High performance size exclusion chromatography with a multiple wavelength absorbance detector for improved dissolved organic matter characterisation and water quality monitoring

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Abstract

This study was to develop better use of chromatographic data for dissolved organic matter (DOM) characterisation using data analytics approach. The findings confirmed that understanding the characteristic and composition of DOM was essential for water quality investigation and this task was highly demanding. Among different DOM analytical techniques, high performance size exclusion chromatography coupled with a photodiode array detector (multi-wavelength HPSEC) for multiple UV-Vis wavelengths measurement was proving to be a simple and informative choice of analytical technique for characterising DOM in the aquatic environments including drinking water supply systems. Compared to using single wavelength detection alone, the additional information imparted by multi-wavelength detection provided better understanding of DOM and yielded more insight into the key information that has more relevant in the field of water research. The development also solved some fundamental problems in multi-wavelength HPSEC analysis including multivariate chromatographic data exploration, resolution optimisation, and information extraction as well as applying the technique for further qualification and quantification of DOM in complex environmental and engineering aquatic samples.

In the early phase of this study, one of the aims was to access the suitability in using an open and free software program, R (version 3.1.0, R Development Core Team) to handle the complex chromatographic data. The data analytics procedures and program codes were developed in the direction of improving multi-wavelength HPSEC data exploration, interpretation and information extraction. This study also explored the applicability of this approach for DOM characterisation in water supply management. The benefits of this approach were demonstrated in two case studies. The case studies were carefully selected to bridge the knowledge gap of exploring the use of HPSEC as an environmental monitoring tool in a stormwater catchment and a drinking water distribution system. Both case studies shared a common aim of using HPSEC as a characterisation tool to determine the molecular profile of DOM and using the analytical information to understand the impact of DOM on 1) stormwater and 2) drinking water quality. Case study one demonstrated the proposed approach could provide better understanding the complexity of DOM in stormwater and the influences of environmental conditions (seasonal variations) and storm event characteristics (rainfall-runoff process) on stormwater DOM characteristics. Case study two demonstrated the proposed approach could also provide better understanding of the association between DOM molecular weight distribution and nitrification occurrence in a chloraminated drinking water distribution system. Results confirmed the usefulness of multi-wavelength HPSEC analysis for providing additional knowledge of DOM characterisation and it can also allow the examination of changes to the molecule weight profiles in the lower wavelength region (below 254 nm). Correct selection of the UV wavelengths can be an important factor for providing appropriate indicators for water quality analysis. The detector wavelengths, 210 and 254 nm were found to provide useful information on the physiochemical properties of DOM in both stormwater and chloraminated drinking water distribution system. The ratio A_{210}/A_{254} could be used to estimate the proportions of functional groups and conjugated carbon species in the stormwater-associated DOM.

In addition, a package of simple analytical techniques, such as dissolved organic carbon (DOC), UV absorbance at 254 nm (UV_{254}), colour, turbidity, nitrate, nitrite, ammonia, total disinfectant residual and microbiological technique (flow cytometry) for bacterial level analysis were also selectively applied and their applicability to indicate water quality changes were evaluated. The correlations of water quality parameters derived from different methods were also statistically analysed using the R software. Pearson's Product Moment Correlation (PPMC) was used to evaluate the correlations among generate water quality parameters, HPSEC-UV profiles and microbiological analysis. The standard analysis of variance (ANOVA) was utilised to evaluate the significant influence of seasonal variation on DOM characteristics.

The findings of this research contributed to new knowledge in DOM characters and demonstrated the potential of using multiple water quality parameters combined with DOM characterisation tool for monitoring stormwater quality, understanding the performance of conventional treatment processes and the assessment of water quality in a water distribution system. This research also highlighted the useful application of chemometric approach provided by the R software program for improving HPSEC-UV analysis of DOM in aquatic systems. In addition, this development can lead to the inclusion of using multi-wavelength HPSEC as monitoring technique for the water quality management system

Declaration

I declare that this thesis presents work is carried out by myself. This work contains no materials which have been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution; and to the best of my knowledge, contains no material previously published or written by another person, except where due references have been made in the text. Additionally, I certify that no part of this work will, in the future, be used in any university or other tertiary institution without the prior approval of The University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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All substantive contributions by others to the worked presented, including jointly authored publications, is clearly acknowledged.

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Table of contents

Chapter 4: Characterisation of dissolved organic matter in stormwater using high-performance size exclusion chromatography 78

Chapter 5: High-performance size exclusion chromatography with a multi-wavelength absorbance detector study on dissolved organic matter characterisation along a water distribution system 104

List of Figures

Chapter 3

Chapter 4

Chapter 5

Appendix

List of Tables

Chapter 4

Chapter 5

Appendix

Chapter 1: **Introduction**

1. Background

Over the last couple of decades, there is an observable trend of dissolved organic matter (DOM) concentration increase in water sources reported in the literature (Matilainen et al., 2011; Nebbioso and Piccolo, 2013; Ritson et al., 2014). DOM is a heterogeneous molecular mixture of naturally occurring organic compounds found abundantly in natural waters and originates from living and dead plants, animals and microorganism, and from the degradation products of these sources. The amount / concentration and character of DOM differ in water sources and are influence by climate, geology and topography (Sharp et al., 2006; Fabris et al., 2008; Wei et al., 2008; Delpla et al., 2009; Chong et al., 2013; Ritson et al., 2014). Originated from a rather wide range of sources, DOM can be classified into allochthonous, transported from the surrounding landscape, and autochthonous, derived from organisms within the water body. Owing to the presence of DOM being undesirable in potable water, DOM removal is always the most important target in water treatment plant operations. Most recent studies have indicated that DOM removal optimisation requires an understanding of DOM specific characteristics and its seasonal variability in water sources (Fabris et al., 2008; Chow et al., 2008; Liu et al., 2010; Xing et al., 2012; Ho et al., 2012; Sanchez et al., 2013). These studies have also confirmed that no treatment process can achieve total DOM removal. Recent studies have also indicated that the physiochemical properties of DOM, including hydrophobicity, aromaticity and molecular weight (MW) distribution have strongly affected the reactivity, mobility and treatability of DOM in aquatic systems (Al-Reasi et al., 2013; Cui et al., 2013; Wei et al., 2013). Information linking changes in DOM characteristics in different stages of water supply system is hence essential to enable the water industry to setup a response for future water quality change by planning a suitable water treatment plant improvement program and adopting optimisation strategy to improve drinking water treatment operations of the existing plants.

