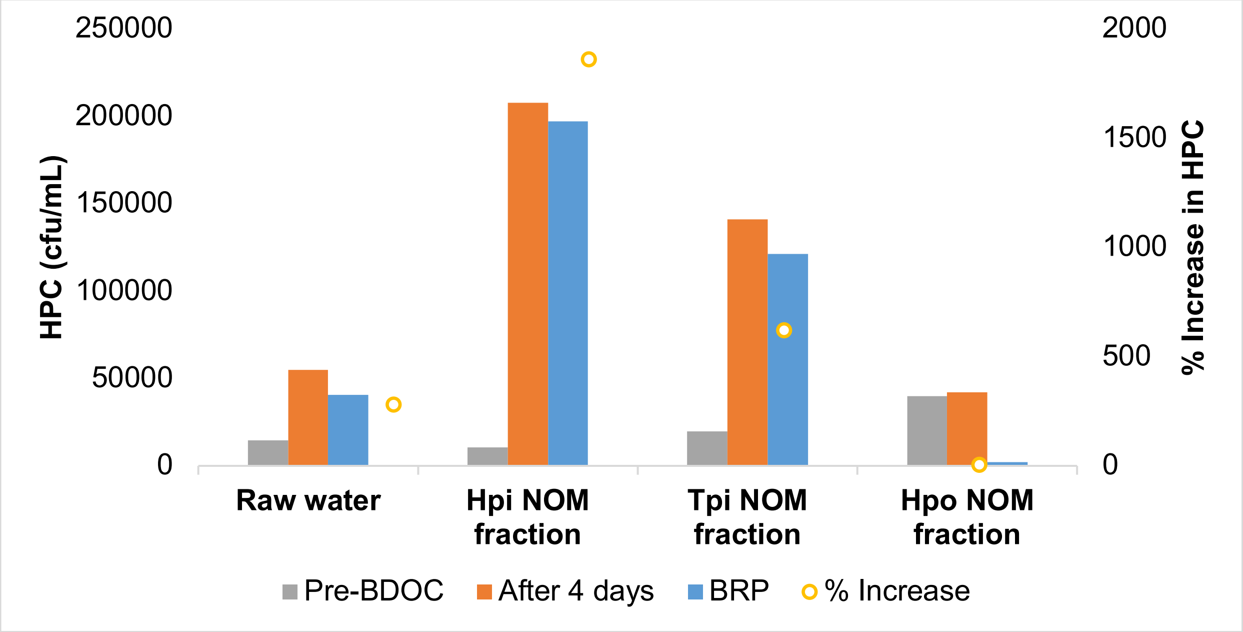

Figure 5-4: The effect of BDOC on bacterial regrowth in various NOM fractions - Raw water

5.4 The impact of various NOM fractions on the formation potential of trihalomethanes

5.4.1 THMs formation within the Rand Water distribution network

Total trihalomethane (TTHM) consists of a combination of four THM species, namely: dibromochloromethane ($CHBr_2Cl$), bromoform ($CHBr_3$), bromodichloromethane ($CHBrCl_2$), and chloroform ($CHCl_3$). All of the four THM species were monitored along the selected line in the distribution system; that is, the Vereeniging to Zuikerbosch booster station (M_A6, D_DA6, D_ZK_A6). This is a single pipeline system that commences just after disinfection from Vereeniging WTP to the booster station, with M_A6 immediately after disinfection.

While D_DA6 is situated at about 5 km from the point of disinfection, D_ZK_A6 is situated just before the chloramination process at Zwartkopjies.

Data collected between August 2018 and September 2019, which shows the occurrence and distribution of the various THM species within the distribution pipeline, is presented in **Figure 5-5**. The data was collected with the aim of gaining insights into the formation of THMs on the network. At the M_A6 sampling point, only bromodichloromethane ($CHBrCl_2$) appears to be consistently formed. The other THM species only appear to have formed at the 5 km mark. Once formed, THMs show persistence and continue to increase within the distribution system. THMs have been reported to persist in water supplies for ±65 days with chloroform being the most persistent species (Fakour and Lo, 2018; Pavelic *et al.*, 2005; Morrison and O'Sullivan, 2014). The SANS 241:2015 standard requires that the ratio of TTHM should not exceed 1. The THM ratio is calculated from all the THM species as fractions of their acceptable limits in drinking water. The acceptable limits for $CHBr_2Cl$, $CHBr_3$, $CHBrCl_2$ and $CHCl_3$ are ≤100µg/L, ≤100µg/L, ≤60µg/L and ≤300µg/L, respectively. The Vereeniging to Zwartkopjies booster station (M_A6, D_DA6, D_ZK_A6) pipeline is continuously in compliance with the requirements specified by SANS 241 for drinking water (**Figure 5-5**).

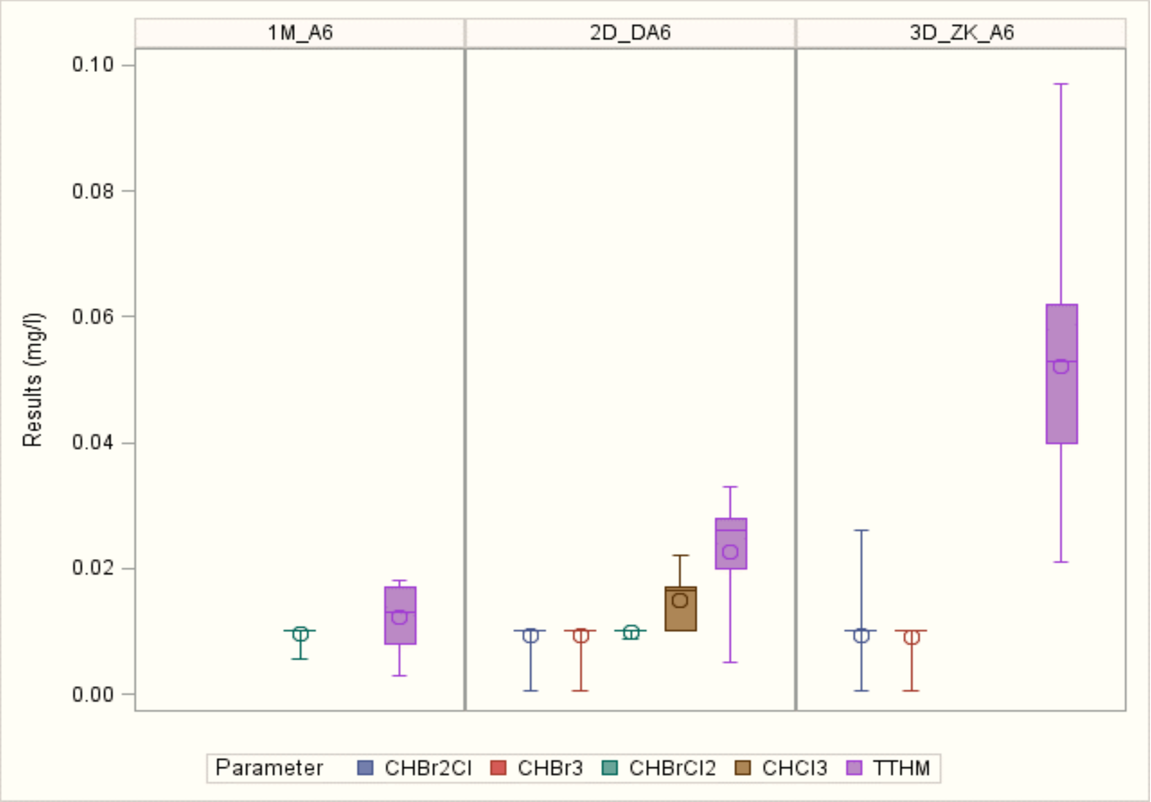

Figure 5-5: The formation of THMs and its species within the Rand Water distribution network

5.4.2 The formation potential (FP) of various THMs species by various NOM fractions

The mean SUVA values for the raw and treated water and their respective fractions are presented in **Table 5-5**. The SUVA results reaffirm what has already been mentioned in **Chapter 4** of this report. The NOM in the raw water is characterized by the huge presence of the Hpo NOM fraction. The high content of aromatic organics in the raw water can be interpreted as a greater tendency of the NOM to react with disinfectants and ultimately form DBPs. However, the low SUVA values of the treated water point to the efficiency of the water treatment processes towards the removal of NOM. Not only does the transformation of the NOM distribution through the water treatment train indicate NOM removal and efficiency, the SUVA of the NOM and its fractions can also be utilized to ascertain the impact of each fraction on the formation potential of DBPs (Fakour and Lo, 2018).

Table 5-5: Mean SUVA for the various NOM fractions-THMFP assessment

Sample ID	DOC	UV_{254}	SUVA (L/mg.m)
Raw water	**4.60**	**0.2202**	**4.79**
Hpi	5.90	0.2263	3.84
Tpi	11.00	0.0815	0.74
Hpo	5.90	0.2745	4.65
Treated water	**4.1**	**0.0648**	**1.58**
Hpi	5	0.0625	1.25
Tpi	10	0.0407	0.41
Hpo	5.7	0.1100	1.93

Figures 5-6 to **5-9** gives an account of the formation potential (FP) of the various THMs species by various NOM fractions. During the 7-day period, only bromodichloroform (**Figures 5-6** and **Figure 5-7**) and chloroform (**Figures 5-8** and **Figure 5-9**) were detected in the raw and treated water samples respectively. The TTHM ratio (**Figure 5-8**) was calculated as a fraction of the detected bromodichloroform and chloroform relative to their acceptable limits in drinking water.

The formation potential for bromodichloroform is low, whether in raw, treated water NOM samples or their respective NOM fractions **(Figures 5-6** to **5-7)**. Both raw and treated water NOM samples had an initial and final concentration of 10 µg/L and 21 µg/L for bromodichloroform, resulting in a formation potential of 11 µg/L. The Hpi fraction in the raw and treated water respectively had formation potentials of 14 µg/L and 11 µg/L. On the other hand, the Tpi fraction had 0 µg/L and 6 µg/L as formation potentials for the respective raw and treated waters. Lastly, the Hpo fraction had a relatively higher formation potential for both the raw and treated water as denoted by 16 µg/L and 13 µg/L respectively **(Figures 5-6** to **5-7)**. The overall contribution to the formation potential of bromodichloroform by each NOM fraction was in the order; Hpo>Hpi>Tpi (**Figures 5-6** and **5-7**). The formation of bromodichloroform in chlorinated water highly depends on the presence of bromide in the raw water and typically, raw waters contain low bromide levels; hence the low levels in bromodichloroform formation (Ristoiu *et al*., 2009). Similar observations where bromodichloroform levels were constantly low were made in a study where the water samples were collected from nine (9) different Districts and monitored for THM concentrations (Budziak and Carasek, 2007). In relation to the local acceptable limit (i.e. ≤60 µg/L) of bromodichloroform in drinking water as per SANS 241:2015 requirements (SABS, 2015), the formation potential of this compound is low.