The analytical challenges are to develop analytical technique which is simple to perform but generate useful analytical information, such as looking for less complex fractions but more amenable for analysis. There is no universal technique suitable for DOM separation and identification, because DOM may contain thousands of different chemical constituents, it is not practical to characterise DOM on the basis of individual compounds but more feasible and the general practice to characterise it according to chemical groups having similar properties. A series of analytical techniques have been widely used for understanding DOM composition and character.

Ultraviolet (UV) and visible spectrometry is probably the most widely applied analytical method for DOM in various matrices, since the major components of DOM are chromophores and which are capable of absorbing ultraviolet and visible radiation. Even though it may not provide an unambiguous identification of an organic compound, an absorption spectrum in UV and visible regions is nevertheless useful for detecting the presence of chromophores.

As an application of quantitative analysis, UV absorption method is widely used for quantity and quantity of DOM. Many studies have indicated that the absorption intensity is proportional to DOM concentration in an aquatic sample. The use of absorbance at 254 nm (UV_{254}) as a surrogate parameter for DOC has been commonly employed in water quality investigations. However, recent work has suggested that DOC measured at single 254 nm may be more representative of aromatic DOC groups rather than total DOC concentration (Her et al., 2008; Korshin et al., 2009; Yan et al., 2012). In another words, single wavelength detection may only be able to provide limited analytical information. Besides absorbance at 254 nm, some studies have also applied absorbance at other wavelengths to remove interference and highlight the absorbance of the compound of interest. Consequently, apart from the surrogate parameters developed using a single wavelength, more than one wavelength and/or multi-wavelength absorbance detection have been introduced and applied by several researchers (Her et al., 2008; Helm et al., 2008; Korshin et al., 2009; Yan et al., 2012).

Column chromatography with UV detector is also the most common spectrometry analyses used in the field of water chemistry. In particular, high performance size exclusion chromatography coupled with UV detection (HPSEC-UV) based on physiochemical molecular weight separation has been proposed as a promising monitoring technique and used reliably to compare natural water source quality, assess water treatment process performance, predict operation condition and determine potential disinfection by-product

(DBP) formation (Fabris et al., 2008; Chow et al., 2008; Korshin et al., 2009; Liu et al., 2010; Xing et al., 2012). It requires only minimum sample pre-treatment, small injection volume as well as ease and speed of analysis, are also the attractive advantages provided by HPSEC application. Higher MW fractions are generally easier to remove using conventional coagulation treatment than lower MW species (Chow et al., 2008); whist lower MW substances are generally more bioavailable but are likely to be linked with DBP formation (Korshin et al., 2009; Kristiana et al., 2010, 2013).

HPSEC coupled with a multi-wavelength detector (multi-wavelength HPSEC) has also been increasingly popular due to the ability to conduct more extensive DOM characterisation (Her et al., 2008; Korshin et al., 2009; Yan et al., 2012). Due to the availability of multi-wavelength UV detection, HPSEC-UV prolife has been improved to give a more comprehensive perspective on the relative absorbance intensity of various apparent molecular weights (AMWs) at different wavelength measurements. Not only does this combination provide more informative knowledge of DOM composition and characteristics, but also promote additional and potential vital information concerning the importance of UV wavelength selection and the association between the properties of DOM AMW distribution and the sensitivities of their UV absorptions.

As a result of these trends, this method for DOM characterisation is becoming increasingly important. It is hence interesting to employ this method to understand greater insight into DOM characteristics and composition in multiple stages in the drinking water treatment plant, including feed water (reservoir and river), during treatment stages and post treatment / prior to entering the distribution system, in order to improve our knowledge in drinking water supply management. To date, minimal studies have evaluated the application HPSEC-UV technique to monitor stormwater (as supplement to river water source which associated with a water source protection study) and product water quality within an operating distribution system (to study the impact of DOM on chloramine residual lost / nitrification occurrence).

Application of HPSEC-UV for treatment optimisation has drawn most attentions in previous research (Chow et al., 2008; Korshin et al., 2009; Liu et al., 2010; Matilainen et al., 2011; Xing et al., 2012), whilst source water protection and drinking water distribution system management have recently obtained a lot of attentions (Chow et al., 2009; Storey et al., 2011; Kristiana et al., 2013; McElmurry et al., 2013). Environmental risk assessment, catchment management and appropriate monitoring methodology development are key activities relevant on source water protection and to prevent and/or minimise chemical pollution loads on surface or groundwater sources of drinking water supply. Monitoring and understanding changes in product water quality within a distribution system is also required to ensure water supply of safe and consistency in quality for the consumer. Assessment of potential distribution system contamination and water quality monitoring are the main emphasises in modern distribution system management (Hall et al., 2007; Storey et al., 2011; Shaw et al., 2014).

Traditionally, HPSEC-UV data are standardised as a plot of absorbance at a particular wavelength against retention time or calibrated into apparent molecular weights using molecular weight standards. A possible explanation for choosing this way of presenting the data could be partially due to software limitation. The generation of three-dimensional data from HPSEC coupled with multi-wavelength detection results in increased in the size of the data set with at least hundreds of variables. This huge amount of data set requires effective and useful interpretation. Extracting additional information and knowledge of DOM characteristics from this method is attractive but challenging.

Being a freely and relatively well-developed programing language, R software (version 3.1.0, R Development Core Team) appears an integrated suite for statistical computing and graphical displaying. R software is not only capable for large quantities data processing and modelling, but also its ability enables to generate well-designed graphics routinely. Applying this software is an innovative way to visualise the data for analysis being more accessible. In fact, data visualisation has been promoted as a scientific and engineering tool for raw data mining. The purpose of visualisation study is not to try to receive information simply by visual inspection, rather, is to learn, develop and improve the effectiveness of data interpretation. In addition, visualisation for water research is attractive, such as more effective data interpretation across all stages of water treatability, quicker and greater number of ideas giving from well-designed figures than reading numbers and notes, associations of predicted probabilities and gaining more insight into the key information that is of importance in water quality monitoring.

2. Aims and Objectives

Enhancement chromatographic multivariate data exploration and interpretation

- Explore the R software program and determine its ability for multivariate data of multi-wavelength HPSEC profile processing
- Develop data analytics procedure and programs codes for data presentation, information extraction and further DOM qualification and quantification
- Determine the benefits of this approach for DOM characterisation in multi stages of water supply management

Case one: semi urban stormwater monitoring

- Extend the use of advanced analytical technique, HPSEC-UV as an environmental monitoring method to characterise stormwater-associated DOM and understanding DOM transformation and mobility based on molecular weight distribution
- Rather than using traditional composite sampling methodology, this study utilised sequential sampling to collect samples automatically. One hypothesis of this work is based on flow conditions sampling would provide more comprehensive and representative results, such as estimating appropriately rapid changes of water quality during each storm event
- Determine stormwater quality uses a series of conventional techniques associated with water quality and DOM properties, and describe their sensitivity and potential relationships
- Estimate influences of various environmental conditions, including storm event characteristics as well as seasonal variations on stormwater DOM character using statistical methods

Case two: chloraminated drinking water monitoring

- Apply organic characterisation tools for monitoring and understanding how DOM impacts on water quality in a chloraminated drinking water distribution system

- Evaluate the roles of DOM, chemical nitrification process and heterotrophic bacterial population towards chloramine residual decay in distribution system by adapting advanced molecular and microbiological analysis techniques
- Study if there are any changes in water quality associated with nitrification occurrence and their associations with changes in DOM molecular weight distribution based on HPSEC-UV profiles
- Determine relationship between general water qualities, microbiological activity and DOM characteristics using statistical methods

3. Outline of the thesis

This thesis is organised in five chapters and a brief description of each is presented in this session.

Chapter 1 presents a background of DOM in drinking water and the problems associated with it. It presents the research gap that is required to improve our understanding of the character of DOM and described the main aims and objectives of this Master research.

Chapter 2 presents a review of the characterisation and influence of DOM in the environments and drinking water systems. A review of the different method that have been applied for the quantification and characterisation of DOM is also presented.

Chapter 3 presents an enhancement to R, open and free software for chromatography and investigates its ability for data-rich multi-wavelength HPSEC profile processing, exploring and information extracting.

Chapter 4 and 5 are the two cases demonstrate the benefits of the data chemometrics approach developed in chapter 3.

Lastly, Chapter 6 presents a summary of the main findings and conclusions of the research study and some recommendations for practice and further research.

The contents of the chapters are organised in such a manner that the two case studies, chapter 3 and 4 have been (or will be) published in international peer reviewed journals. All the chapters are generally so presented that they can be read nearly independently.

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Chapter 2: **Literature Review**

Introduction

Most natural water sources throughout the world contain natural dissolved organic matter (DOM), and the range of these organic components varies from water to water as well as seasonally, and there is an observable trend of these organic components concentration increase in feed water (reservoir and river), which are slowing turning brown as a result of increased levels of organic carbon being released from surrounding catchments and/or terrestrial systems. These indications are that high levels of organics are here to stay and natural source water protection is becoming an essential task in drinking water supply management. This chapter gives a discussion of various challenges associated with the presence of DOM in environmental and engineering aquatic systems. Also, this chapter gives an overview of various challenges of water source management, potential risk assessments as well as water treatability and distribution system management on the basis of literature reviews, and the requirements for DOM characterisation in water with various analytical methods. Improving analytical method for DOM detection and characterisation drives continuously the most efforts in water research. This chapter is hence with a special emphasis on DOM analysis and characterisation methods.

1. Natural dissolved organic matter

Natural organic matter (NOM), a group of carbon-based compounds, is ubiquitous in natural water sources derived from plants, animals, microorganisms and their waste and metabolic products (Nebbioso and Piccolo, 2013). NOM is a heterogeneous mixture and is not fully characterised with various functional groups and aromatic contents and with a broad range of molecular weights from a few hundred to 100,000 Daltons (Da). The source of organic matter, the water matrix, environmental conditions and biological activity in the water source are the main factors considered affecting on the composition and characteristics of NOM in the aquatic systems (Leenheer and Croué, 2003; Nebbioso and Piccolo, 2013).

There are many ways for categorising NOM. NOM can be categorised into two groups depending on the origin: allochthonous DOM comes from soils and plants and autochthonous DOM is organism by-product within the water body. NOM can be operationally separated into two phases, dissolved organic matter (DOM) and particulate organic matter (POM), pass through a 0.45-um pore filter (Nebbioso and Piccolo, 2013). The former appears a very fine colloidal suspension whist the latter is blocked after filtration. Based on chemical composition, DOM includes mainly dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and dissolve organic phosphorus (DOP). In the drinking water industry, the quantity of NOM is generally inferred from DOC and total organic carbon (TOC) measurements, since 90% of NOM is present as DOC (Amy et al., 1992). DOM can also be generally classified into six groups, including humic substances, fuvlic substances, carboxylic acids, carbohydrates, amino acids and hydrocarbons (Leenheer and Croué, 2003). Relevance to DOM in drinking water treatment, DOM is generally separated into two groups, humic fraction and non-humic fraction. The former substances have large aromatic carbon contents and arise largely from biological activity in catchment or terrestrial systems. The latter is relatively more polar properties and derived typically from biological precursors such as proteins and carbohydrates. The origin of the non-humic compounds is attributed to fresh-water autochthonous life; the sources of the humic materials are believed to be from terrestrial systems (Stolpe et al., 2010). Boehme and Wells (2006) reported that there is a strict correlation between the size fraction and the composition of the colloidal phase, with protein-like materials occurring primarily in the smaller size fraction and humic-type materials in the larger size.

DOM transportation and transformation have also been obtaining interest, since accumulated knowledge for understanding DOM characterisation is not just emphasised on DOM itself and its fate, but also on the constituents in the system, such as its environmental domains and surrounding biota.