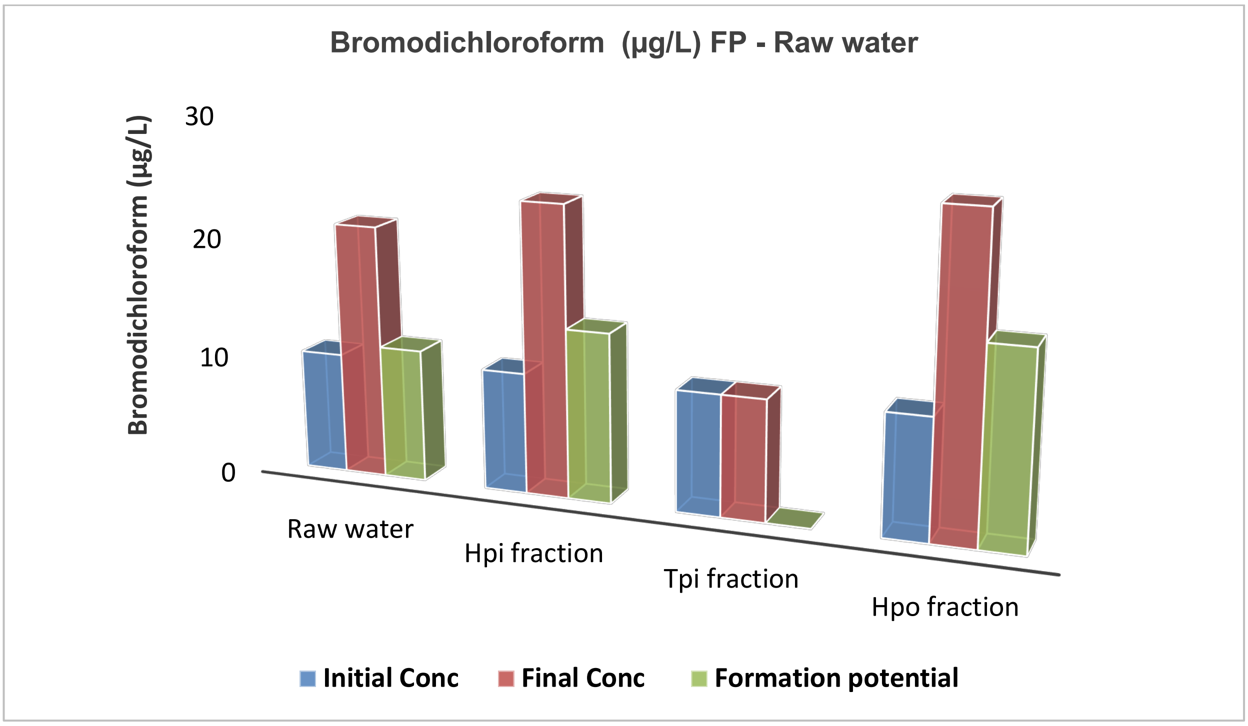

Figure 5-6: The formation of Bromodichloroform and the various NOM fractions in raw water

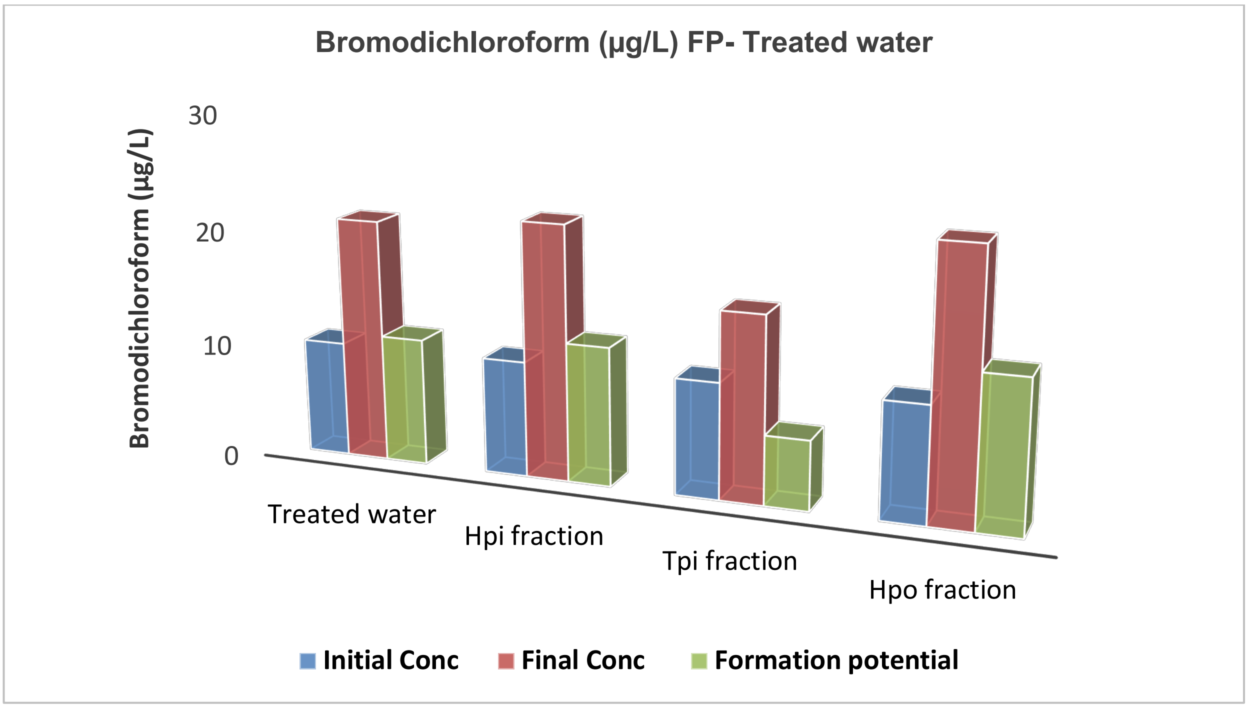

Figure 5-7: The formation of Bromodichloroform and the various NOM fractions in treated water

With regards to chloroform formation, the formation is very high when compared to the other TTHM species (i.e. $CHBr_2Cl$, $CHBr_3$, $CHBrCl_2$) as the overall formation potential ranged between 195 – 708 ug/L and 290 – 611 ug/L for the raw water and treated water respectively (**Figures 5-8** to **5-9**). The raw water NOM sample had 490 µg/L and 475 µg/L as the maximum recorded concentration and increase (formation potential) respectively, whilst the treated water recorded a maximum and an increase (formation potential) of 630 µg/L and 611 µg/L respectively (**Figures 5-8** to **5-9**). In relation to the other TTHMs species, chloroform is the most dominant. The contribution to the formation potential of chloroform by each of the NOM fractions, in both raw and treated water was in the order; Hpo>Hpi>Tpi (**Figures 5-8** to **5-9**).

The Hpi fraction in the raw and treated water respectively had formation potentials of 392 µg/L and 383 µg/L, whilst the Tpi fraction had the least formation potentials for the raw and treated water as respectively denoted by 195 µg/L and 290 µg/L. A major contribution to the formation of chloroform in both raw and treated water was observed through the Hpo fraction which respectively resulted in formation potentials of 475 µg/L. and 420 µg/l. According to Ristoiu *et al*., (2009), the formation of chloroform does not depend on the presence of bromide in water, therefore even at low concentrations of bromide chloroform can still optimally form. The contribution to the formation of chloroform by each NOM fraction was in the order; Hpo>Hpi>Tpi (**Figures 5-8** and **5-9**). These results are aligned to findings in various studies (Wang *et al*., 2013; Chen *et al*. 2008) where the Hpo fraction of NOM was found to be the major precursor to chloroform formation. The Hpo fraction has a higher aromatic content, UV absorbance and phenolic acidity which results in a higher halogenated organics-formation potential (Wang *et al*., 2013; Chen *et al*. 2008). Of all the TTHMs species, chloroform has been found to be the most abundant (Budziak and Carasek, 2007). In relation to the acceptable limit (i.e. ≤≤300 µg/L) of chloroform in drinking water as per SANS 241:2015 requirements (SABS, 2015), the formation potential of this compound is extremely high. In the presence of a higher chlorine residual, chloroform may easily form and exceed the acceptable limit. As previously stated chloroform can persist for ±65 days within the distribution system, therefore careful operation is required when controlling DBPs, particularly the chloroform formation (Fakour and Lo, 2018; Pavelic *et al*., 2005; Morrison and O’Sullivan, 2014).

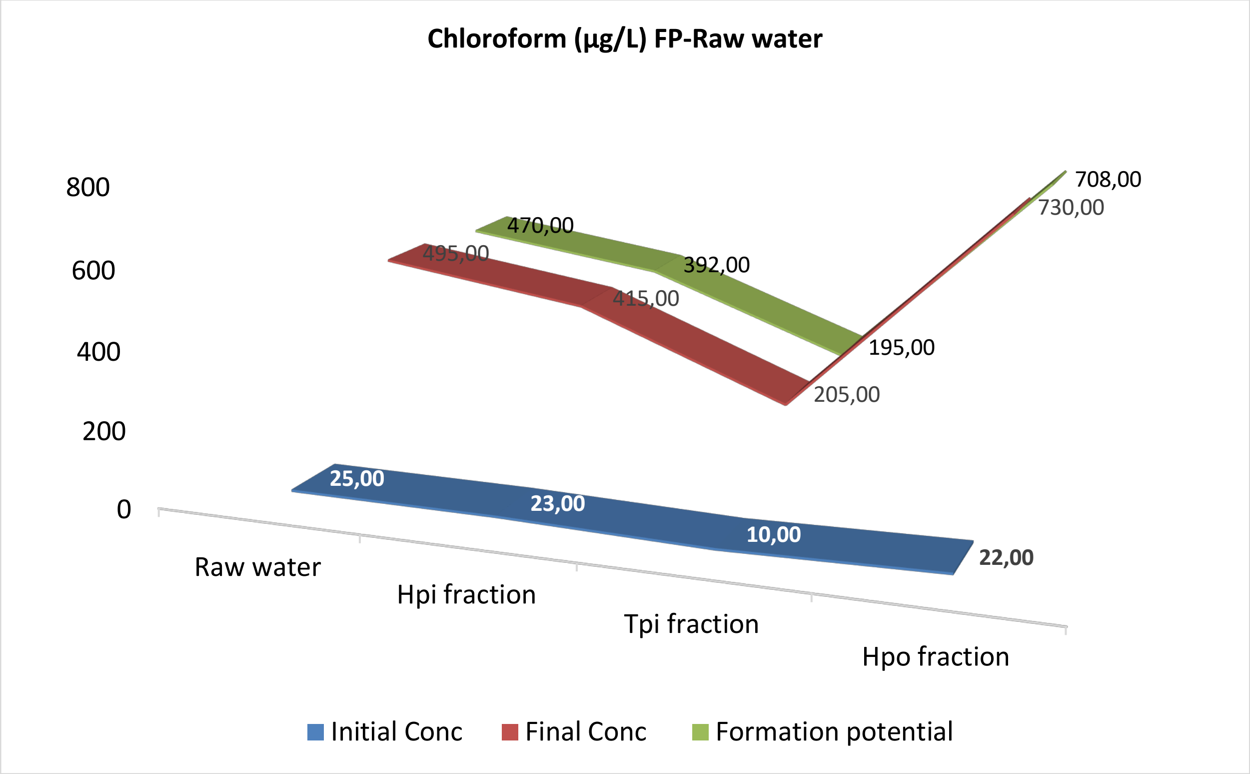

Figure 5-8: The formation of Chloroform and the various NOM fractions in raw water