The major fraction of surface water DOM is allochthonous inputs, which are commonly from terrestrial soil organic matter (SOM) and its increases, is because of SOM undersetting specific transportation and transformation into environmental aquatic phases. Goñi et al. (2003) study have also demonstrated surface water DOM shares similar genesis with SOM, as these authors found that the correlation between river sediment and terrestrial plants are highly strong according to the results of δ13 measurements. Also, other studies associated with different analytical techniques support the hypothesis that the composition of surface water DOM depends on the transformation of plant materials into humic-like substances (Nebbioso and Piccolo, 2013). Nevertheless, unlike SOM deposited as solid, DOM transport and transformation is in larger amount than SOM, since it is dissolved in water body with greater dynamics. Also, the availability of DOM for enzyme activity, oxidation, or metal complexion is significantly greater than SOM. The correlation between riverine and marine DOM has also been studied, in order to better understanding insight into the DOM transportation in the environments. Jaffé et al. (2004) focused on the analysis of estuarine systems distribution and found that riverine DOM is highly preserved in the ocean, despite structural rearrangements, which occur mostly because of changes in salinity. In a review of recent breakthroughs in arctic biogeochemistry, Dittmar and Kattner (2003) observed that terrestrial DOM is persistently refractory in DOM pools of the Arctic Ocean, being the result of long-term carbon sedimentation and the end product of a natural carbon sink.

Within nature, DOM is harmless; however, increase in its concentration introduces potential risks to aquatic ecosystem. As a general concern in the course of water treatment and recycle process, its amount and characteristics variation introduces challenges for the water treatment industry.

Although being an important source of energy in ecosystem, a continuing increase in the presence of DOM influences additionally on natural aquatic systems and leads to potentially cascading impacts on eco-toxicological issues (Al-Reasi et al., 2013; Cui et al., 2013). Its export affects not only organic pollutants mobility and bioavailability, but also being referred as strong chelating agents for metals, it influences on inorganics solubility, transport and toxicity, and also microbial community alternation due to as nutrient resource for microorganism growth (Al-Reasi et al., 2013; Cui et al., 2013). As DOM composited with various functional groups and variations in charge densities, it serves pH-buffering and impacts on ions distribution between aqueous and solid phases.

2. Dissolved organic matter in stormwater

During storm/rain events, DOM enters into natural water sources increases significantly due to flushing of the surrounding organic matter from catchment and/or terrestrial systems. Positive relationships between DOM concentrations and river discharge have been reported (Sharp et al., 2006). Stormwater discharge enters directly or indirectly into the environments having potential impacts on ecosystem balances as well as human health (Chong et al., 2013; Tang et al., 2013). Since stormwater discharges contain various pollutants, including pathogen, heavy metals, nutrients and synthesised organic matter are mostly and typically assigned from moderate to high risk levels. Additionally, stormwater commonly carrying with various levels of suspended solids, terrestrial organic material and vegetation is generally discharged into rivers and transported continuously to oceans (Eriksson et al., 2007; Worrall and Burt, 2007). These NOM also pose pivotal effects on the geochemical carbon balance (Monteith et al., 2007).

Pollutant loadings are most likely to originate from surface runoff or waste discharges from household, industrial or agricultural processes. They are increasingly introduced into the environments and lead to serious problems for safe drinking water production. Hurst et al. (2004) reported an up to 40% increase in DOC concentration is found in surface water because of surface runoff and near surface lateral flow. Yoon and Stein (2008) reported that natural catchments are as sources of background levels of storm-water metals, nutrients, and solids. These inputs once enter into aquatic system can distribute to different degrees throughout the environment and thus remain within solution or sediment to interact with organics, inorganics and microorganisms. Synthetic contaminants such as pesticides and biocides applied for agricultures, for instance, have been detected as leak pollutants (Skarzynska et al., 2007; Burkhardt et al., 2011). A couple of reports given from Baun et al. (2006) and Eriksson et al. (2007) indicated that there are hundreds of different organic and inorganic chemicals which have been clarified as potential pollutants in stormwater. Owing to some of these presenting poorly aquatic solubility, their final repository have been observed in environmental solid matrices, and particular in sediments, where eco-toxicological impacts are closely regulated by sorption with the associations of bioavailability (Cui et al., 2013). Since sediment performing as integrators of inputs within a catchment, it has been considered to be excellent archive of environmental contaminants, which generally can adsorb and persist over time. The assumption is the chemicals reached the environment are totally available for organism uptake (Katayama et al., 2010). However, the partitioning of chemical contaminants associate with various environmental phases, which are divided into separated components based on the particle size or chemical qualities (Cui et al., 2013). For instance, it has revealed that due to the presence of DOM, competition between DOM and bacteria can occur to cause bioavailable decrease (Robinson and Novak, 1994; Katayama et al., 2010). Harms and Bosma (1997) have also indicated the binding between chemicals and DOM can be either reversible or irreversible. The amount of chemicals uptake by biota is less than the total concentration in sediment thus resulting in bioavailability reduction (Katayama et al., 2010). Strong hydrophobicity of hydrophobic organic carbons (HOCs) is an additional critical factor for assessing bioavailability of HOC-contaminated sediments, since chemicals must form as freely dissolved to be bioavailable because only free molecules enable to cross a cell membrane and lead to a biological response (Ehlers and Luthy, 2003).

Moreover, DOM carried in stormwater can result in its increase in surface waters, which may have also cascading impacts on human health through the drinking water supply system, since its presence may increase toxic disinfectant by-product (DBP) formations and the levels of synthetic organic contaminants sorbed to DOM entering water treatability. Avellaneda et al (2009) represented a report consists of rainfall, flow, and runoff samples measurement covering over 26 storm events, and demonstrated the total suspended solids (TSS) is quantified higher in magnitude compared with other pollutants. Beck and Birch (2012) observed the interactions between TSS and metal are stable over inter-annual periods. These authors also determined accurate estimations of pollutant loading according to the relationship between TSS and contaminants and monitoring TSS and flow (Beck and Birch, 2013). An earlier study proposed by Leecaster et al (2002) have mentioned that a 20% trend of the TSS deliveries can be observed when only three events sampling per year. Detailed mechanistic insight into the interactions between DOM and emerging contaminants has, hence, also been received attention recently in order to enable to evaluate the bioavailability of the contaminants in the environments and their fate during the drinking water supply management.

Recent research into the influence of DOM composition and character has focused on the seasonal changes observed in source waters. Sharp et al. (2006) determined the impacts of seasonal changes on treatment processes and found strong influences of rainfall on changes in DOC concentration. Fabris et al. (2008) confirmed the presence of NOM in source waters undergoes seasonal changes and depends on the specific catchment characteristics and combination of hydrological and climatic factors. A study of the effects of seasonal variation on DBP formation was conducted by Courtis et al. (2009). These authors found that trihalomethanes (THM) formation is more prevalent in summer months because of higher temperatures.

Stormwater management is not only to focus on its discharges causing potential environmental impacts but also is to consider stormwater as an alternative water sources for indirect potable reuse. With the concerns of potential environmental impacts, as an alternative water sources for indirect potable reuse, stormwater quality and discharges has drawn a lot of attention, however, knowledge of stormwater-associated DOM characterisation are still limited. Current knowledge about urban stormwater management, potential public risks and monitoring developments would be briefly reviewed in order to provide better insight on the stormwater quality evaluation (Francey et al., 2010).

Limitation of water sources, unpredictable rainfalls as well as population growth, are the main factor to lead to increase demand and pressure on water sources in urban areas. Consequently, considered as a new and alternative water source, stormwater reuse has gained interest recently. Urban runoff has been safely treated for non-potable use, such as utilised for toilet flushing, car washing, agricultural irrigation and industrial applications

(Hatt et al., 2006, Mitchell, 2006; Mitchell et al. 2006; Vanderzalm et al., 2014). Vanderzalm et al. (2014) has summarised current stormwater harvesting programs involved in quality monitoring and treatment requirement, including not only each state in Australia but also compared and contrasted to other countries over the world. In Australia, treated stormwater for both non-potable and potable use have also been reported (Page et al., 2013; Vanderzalm et al., 2014).

However, due to various factor impacts, such as climate changes (e.g. dry and wet weather, temperature, rainfall intensity), anthropogenic activists (urbanization and industrial processes) as well as surrounding land uses (agricultural applications and sewage utilisations), and also open channels/streams or pipes designs, variability of stormwater quality and quantity is very variable and dynamic (Zgheib et al., 2011, 2012). Different sites also contribute various orders of magnitudes. Tang et al (2013) confirmed land use plays an important role in affecting the stormwater toxicity in accordance with bio-analytical tools investigation. Results of stormwater DOM characterisation varies depending on weather, dry and wet conditions (Chong et al., 2013).

As a result, development of effective stormwater treatment system is full of challenges, appropriate and better treatment designs, techniques and facilities are vitally needed. It is compulsory to remove various hazards and optimise water quality so as to meet the requirements for the intended use or for higher value use, to meet the health based targets for potable use.

Hence, there is a requirement to improve our knowledge of DOM characteristics in source water, how that composition and character varies over the seasons and flow conditions and how this impacts on drinking water management. Analytical capability for identification of individual DOM compounds is limited because of the complexity and variability of DOM species. Prediction of DOM characteristics is challenging. Water treatment industry must have adequate analytical techniques for DOM characterisation to keep the operation of the water systems in response to the constantly changing environmental conditions.

3. Dissolved organic matter in water treatment

The presence of DOM is problematic from a water treatment perspective, since DOM is responsible for colour, odour, hazardous DBP formation and microbial activity. Every effort that has been taken by the drinking water industry was mainly focussed on DOM removal processes and how to optimise the process.

DOC measurement has traditionally been used to infer the quantity of total NOM concentration in the drinking industry (Amy et al., 1992). Other general water quality parameters, such as colour, turbidity, the absorbance of ultraviolet light at 254 nm (UV_{254}) and the specific UV absorbance (SUVA) are also widely used to indicate the performance of water treatment processes, before and after each process on both the concentration and character of DOM (Leenheer and Croué, 2003). The SUVA is an indicator of the organics and is also a useful treatment indicator to determine treatability of DOM, including coagulation requirements and the potential for coagulation processes to provide effective removal (Korshin et al., 2009). However, these analyses provide only limited information regarding DOM characteristics with respect to drinking water treatment process design and optimisation. Understanding and management of DOM removal has remarkably improved over the last decade, largely due to advancements in analytical technique development to improve organic characterisation techniques, which have the ability to isolate or separate complex DOM mixture into different fractions based on physiochemical properties prior to analysis as pre-treatment step. Most studies focused on DOM characterisation have provided greater insight into the knowledge of DOM composition, structures and reactivity, and some of them have also provided improvement to the water treatment practice, particularly applying the knowledge at the operational level of managing water treatment plant (Matilainen et al., 2011).

Coagulation processes, membrane filtration, advance oxidation and granular activated carbon (GAC) systems have been successfully demonstrated and employed to remove undesirable substances during water treatment. The presence of DOM can, however, have various adverse impacts on the effectiveness of treatment operations, such as membrane fouling, filters clogging and uptake of adsorption sites within GAC systems.

There is a fairly large portion of the available literature focused on DOM removal by the conventional water treatment processes consisting of coagulation, flocculation and sedimentation, since this is still the most widely applied process for treatment of water both in Australia and worldwide. Most recent studies with the focus of organic characterisation applications have indicated and confirmed that no coagulation processes could achieve total DOM removal (Chow et al., 2008; Korshin et al., 2009; Ho et al., 2012; Sanchez et al., 2013). These studies have also allowed understanding of the types of NOM that can be removed by coagulation and have also determined when water quality will prevent further NOM removal by conventional treatment, regardless of optimization of the coagulation process (Chow et al., 2008; Fabris et al., 2008). These studies have also led to the development to new characterization techniques to improve DOM removal effectiveness. Sanchez et al. (2013) reported approximately 50% of humic-like fluorophores are the most amenable component to be removed, whereas about 30% of protein-like components are removed in a full scale water treatment plant with coagulation – filtration processes. Drikas et al. (2011) has also shown the performance of magnetic ion exchange (MIEX) pre-treatment to microfiltration or conventional coagulation treatment over a 2 year period.