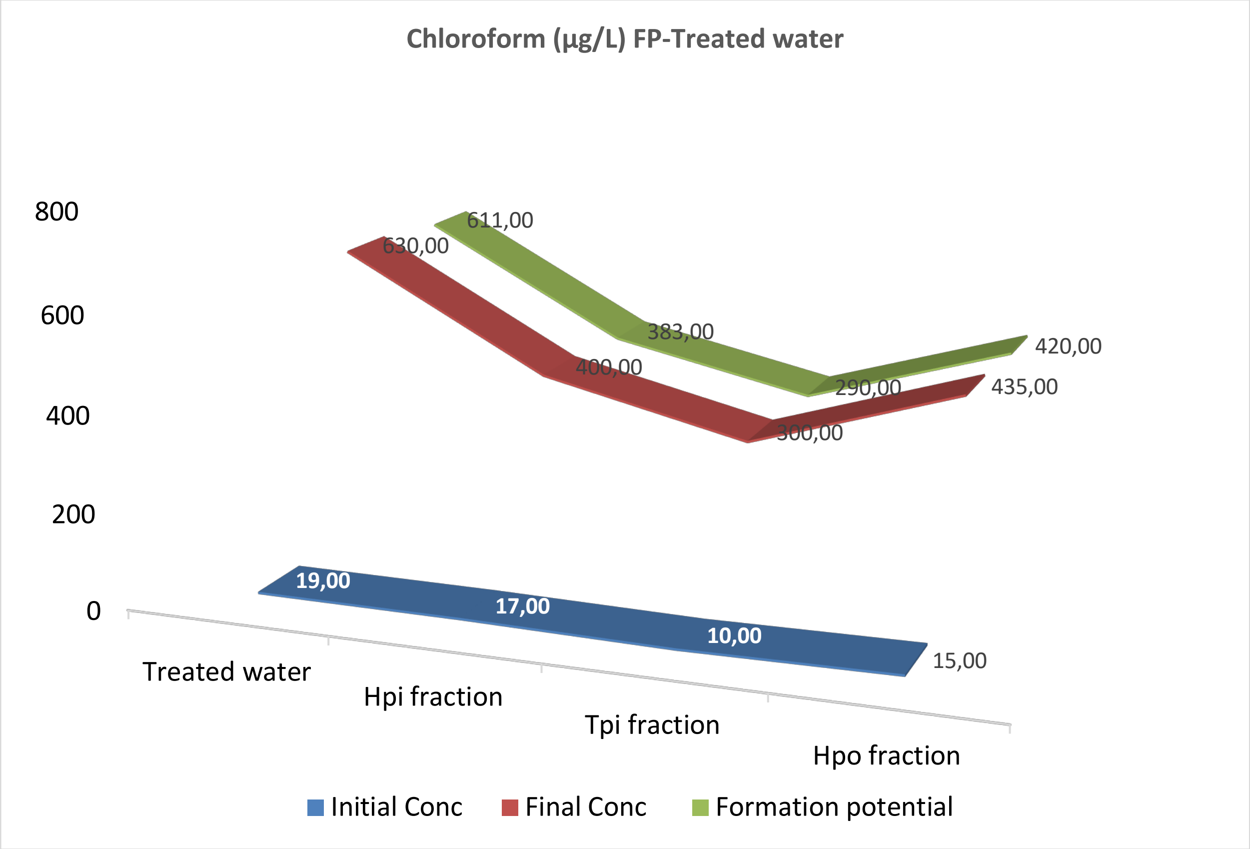

Figure 5-9: The formation of Chloroform and the various NOM fractions in treated water

The results indicate that the most dominant THM species is chloroform, increasing up to 708 µg/L and 611 µg/L for the raw water and treated water, respectively, contributing largely to the overall TTHM ratio (**Figure 5-10** and **Figure 5-11**). The Hpo NOM fraction is largely responsible for the overall formation of TTHM. This suggests that the formation of TTHM and more specifically chloroform was primarily due to the HMW NOM. The HMW Hpo NOM fraction is the main precursor to chloroform formation (Lu *et al*., 2009). The Tpi fraction contributes the least to the formation of THM. It is worth noting that the amount of DBPs formed is dependent on the varying characteristics of the NOM (Chang *et al*., 2001).

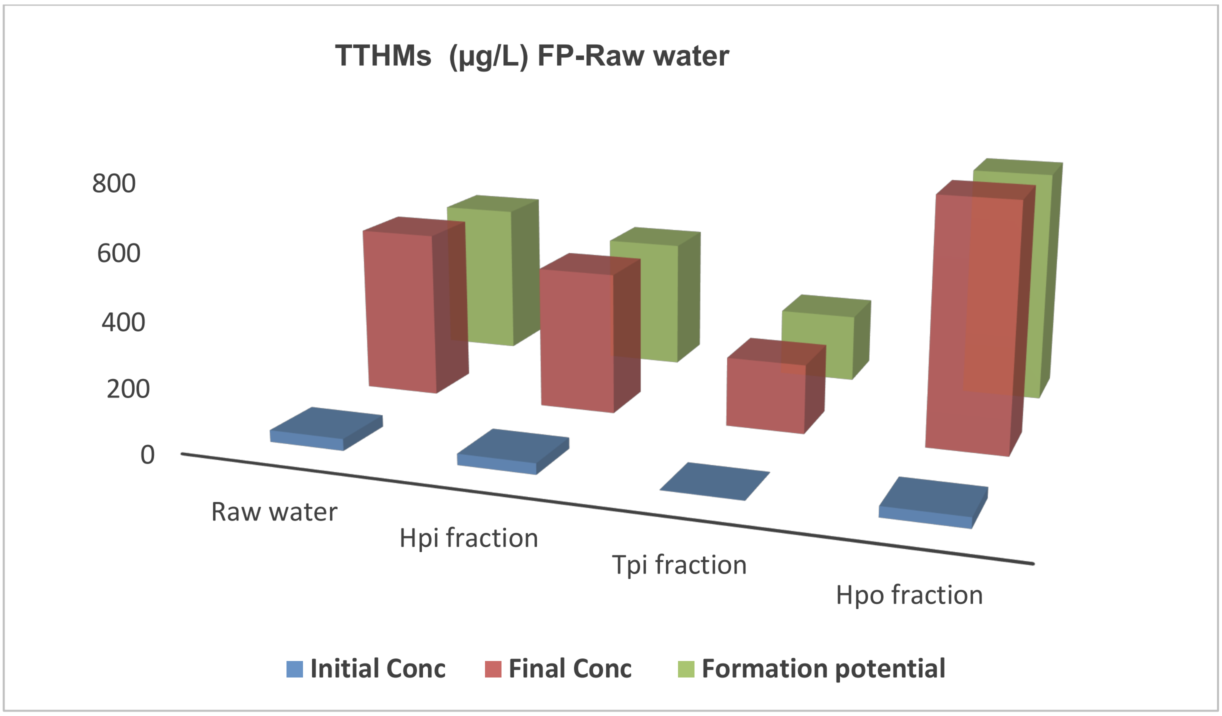

Figure 5-10: The formation of TTHM and the various NOM fractions in raw water

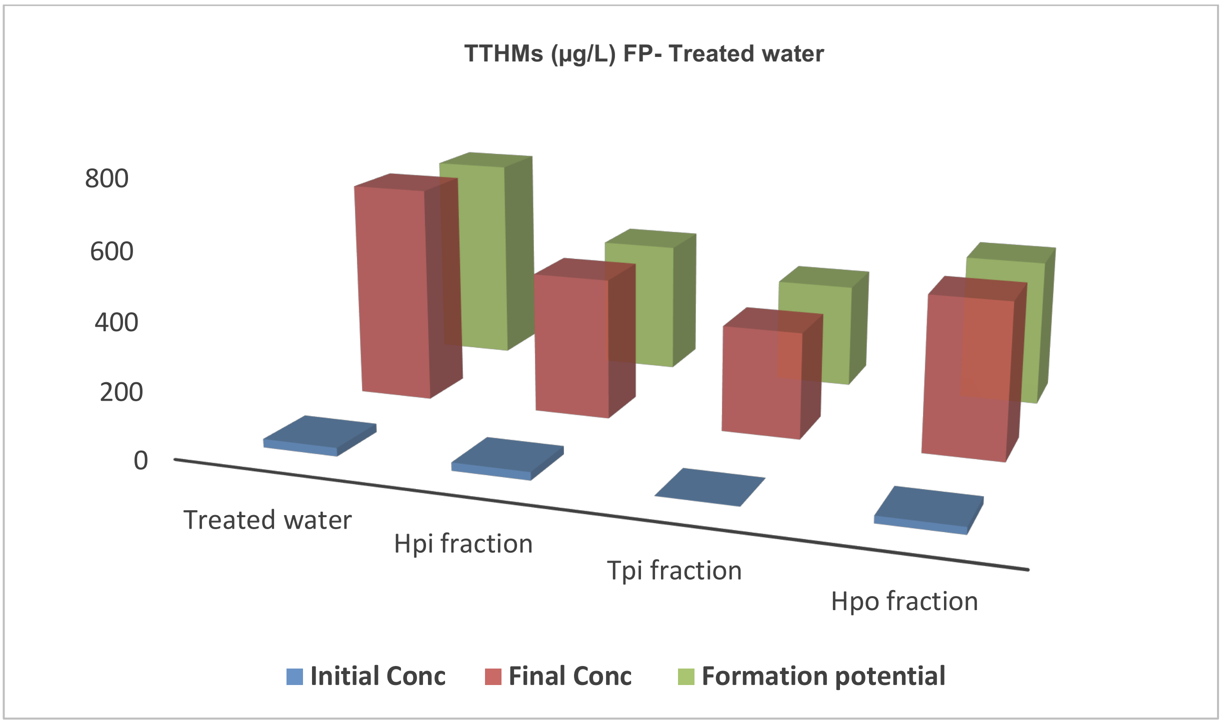

Figure 5-11: The formation of TTHM formation and the various NOM fractions in treated water

As evidenced by the respective R^2 values of 0.91, 0.84 and 0.83, a good correlation between SUVA-bromodichloroform, SUVA-chloroform, and SUVA-TTHM (**Figures 5-12** and **5-13**). Similar results were observed for the HMW organic matter, which was associated with the increased reaction of chloroform formation during chlorination (Chowdhury, 2013). Also, according to Fakour and Lo (2018), aromatic organics are more amenable to the formation of DBPs through the reaction with disinfectants; therefore, a high SUVA value is typically indicative of a high potential for the formation of DBPs.