Subsequently research extends to assess removal of DOM by alternative treatment processes. Ho et al. (2012) compared and contrasted the performances of six types of water treatment processes on the removal of DOM from an Australia water source, including alum coagulation, MIEX resin treatment, chlorination, ozonation and powdered activated carbon (PAC) adsorption as well as biological sand filtration. This case study has identified which processes may be useful for implementing as upgrades to current treatment plants, and their limitations. Effectiveness of DOM removal benefits also DBPs reduction, since DOM is confirmed as a source of DBP formation. Warton et al. (2007) confirmed that MIEX treatment results in reduction of THM formation potential of the treated water. Ozone and activated carbon absorption are other water treatment strategies implemented in Australia that have advanced for DBPs formation research (Kristiana et al., 2011).

4. Dissolved organic matter in water distribution

Ideally, the quality of treated water it leaving from the water treatment plant should be stable to the customer tap for consuming. In reality, substantial changes can occur to treated water as a result of complex physical, chemical and biological reactions. Reservoirs, storage tanks, hydraulic appurtenances, pipes and pumps are also the potential operational factors relevant on treated water quality degradation.

DOM has been commonly detected in drinking water distribution system surveys and has also been demonstrated to associate with biological activity resulting in water quality degradation (Zacheus et al., 2001; Lipponen et al., 2002; Beech and Sunner, 2004; Chow et al., 2009; Motzko et al., 2009; Zhang et al., 2009; Bai et al., 2015). The presence of DOM in distribution system can also be one of the principal causes of microbes' growth and regrowth (Shaw et al., 2014). Harmful DBP formation and disinfectant residual decay have also been suggested as a result of interaction between disinfectant and DOM passage throughout water distribution system (Wilczak et al., 2003; Shah and Mitch, 2012; Kristiana et al., 2013; Shaw et al., 2014).

Disinfection is virally essential for control of waterborne infectious disease. Maintaining a disinfectant residual is also a protection against microbial contamination in drinking water distribution system. Chlorine is the most commonly disinfectant in the world. Chlorination has been used to ensure the safe supply of drinking water for over 100 years and was introduced into South Australia in 1953. With the discovery of THM as a by-product of chlorination in 1974 (Rook, 1974), attention around the world focused on the cause of formation of this and other DBP compounds arising from chlorination (Hrudey and Charrois, 2012). This has led to many studies into understanding the precursors forming DBP with significant focus on the key precursor - DOM. Understanding DOM and its reaction with chlorine, developing ways of controlling the reaction to minimise DBP and identifying effective processes to remove DOM have been the key focus of researchers and utilities worldwide (Korshin et al., 2009).

One mean of avoiding high level of chlorinated DBPs formation, such as THM, has hence been to use alternative disinfectants. Chloramination to form monochloramine has been one of the most popular approaches. The benefits of monochloramine include its greater persistence in long distribution systems, greater stability to consumers and lower DBP formation. Chloramine as a secondary disinfectant has been widely used in many drinking water utilities to comply with DBP regulatory limits. Reduction DBP formation in the US, use of chloramine in place of chlorine as a disinfectant has been a popular strategy (Richardson, 2003). Chloramination was introduced into country supplies in South Australia in the early 1980s to ensure effective disinfection to prevent the occurrence of the amoeba Naegleria Fowleri. However, evidence that nitrogenous DBP such as N-nitrosodimethylamine (NDMA) which is a probable carcinogen can also be formed (Charrois et al., 2007; Luo et al., 2012). Krasher et al. (2013) has summarised major findings over the last decade related to nitrosamines in drinking water, with a particular focus on DBP formation.

Association of chloramine residual control and maintenance is also a major challenge to a water authority's ability to deliver high quality water to the customer tap. Although offering greater stability over chlorine, chloramine still degrades over time. Biofilm formation, bacterial regrowth and nitrification occurrence can contribute to the loss in disinfectant in chloriminated systems. Understanding mechanism behind chloramine residual decay is hence important requirements for distribution system management strategies (Wilczak et al., 2003). Nitrification is the most important pathway for chloramine residual loss. Nitrification occurs when free ammonia is oxidised to nitrate and subsequently nitrate by microorganisms. These substances formation may lead to non-compliance of the levels set in the drinking water guidelines, and adversely affect the disinfectant residual needed to ensure a safe drinking water supply (Hoefel et al., 2005).

High levels of DOM in treated drinking water increases the rate of moochloramine residual decay (Wilczak et al. 2003). The auto-decomposition rate of monochloramine is known to increase with decreasing pH, inorganic carbon content and temperature, as well as initial chloramine concentration. Several studies have investigated the association between the presence of organic carbon and bacterial activity in drinking water distribution system. LeChevallier et al. (1990) and Volk and LeChevallier (1999) have suggested that the reduction of nutrients, organic carbon is essential for bacterial regrowth inhibition. Shaw et al (2014) have also indicated that the reduction of organic components resulted in bacterial biofilm regrowth inhibition by comparing four different water treatment processes. This study also illustrates that the importance of disinfectant residual control in treated water, since biofilm converge towards a common stablished community equilibrium regardless of the effectiveness of the treatment method at the beginning of the system when the disinfectant residual has decayed within the distribution system. Understanding the factors relevant on controlling chloramination and chloramine residual decay was undertaken at the same time, such as monochloramine degradation through reaction with organic and inorganic substances in water and auto-decomposition during travel/retention time in distribution systems (Richardson and Postigo, 2012). Higher levels of DOM in treated water increase the rate of disinfectant residual decay. In addition, loss of chloramine residual makes the distribution system more vulnerable to microbial contamination. Distribution systems must hence be monitored continually to verify a chloramine residual. Chen and Valentine (2007) have determined the association between NDMA formation and hydrophobicity and acidic fractions of DOM. These authors reported that the hydrophilic acid fraction of DOM tends to form more NDMA than the hydrophobic fraction of DOM and that the basic fractions tend to form more NDMA than the acidic fractions. These observations are associated with the previously reported higher nitrogen content in the hydrophilic and basic fractions of DOM (Croué et al., 2000). Dotson et al. (2009) also found that dissolved organic nitrogen (DON) contained in DOM isolated from surface waters is a source of nitrogen in the formation of NDMA, and that the formation of NDMA is greatly increased when waters with high DON content are chloraminated. The mechanism between the monochloramine decay and the DOM has also been studied. Woolschlager et al. (2001) showed that the monochloramine in the distribution system can react with DOM with a series of chemical mechanisms. DOM reacts with the monochloramine to produce ammonium, which is oxidised to produce nitrite and then nitrate (AWWA Research Foundation 2004).

The management of chloramines decay and DOM presence and the prevention of nitrification are thus critical and of utmost importance for water utilities with chloraminated drinking water distribution systems.

5. Dissolved organic matter measurement and characterisation

The challenge of DOM analysis is due to incomplete dissolution, improper separation, extreme heterogeneity as well as complex composition and characteristics of the analytes. DOM qualification and characterisation from a range of environmental and drinking water supply aquatic samples have been recurrently demonstrated that they are associated with their physiochemical properties, such as hydrophobicity, aromaticity, molecular weight distributions as well as acidic, basic and neutral contents. This session is to provide a briefly overview on the various approaches applied for DOM molecular isolation and characterization, and also on current advanced analytical instruments performances, strengths as well as limitations.

5.1. DOC, UV_{254} and SUVA

Conventional DOM analyses, such as DOC, UV_{254} and SUVA (normalization UV_{254} to DOC) can provide simple and valuable DOM evaluation, and SUVA can be used to indicate the efficiency of DOM treatability. DOC and UV_{254} are the two fundamental quantitative measurements of dissolved organic constituent in a water sample (Leenheer and Croué, 2003). SUVA offers a more practical characterisation of DOM. All these direct measuring techniques share similar strengths and weaknesses.

DOC measurement is generally utilised as a proxy for the quantitative total DOM concentration. The principle of DOC method is to oxide organic carbon at high temperature or under severe oxidation to form carbon dioxide (CO_2). The amount of carbon present in the sample is proportional to the amount of carbon detected from a CO_2 calibration curve, expressed in mg/L.

Ultraviolet-visible (UV-vis) absorbance spectroscopy is one of the simplest analytical methods and has been used as a water quality indicator by the water industry for many years. Absorbance spectroscopy is the measurement of the light beam attenuation after passage through a medium of interest (Matilainen et al., 2011). A series of surrogate parameters have been developed and applied as water quality indicators based on UV absorbance measurements, either at a specific wavelength determination, or multiple wavelengths detection. Their utilisations associate with water sample characterisation as well as water quality changes monitoring (Leenheer and Croué, 2003; Helm et al., 2008). UV spectroscopy measurement is usually taken at a wavelength of 254 nm (UV_{254}), since which used as a standard parameter represents particularly the aromatic character of the organic species. However, not all NOM components are detectable this wavelength. A single wavelength, 254 nm (A_{254}) considered as a surrogate parameter, is commonly employed in water quality analysis, such as DOC concentration measurement, chlorine demand estimation and DBP formation prediction (Chow et al., 2007; Roccaro and Vagliasindi, 2009). The differential absorbance ($\Delta A\ \lambda$) between before and after an event and/or treatment process has also been considered as an indicator of DOC concentration alternations. A good correlation with regression values of $R^2 > 0.92$ is achieved when studied on Australian waters (Chow et al., 2007). A_{254} has been used as a comparison tool against other surrogate parameters to assess its suitability to apply as NOM reactivity surrogate (Roccaro and Vagliasindi, 2009). However, recent work has suggested A_{254} may be more representative of aromatic DOC groups rather than total DOC concentration (Korshin et al., 2009). Besides absorbance at 254 nm, some studies have also applied absorbance at other wavelengths for interference minimisation and the absorbance of the compound of interest extension (Helm et al., 2008; Korshin et al., 2009). Current researches placed emphasis on the differential absorbance at 272 nm rather than 254 nm. The relationship between A_{272} and DBP formation has found to be significantly correlated (Korshin et al., 2002, Roccaro and Vagliasindi, 2009). Some studies have also focused on the wavelengths other than 272 nm to minimise the effect from interfering compounds. For example, differential absorbance at 280 nm (Iriarte-Velasco et al., 2006) and 405 nm (Fabbricino and Korshin, 2005) were employed for analysis to minimise the interference of quenching agents, which were added to quench residual chlorine in the water sample. In the work by Iriarte-Velasco et al. (2006), A_{280} shows better colrrelation between DOC and THM than A_{254}. Korshin et al. (2009) has utilised A_{220} and A_{272} due to their association with different fractions of DOM. The accuracy of single wavelength detection has currently been questioned, since it provides limited information and also gives errors due to artificial wavelength selection.

SUVA is calculated by dividing the UV absorbance of the sample by the corresponding DOC measurement and the multiplying by 100. SUVA is an indicator used to determine the relative aromaticity of humic fraction. It also gives an indication of the amount of humic fraction against non-humic fraction in the sample. This parameter has been proven as a reliable predictor to evaluate coagulation requirements and performance, NOM removal effectiveness (Korshin et al., 2009; Ho et al., 2013). Higher SUVA is associated with greater treatability using coagulation (Korshin et al., 2009). A good correlation between SUVA and DBP-formed potential has also been reported by Korshin et al (2009) but not been confirmed by others (Ates et al., 2007). Ates et al. (2007) indicated that SUVA does not correlate well with the DBP formation, such as THMs, suggesting that SUVA does not capture the reactive sites on NOM fractions responsible for DBP formation in low SUVA and DOC waters.

Although simple, the main limitation of these methods as an analytical tool is that it only provides one-dimensional value, but does neither reveal further DOM characters, such as compositions, nor even possibly give insight into the molecular characteristics of DOM in the sample. A single wavelength parameter, UV_{254} tends to be representative of aromaticity prediction, which is correlated with DBP formation estimation, whereas rather than total DOC information, these techniques appear to be incomprehensive and inaccurate to predict other fractions, such as non-conjugated and/or non-aromatic structural organic matter (Her et al., 2008; Yan et al., 2012).