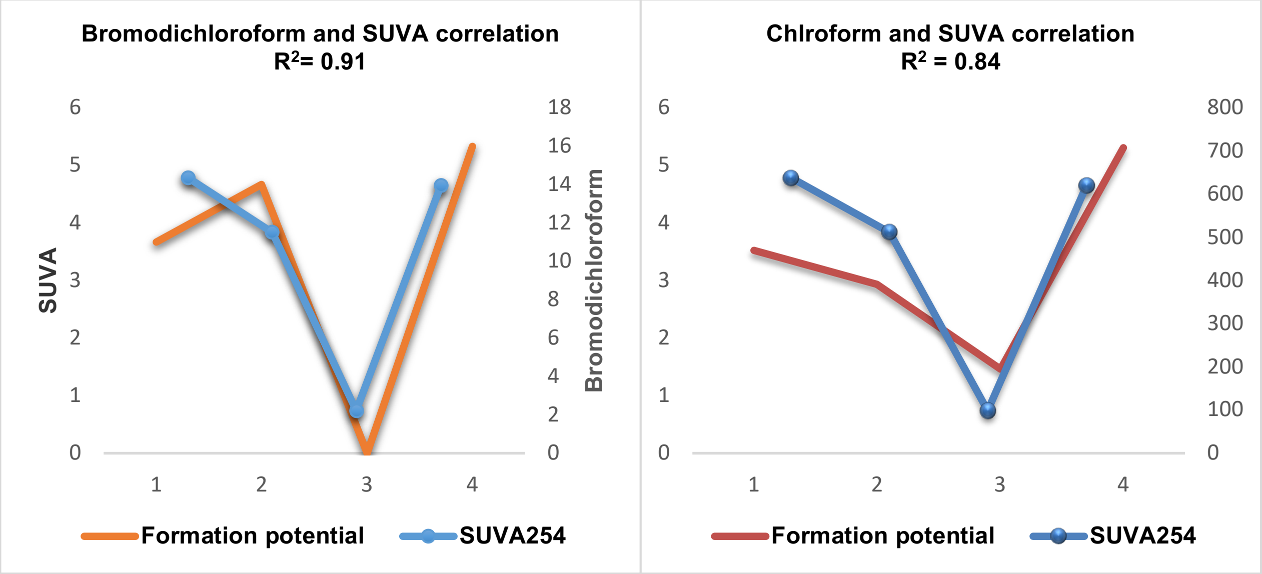

Figure 5-12: Correlation between SUVA and the formation potentials of bromodichloroform and chloroform by each NOM fraction

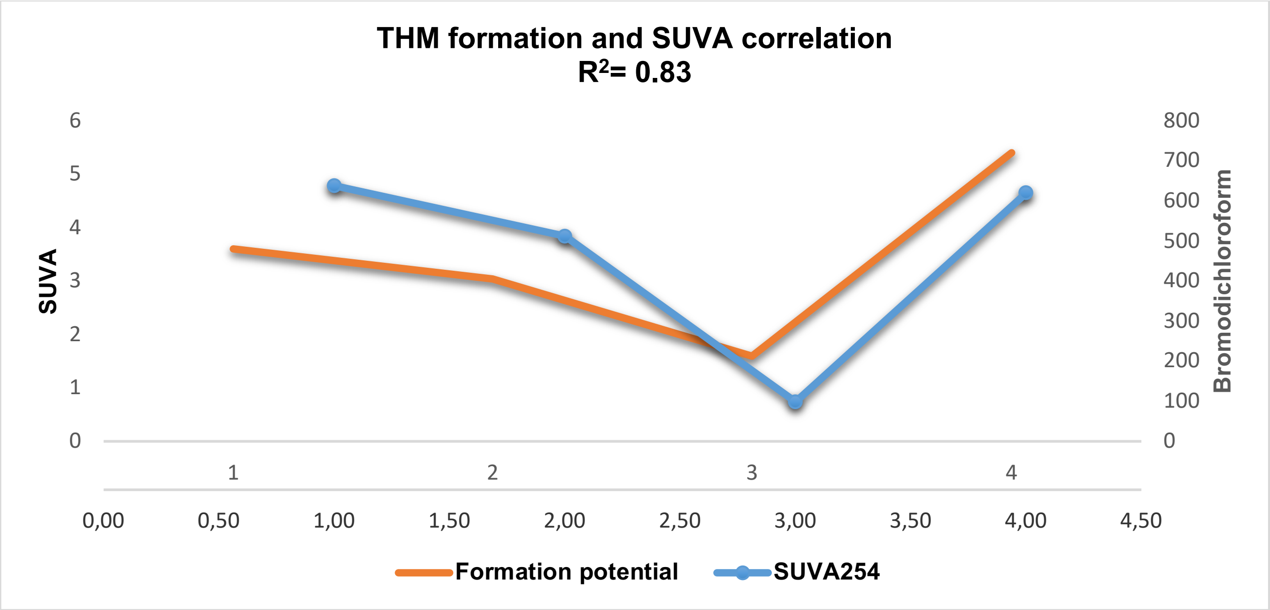

Figure 5-13: Correlation between SUVA and the formation potentials of TTHMs by each NOM fraction

5.5 Conclusions

This chapter assessed the biodegradability of NOM and its various fractions through the enhanced BDOC method in an attempt to study the resulting potential for the BDOC to influence TTHM formation by establishing if there is a relationship between the biodegradability of NOM and THMFPs. Moreover, the chapter assessed the impacts of

BDOC on bacterial regrowth in distribution systems by linking the presence of BDOC on each fraction and the growth in HPC. Not all DOC is biodegradable, as evidenced by the obtained BDOC ranging between 7.25% and 35.75% across all the NOM fractions.

The overall biodegradability was of the order; Hpi >Tpi > Hpo for both the raw and treated water. The BDOC on the raw and treated Hpi NOM fraction account for a mean of 32.07% and 35.22 %, whilst the Tpi NOM fraction account for a mean of 29.22% and 35.75 %. Lastly, the BDOC on the raw and treated Hpo NOM fractions account for a mean of 7.25% and 13.88 %, respectively. The huge variation in BDOC signified by huge standard deviations (i.e. 10.3-28.71%) is one determinant which shows that the biodegradability is heavily influenced by the amount and type of bacterial inoculum and incubation conditions. Nutrient availability has a major influence on the performance of the BDOC. This is particularly true considering that NOM (the substrate) is a complex mixture of organic molecules from freshwater ecosystems with distinctive catchment characteristics.

Not only was the BDOC essential in determining if NOM was biodegradable, but also in establishing if there was a link between BDOC by fractions and bacterial regrowth. The BDOC has proven to be a good water quality indicator for bacterial regrowth as there was a positive correlation between biodegradable NOM fractions and bacterial regrowth. In both raw and treated water, the Hpi fraction was the most biodegradable fraction and the Hpo fraction is the least biodegradable fraction, whilst the Tpi had a BDOC % in between the other two fractions. Similarly, with the BDOC of the various fractions, their contribution to bacterial regrowth was also in the order; Hpi >Tpi > Hpo for both the raw and treated water

The quantification of the BDOC also indirectly helps in the estimation of the non-biodegradable dissolved organic carbon (NBDOC) fraction in water and ultimately its impact on other water quality parameters, particularly its influence on DBPs (THM) formation. The high proportion of the NBDOC to the BDOC observed on the HWM Hpo fraction can be attributed to the higher potential of the Hpo fraction to form TTHMs. A relationship between the biodegradability of NOM and DBPFP exists, the less biodegradable the NOM fraction, the more influence they have on the formation potential of DBPs.

This information is very critical in the management of water treatment and supply systems and can be used for decision making with regards to treatment and optimization needs. For instance, this study revealed that the Hpo is the least biodegradable NOM fraction and inversely has a major contribution to DBP formation. However, linking the BDOC/THMFP findings and findings previously reported in Chapter 3 regarding the removals of the various fractions during treatment, the Hpo fraction is not a threat in terms of DBP formation as most of it is removed during treatment. A comparative analysis of the biodegradability of the bulk NOM and the respective NOM fractions provides insight into the mechanism involved in BOM consumption and quantification. Water systems that have favourable conditions for bacterial regrowth, such as the presence of BDOC, can also result in the loss of disinfectant residuals since bacterial regrowth tends to increase the demand of the disinfectant. Bacterial regrowth within the WDS could lead to biofouling, thereby multiplying the counts of coliforms and expedient pathogens and also increasing non-compliances with respect to the quality standards and ultimately customer complaints.

5.6 References

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CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The biodegradability of NOM and the resulting potential of the biodegradable NOM fractions to form disinfection by-products have been investigated. This was achieved through the applications of bulk NOM characterization techniques (i.e. DOC, UV_{254} and $SUVA_{254}$), the m-PRAM and the enhanced BDOC methods. The application of these various techniques was successful in; determining the character of the raw and treated water within the Rand Water conventional treatment processes, in isolating the NOM into various NOM fractions by means of polarity, in assessing which of the fractions is or are biodegradable, and lastly how each of the fractions influences the THMFP, as well as bacterial regrowth.

- The bulk NOM characterization parameters (i.e. UV_{254}, DOC and SUVA) gave details on the aromaticity and the treatability of the source water. The SUVA values ranged between 3.88 L/mg.m and 4.11 L/mg.m in the raw water, indicating a mixture of non-humic and aquatic humic compounds, comprising both Hpi and Hpo NOM compounds. The characterization of the NOM showed consistency throughout the research study in terms of composition and character. However, the characterization of NOM using SUVA only gives an indication of the aromaticity of the NOM. Solely, such characterization is substandard and insufficient to fully comprehend the character and the actual concentrations of the humic or non-humic substances of the NOM. As a result, the treatability of the NOM is compromised and this commands for the introduction of other sophisticated characterization and isolation techniques; hence the adoption of the m-PRAM application to further isolate the NOM.

- Applying the m-PRAM helped by providing a further discernment of the NOM, thereby allowing for further characterization of the different NOM fractions. Not only did this help in determining the aromaticity of the bulk NOM, but also of the individual fractions. The m-PRAM helped in assessing the character and composition of the NOM and its fractions both quantitatively and qualitatively. The m-PRAM fractionation resulted in the isolation of the NOM into three (3) fractions which resulted in an equal distribution of the Hpi and Hpo fractions confirming the bulk NOM characterization data. The consistency of the m-PRAM technique and its rapid nature validates it as one of the best rapid techniques in NOM isolation and characterization. Furthermore, the use of the m-PRAM also gives a better perspective into the contribution and role by each of the various NOM fractions, particularly with regards to the removal efficiencies of the system, their biodegradability and influence on DBP formation.

- The enhanced BDOC method was successfully optimized and it gave reliable results for NOM biodegradation. The duration of the BDOC was successfully reduced to 4 days, where the minimum and constant DOC levels were also observed. The key factors that controlled the BDOC period were; the highly optimized biological conditions for the BDOC with sufficient nutrients (N and P), a high oxygen flow for aeration, maintaining the right temperature (i.e. 22ºC), as well as the elimination of the impacts of photodegradation.

- The BDOC of the NOM fractions only accounts for a portion of the biodegradable organic matter (BOM) in a DOC sample as represented by BDOC ranging from 7.25% to 35.75%. The least biodegradable fraction is the Hpo fraction, while the Hpi and Tpi are relatively more biodegradable (i.e. ±30%), thereby, substantially contributing to BRP and less on THMFP. On the other hand, the impact by the Hpo fraction on BRP and THMFP is contrary to that of the other fractions. A strong correlation (i.e. R^2= >0.9) between BDOC and BRP has been successfully established and with absolute certainty, the Hpi NOM fraction can be confirmed as the primary cause of bacterial regrowth. This strong correlation allows for the prediction of the BRP in a water sample using the BDOC of each of the NOM fractions. To lessen the BRP in water supply systems, successful reduction of DOC is paramount, particularly the Hpi and Tpi fractions of NOM.

- The high proportion of the NBDOC to the BDOC observed on the HWM Hpo fraction are as a result of the higher potential of the Hpo fraction to form TTHMs. A relationship between the biodegradability of NOM and DBPFP exists, the less biodegradable the NOM fraction, the more influence they have on the formation potential of DBPs. The fraction (i.e. Hpo) that was the least biodegradable has a major contribution to the formation of THMs, however, this fraction is not a major threat to the system as higher removal efficiencies were also observed on this fraction. The information revealed by the BDOC is critical in the management of water treatment and distribution processes, as it is able to pin-point and link specific challenges to specific NOM fractions. This can lead to successful retrofitting of the conventional processes with the aim of addressing a specific challenge.

In summary, this research study has successfully established which of the NOM fractions (i.e. Hpi and Tpi) are easily biodegradable, to what extent, as well as the link between BDOC and the formation potential of THMs in the distribution system. The study has also identified the fractions (i.e. Hpi and Tpi NOM fraction) mostly responsible for bacterial regrowth. With regards to the main aim of the research study which was to investigate the biodegradability of NOM and the resulting potential of biodegradable NOM fractions to form DBPs, the study was successfully conducted. Additionally, the research study looked into the removal efficiency of the Rand Water conventional treatment processes and it was found to be fairly effective in the reduction of NOM, particularly the HMW Hpo fraction. Efficiently reduced NOM will minimize the DBP formation potential and bacterial regrowth potential of water.

6.2 Recommendations for future work

I. The formation potential for TTHM has now been established for the chlorinated distribution system in the Rand Water network, however, NDMA is more likely to form, more than THMs or HAAs in the chloraminated portion of the distribution network. This study recommends an assessment of the formation potential of N-nitrosodimethylamine (NDMA) and Haloacetic acids (HAAs) in the chloraminated and chlorinated distribution systems respectively.

II. The biodegradability of the three (3) major NOM fractions in terms of the polarity have been evaluated. Each of the fractions can be further classified into acids, neutrals and base. Therefore, the study recommends an investigation of the other NOM fractions such as Hpi-A, Hpo-A, Hpi-N, etc., with respect to biodegradability and how they can impact the mechanisms for bacterial regrowth and DBPFP in distribution systems.

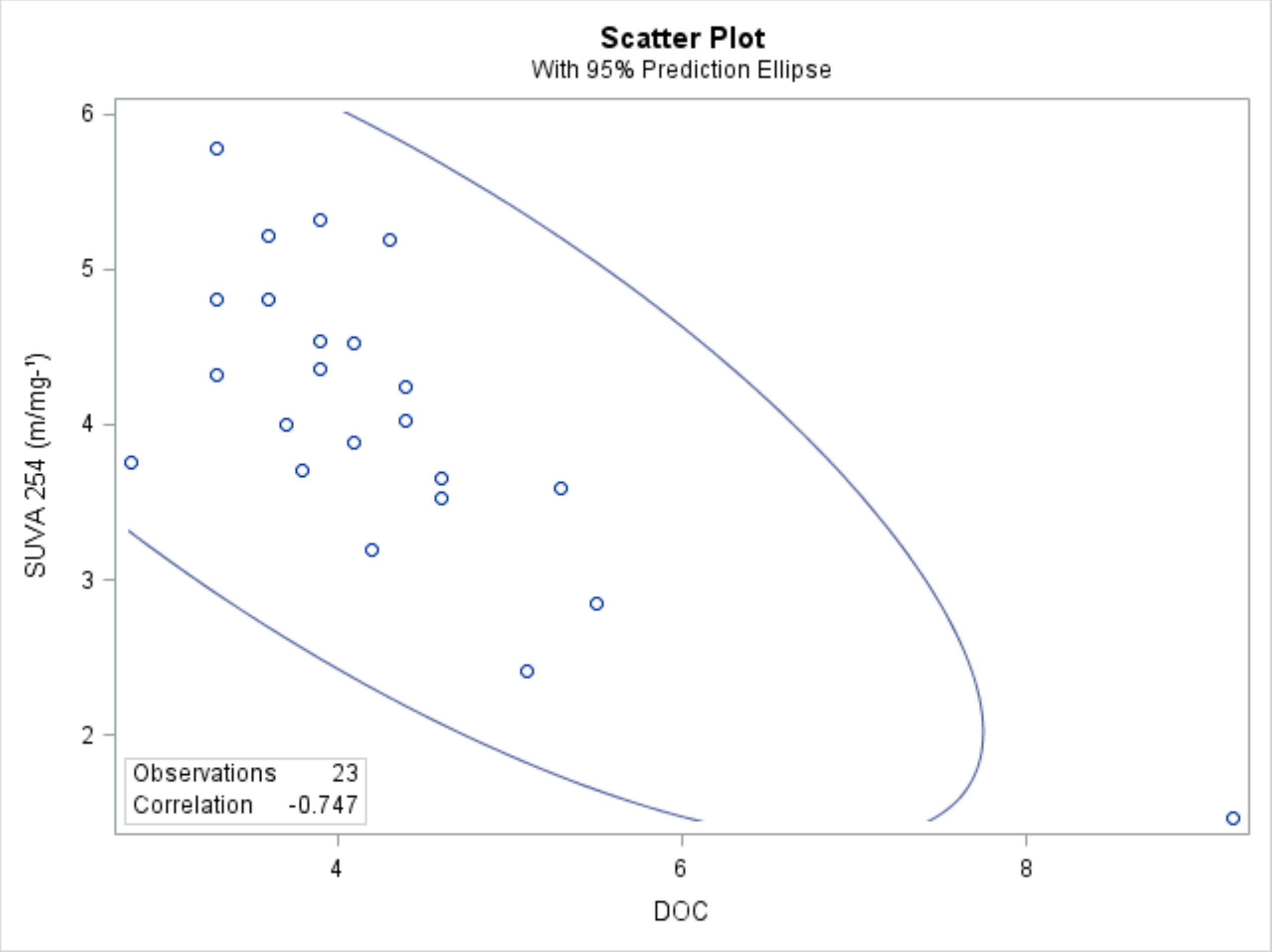
APPENDICES

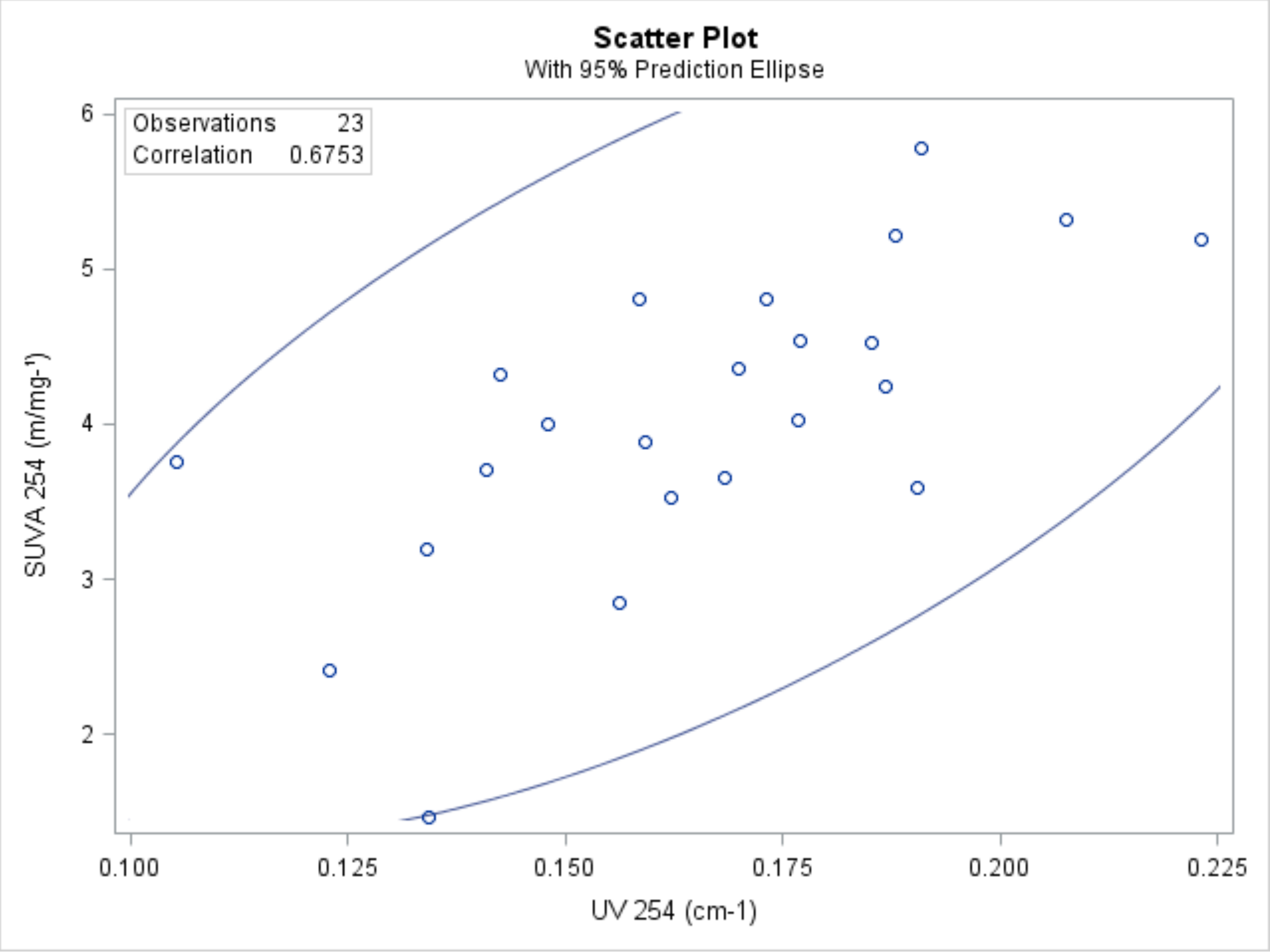
Appendix A: The characterization of NOM and its removal through the water treatment process

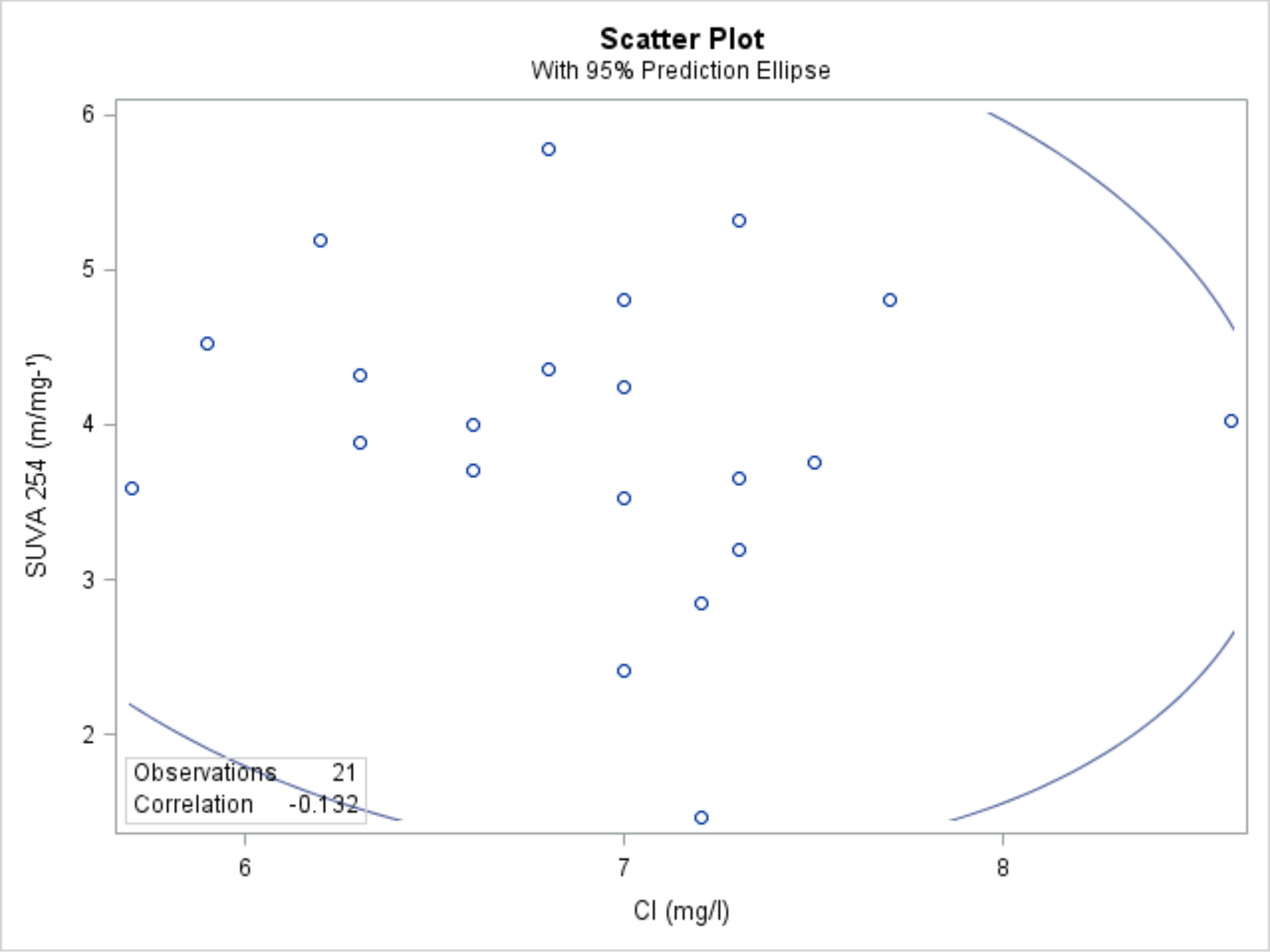
Appendix A1: Bulk NOM parameters monitored between August 2018-July 2019

Date	Cl (mg/l)	DOC (mg/l)	Humic Acids (mg/l)	Turbidity (NTU)	UV_{254} (m-^{1})	UV_{300} (m^{-1})	UV_{254} (cm^{-1})	UV_{300} (cm-^{1})	$SUVA_{254}$ (m/mg-^{1})
01-08-18	7.3	4.6	8.9	24	16.83	8.31	0.1683	0.0831	3.66
15-08-18	7	4.4	3.4		18.68	9.12	0.1868	0.0912	4.25
29-08-18	7.3	3.9	8.9	37	20.76	10.23	0.2076	0.1023	5.32
12-09-18	7.5	2.8	8.8	38	10.52	7.37	0.1052	0.0737	3.76
03-10-18	6.3	4.1	8.7	21	15.93	7.62	0.1593	0.0762	3.89
17-10-18		3.9	8.3	27	17.70	8.78	0.177	0.0878	4.54
31-10-18	6.8	3.9	8.8	26	17.00	8.30	0.17	0.083	4.36
14-11-18	7	3.6	8.4	29	17.32	8.46	0.1732	0.0846	4.81
12-12-18	7.2	5.5	8	23	15.63	7.65	0.1563	0.0765	2.84
16-01-19	7.2	9.2	7.9	33	13.44	6.66	0.1344	0.0666	1.46
30-01-19		3.6	9.4	60	18.79	9.02	0.1879	0.0902	5.22
13-02-19	6.2	4.3	9	64	22.32	11.04	0.2232	0.1104	5.19
27-02-19	6.8	3.3	9.4	59	19.09	9.37	0.1909	0.0937	5.78
13-03-19	6.6	3.7	6.9	83	14.81	7.17	0.1481	0.0717	4.00
03-04-19	7.7	3.3	5	57	15.86	7.60	0.1586	0.076	4.81
17-04-19	7.3	4.2		59	13.40	6.31	0.134	0.0631	3.19
15-05-19	7	5.1	8.6	82	12.28	5.91	0.1228	0.0591	2.41
29-05-19	8.6	4.4	8.7	81	17.69	8.45	0.1769	0.0845	4.02
12-06-19	5.9	4.1	8.2	66	18.53	8.80	0.1853	0.088	4.52
03-07-19	7	4.6	6.7	44	16.22	7.81	0.1622	0.0781	3.53
17-07-19	6.6	3.8	8.1	29	14.09	6.72	0.1409	0.0672	3.71

Appendix A2: Relationships between SUVA254 and other bulk NOM parameters

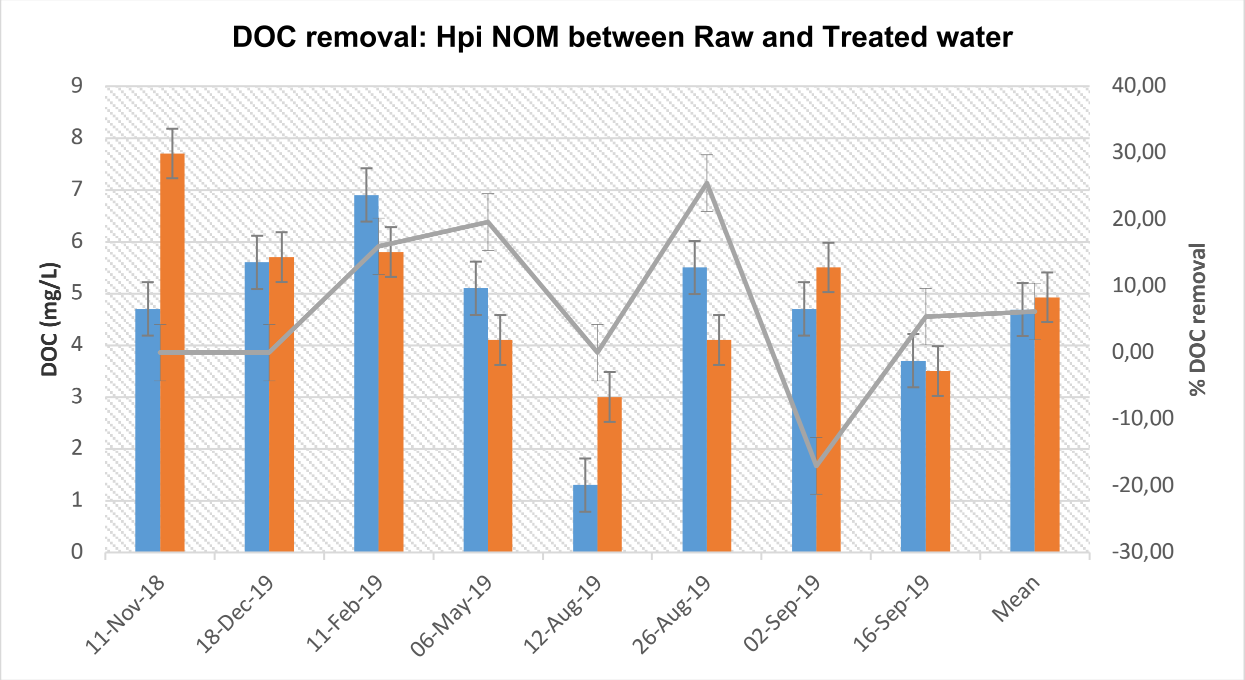


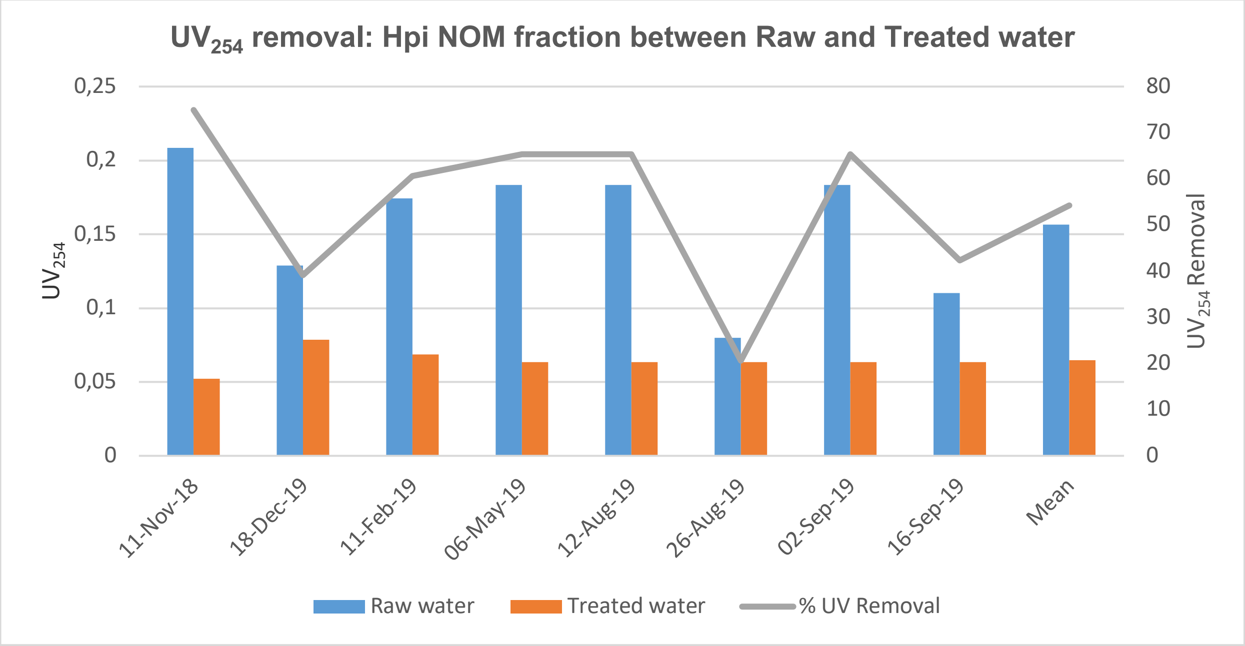

Scatter Plot
With 95% Prediction Ellipse
Observations 23
Correlation 0.6753
SUVA 254 (m/mg-1)
6
5
4
3
2
0.100
0.125
0.150
0.175
0.200
0.225
UV 254 (cm-1)



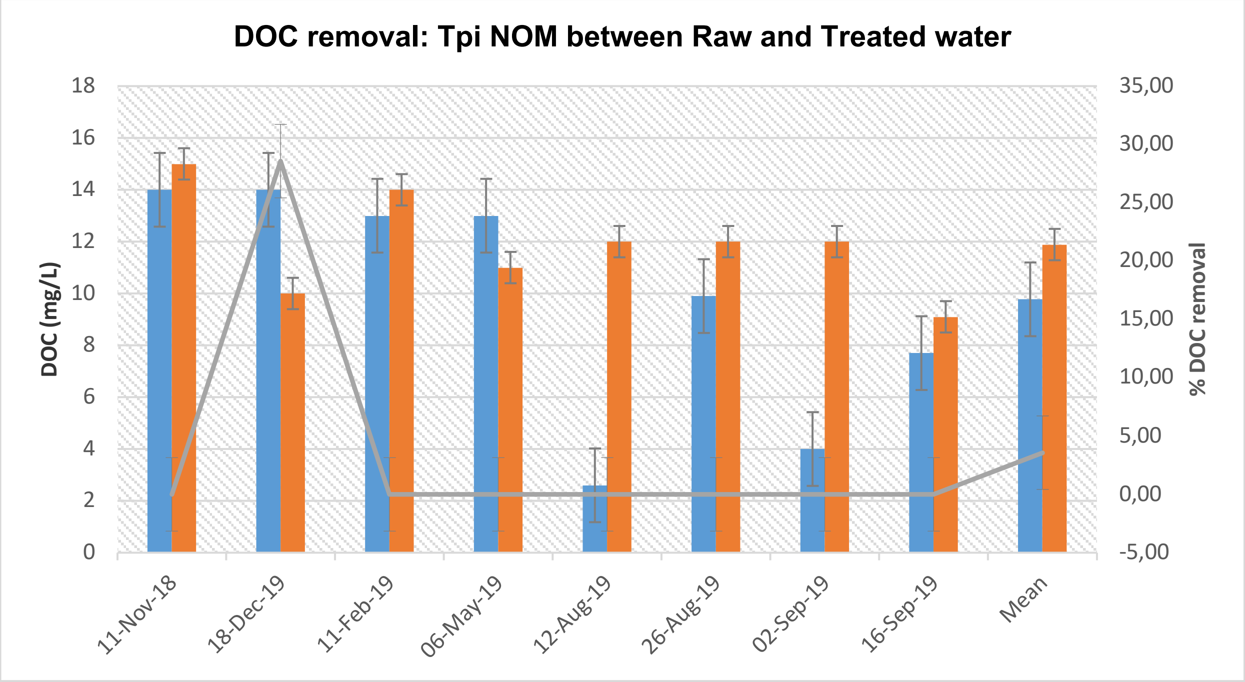
Appendix B*:* The removal of the various NOM fractions through the conventional treatment process- DOC and UV_{254}

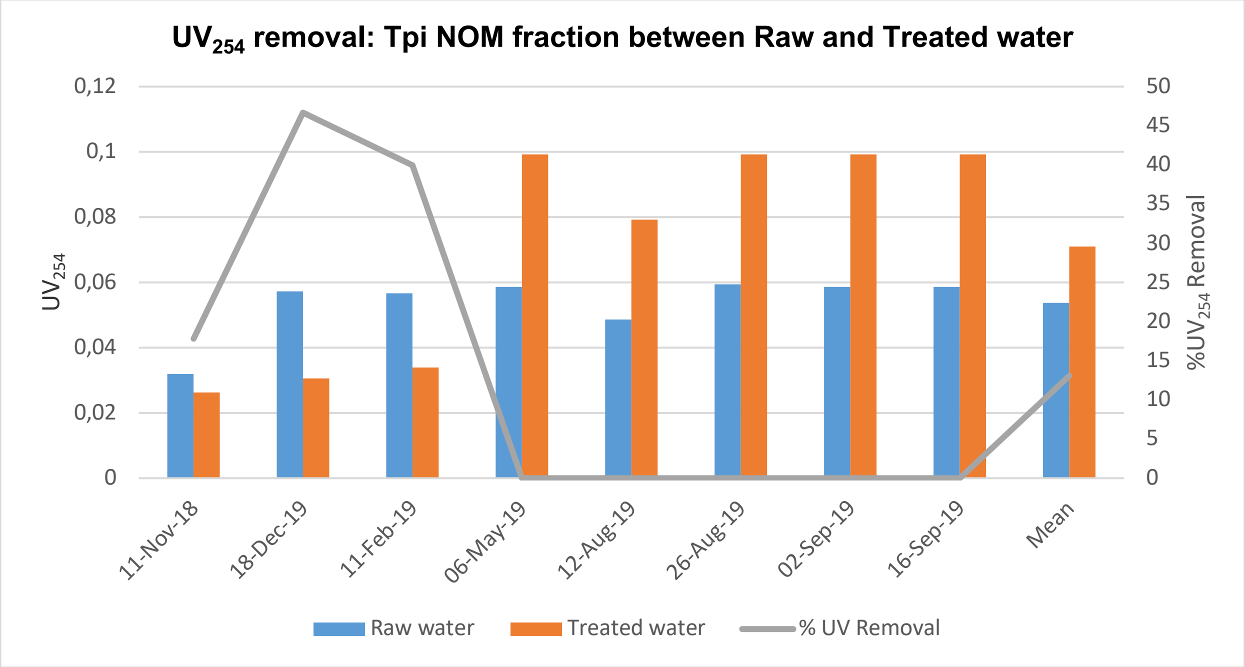
Appendix B1: The removal of the Hpi NOM fraction- DOC and UV_{254} respectively



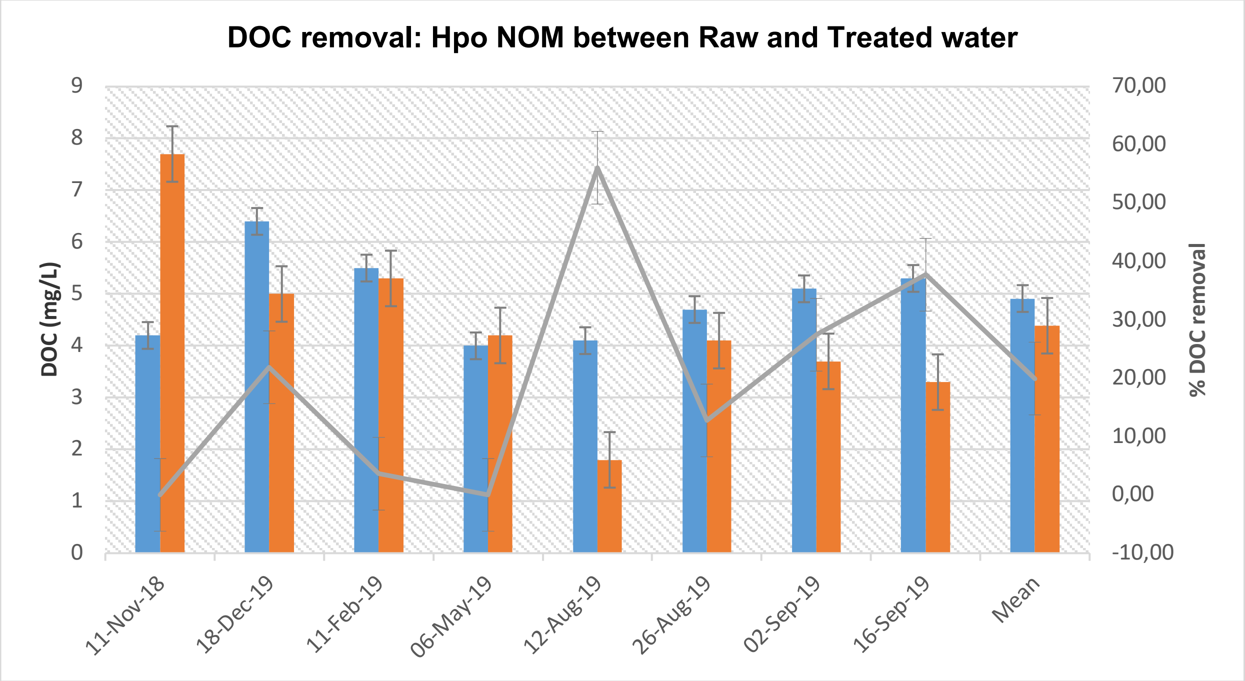


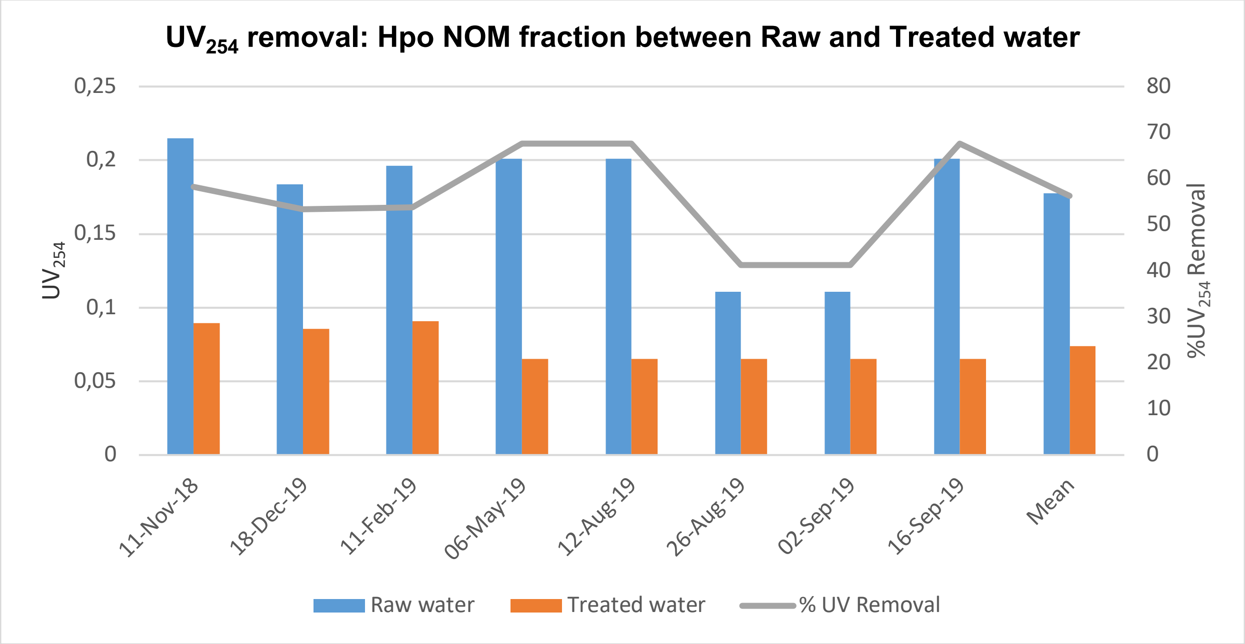
Appendix B2: The removal of the Tpi NOM fraction- DOC and UV_{254} respectively





Appendix B3: The removal of the Hpo NOM fraction- DOC and UV_{254} respectively





Appendix C: The Biodegradability of NOM and the various fraction

Appendix C1: Sodium acetate control sample (5 mg/L)

Biodegradation test no	Initial DOC	Lowest DOC/ NBDOC	BDOC	% BDOC
9.	5.50	2.40	3.10	56.36
10.	6.30	3.60	2.70	42.86
11.	6.40	3.20	3.20	50.00
12.	6.30	3.60	2.70	42.86
13.	4.20	3.00	1.20	28.57
14.	5.70	3.80	1.90	33.33
Minimum	**4.20**	**2.40**	**1.20**	**28.57**
Mean	**5.73**	**3.27**	**2.47**	**42.33**
Maximum	**6.40**	**3.80**	**3.20**	**56.36**
Standard deviation	**0.84**	**0.52**	**0.77**	**10.26**
N	**6**	**6**	**6**	**6**

Appendix C2: Sodium acetate control sample (10 mg/L)

Biodegradation test no	Initial DOC	Lowest DOC/ NBDOC	BDOC	% BDOC
1.	11.00	9.70	1.30	11.82
2.	9.80	1.30	8.50	86.73
3.	12.00	7.00	5.00	41.67

4.	9.90	0.70	9.20	92.93
5.	12.00	2.70	9.30	77.50
6.	8.00	5.40	2.60	32.50
7.	8.50	7.40	1.10	12.94
Minimum	**8.00**	**0.70**	**1.10**	**11.82**
Mean	**10.17**	**4.89**	**5.29**	**50.87**
Maximum	**12.00**	**9.70**	**9.30**	**92.93**
Standard deviation	**1.59**	**3.40**	**3.71**	**34.52**
N	**7**	**7**	**7**	**7**

Appendix C3: Raw water-Hpi NOM biodegradation results in August 2018-September 2019

Biodegradation test no	Initial DOC	Lowest DOC/ NBDOC	BDOC	% BDOC
1.	5.90	4.00	1.90	32.90
2.	6.20	5.10	1.10	17.74
3.	5.20	4.20	1.00	19.23
4.	4.60	1.00	3.60	78.26
5.	13.00	4.20	8.80	67.69
6.	6.40	3.00	3.40	53.13
7.	5.50	4.40	1.10	20.00
8.	4.00	3.00	1.00	25.00
9.	4.10	3.70	0.40	9.76
10.	5.10	3.90	1.20	23.53hf
11.	5.40	5.10	0.30	5.56
Minimum	**4.00**	**1.00**	**0.30**	**5.56**
Mean	**5.95**	**3.78**	**2.16**	**32.07**
Maximum	**13.00**	**5.10**	**8.80**	**78.26**
Standard deviation	**2.47**	**1.15**	**2.45**	**23.85**
N	**11**	**11**	**11**	**11**

Appendix C4: Raw water-Tpi NOM biodegradation results in August 2018-September 2019

Biodegradation test no	Initial DOC	Lowest DOC/ NBDOC	BDOC	% BDOC
1.	5.90	2.30	3.60	61.02
2.	8.00	6.10	1.90	23.75
3.	15.00	5.30	9.70	64.67
4.	12.00	9.70	2.30	19.17
5.	14.00	12.00	2.00	14.29
6.	14.00	12.00	2.00	14.29
7.	13.00	8.00	5.00	38.46
8.	13.00	8.40	4.60	35.38
9.	9.90	9.20	0.70	7.07
10.	4.00	3.10	0.90	22.50
11.	7.70	6.10	1.60	20.78
Minimum	**4.00**	**2.30**	**0.70**	**7.07**
Mean	**10.59**	**7.47**	**3.12**	**29.22**
Maximum	**15.00**	**12.00**	**9.70**	**64.67**
Standard deviation	**3.70**	**3.23**	**2.59**	**18.91**
N	**11**	**11**	**11**	**11**

Appendix C5: Raw water-Hpo NOM biodegradation results in August 2018-September 2019

Biodegradation test no	Initial DOC	Lowest DOC/ NBDOC	BDOC	% BDOC
1.	5.90	5.90	0.00	0.00
2.	6.20	5.70	0.50	8.06
3.	7.50	7.50	0.00	0.00
4.	5.50	5.50	0.00	0.00
5.	4.70	4.70	0.00	0.00
6.	5.60	5.40	0.20	3.57
7.	6.90	5.20	1.70	24.64
8.	5.10	3.90	1.20	23.53
9.	5.50	4.40	1.10	20.00
10.	4.70	4.70	0.00	0.00

11.	3.50	3.50	0.00	0.00
Minimum	**3.50**	**3.50**	**0.00**	**0.00**
Mean	**5.55**	**5.13**	**0.43**	**7.25**
Maximum	**7.50**	**7.50**	**1.70**	**24.64**
Standard deviation	**1.10**	**1.09**	**0.62**	**10.29**
N	**11**	**11**	**11**	**11**

Appendix C6: Treated water-Hpi NOM biodegradation results in August 2018-September 2019

Biodegradation test no	Initial DOC	Lowest DOC/ NBDOC	BDOC	% BDOC
1.	7.70	3.80	3.90	50.65
2.	5.00	3.00	2.00	40.00
3.	5.30	4.50	0.80	15.09
4.	4.20	3.40	0.80	19.05
5.	3.10	1.80	1.30	41.94
6.	7.10	6.30	0.80	11.27
7.	37.00	4.20	32.80	88.65
8.	3.30	2.80	0.50	15.15
Minimum	**3.10**	**1.80**	**0.50**	**11.27**
Mean	**9.09**	**3.73**	**5.36**	**35.22**
Maximum	**37.00**	**6.30**	**32.80**	**88.65**
Standard deviation	**11.40**	**1.34**	**11.14**	**26.20**
Range	**33.90**	**4.50**	**32.30**	**77.38**
N	**8**	**8**	**8**	**8**

Appendix C7: Treated water-Tpi NOM biodegradation results in August 2018-September 2019

Biodegradation test no	Initial DOC	Lowest DOC/ NBDOC	BDOC	% BDOC
1.	15.00	13.00	2.00	13.33
2.	10.00	9.70	0.30	3.00
3.	14.00	11.00	3.00	21.43
4.	11.00	7.30	3.70	33.64
5.	12.00	2.20	9.80	81.67

6.	12.00	5.60	6.40	53.33
7.	12.00	3.90	8.10	67.50
8.	9.10	8.00	1.10	12.09
Minimum	**9.10**	**2.20**	**0.30**	**3.00**
Mean	**11.89**	**7.59**	**4.30**	**35.75**
Maximum	**15.00**	**13.00**	**9.80**	**81.67**
Standard deviation	**1.94**	**3.63**	**3.44**	**28.71**
Range	**5.90**	**10.80**	**9.50**	**78.67**
N	**8**	**8**	**8**	**8**

Appendix C8: Treated water-Hpo NOM biodegradation results in August 2018-September 2019

Biodegradation test no	Initial DOC	Lowest DOC/ NBDOC	BDOC	% BDOC
1.	6.20	6.20	0.00	0.00
2.	5.70	5.70	0.00	0.00
3.	5.80	4.40	1.40	24.14
4.	4.10	4.10	0.00	0.00
5.	3.00	1.20	1.80	60.00
6.	4.11	3.90	0.21	5.11
7.	5.50	4.30	1.20	21.82
8.	3.70	3.70	0.00	0.00
Minimum	**3.00**	**1.20**	**0.00**	**0.00**
Mean	**4.76**	**4.19**	**0.58**	**13.88**
Maximum	**6.20**	**6.20**	**1.80**	**60.00**
Standard deviation	**1.18**	**1.50**	**0.76**	**21.19**
Range	**3.20**	**5.00**	**1.80**	**60.00**
N	**8**	**8**	**8**	**8**