Due to the chemical complexity and heterogeneous nature of DOM, characterisation of DOM can help reduce the molecular heterogeneity of DOM and provide better insight into its chemical structure and composition. Adequate analytical techniques for DOM characterisation requires to optimise and maintain the operation of the water supply and treatability adjusted to constantly changes in environmental conditions. This leads to the development of extending the need of DOM characterisation, a series of advanced analytical techniques, including resin fractionation, fluorescence excitation-emission matrix (EEM) and size exclusion chromatography (SEC) have been widely and dominantly used in water research and also water industry. The results generated from these instrumentations are associated with hydrophobicity, molecular weight and aromaticity, which have been considered as being more informative DOM character indicators enabling to leap to deliver more insight on chemical qualitative and structural features of DOM.

5.2. Resin fractionation

Resin fractionation (RF) is a technique used to fractionate DOM into different components based on polarity and hydrophobicity. The RF is that its process can be changed by different resin applications (Croué et al., 1994). Traditionally, organic fractionation is generally used to isolate organic matter (Croué et al., 1994). Croué et al. (1994) used this technique by applying the two columns of XAD-8 and XAD-4 resins for isolation of four fractions of organics, i.e. humic acids, fulvic acids, hydrophobic neutral (adsorbed on XAD-8 resins) and hydrophilic acids (adsorbed on XAD-4 resins from the effluent of XAD-8 resins). There are many drawbacks of this conventional fractionation, such as time consuming, high level of skill requirement, advance detection requirement and difficulties in result interpretation. Rapid fractionation technique based on the full fractionation scheme developed by Chow et al (2004) is able to improve not only time assumption, but also to simplify the operational conditions. Rapid fractionation has been widely used in helping understand water treatment processes, including coagulation and advanced oxidation (Fitzgerald et al., 2006; Liu et al., 2008, 2010; Bazrafkan et al., 2012).

The principle of rapid fractionation technique is to measure DOC concentration before and after contact with the resins DAX-8, XAD-4 and IRA-958 (Chow et al., 2004). This is specifically designed to study water treatment processes. Based on subtraction of the DOC concentration of subsequent resin effluents, the organic carbon concentration of four fractions of DOM can be determined.

Very hydrophobic acids, (adsorbed by DAX-8)

Slightly hydrophobic acids (adsorbed by XAD-4)

Charged hydrophilic (bound to the anion exchange resin IRA-958)

Neutral hydrophilic (passed through all columns)

Results can be presented as actual DOC concentrations of each fraction or as a relative percentage. Hydrophobic acids are predominantly composed of higher molecular weight humic substances, including humic and fulvic acids. Hydrophilic acids are ascribed to proteins, amino acids and anionic polysaccharides, and neutral fractions ascribed to carbohydrates, ketones and alcohols as well as aldehydes.

5.3. Fluorescence excitation emission matrix (EEM)

Over the decades of research applying fluorescence spectroscopy to analytical studies, the simultaneous collection of florescence data over a full range of different excitation and emission wavelengths instead of simple measurements of selected wavelengths have been widely applied for DOM characterisation (Matilainen et al., 2011). Since NOM is the main contributor for fluorescence responses, fluorescence methods have been widely used for DOM analysis in waterways. Fluorescence excitation emission matrix (EEM) spectroscopy has been drawn much interest, because it is capable of DOM analysis in a wide range of water samples and its specific spectrum obtained from each known fluorophore.

EEM has emerged as a rapid characterisation tool for DOM determination. This technique has been widely applied for determining DOM in a number of water treatment areas (Wei et al., 2013; Sanchez et al., 2013; Jiao et al., 2014; Xing et al., 2015) and has been suggested as a potential online monitoring tool for real-time water quality analysis (Henderson et al., 2009). EEM can also provide semi-quantitative characterization information of dissolved organic matter. Four components, humic-like, fulvic-like, microbial protein-like and aromatic protein-like substances have been identified from fluorophores described in the literature (Chen et al., 2003; Henderson et al., 2008). Location and intensity change of the peaks in EEM before and after coagulation have been used to determine the effectiveness of this process (Bieroza et al., 2009). EEM has been demonstrated is capable of determining environmental samples. Wei et al. (2013) used Micro-volume resin fractionation coupled with EEM to assess the impacts of urban pollution on natural source waters. Chong et al. (2003) also used EEM for characterisation of DOM in stormwater. Xing et al. (2015) extended the EEM application for determining the coagulation performance for extracted allogenic organic matter. These authors found that this technique is sensitive enough to indicate the changes in aromatic protein compositions.

Although EEM provides a wealth of information about DOM, data exploration and information extraction are challenged. Multivariate data analysis techniques need to be applied to study the complex mixture of fluorescence signals. The initial stage in data processing is to standardise the data, which might include correcting the instrument bias and the inner filter effects. Method validation and calibration development are additionally required to ensure data accuracy.

5.4. High performance size exclusion chromatography (HPSEC)

Liquid chromatography (LC) is an analytical technique used in several different research areas to aid the separation, qualification and quantification of analytes in various matrices. A chromatographic process can be defined as the separation of a mixture of analytes by sequential elution. This technique in conjunction with various detectors has widely been used for the sensitive and selective determination of various chemical compounds, organic and inorganic substances. Effort has been made to improve its efficiency and performance. Smaller columns, higher flow rates, and higher working temperature are applied to increase the diffusion, but then a pressure problem occurred and thus limitations were soon reached. Considering all these purposes, a completely new system design with advanced technology in the pump, instrumental performances have been improved and called high performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC). These improved techniques allow higher resolution, better peak shape, reproducible responses and the speed of analysis. Their columns can be used again without repacking or regeneration, with better control of parameters affecting the efficiency of separation, easy automation of instrument operation and data analysis.

HPLC technique, particular reverse phase HPLC has been reported as a characterisation tool for NOM (Owen et al., 1995; Xing et al., 2012b). This technique is potentially useful for characterising NOM in relation to the water treatment process. A recent work conducted by Xing et al. (2012b) was to investigate reverse phase HPLC as a rapid analysis of the hydrophobicity and hydrophilicity of DOM. These authors found that the changes in peak areas generated from HPLC are correlated positively with DOC and UV measurements for DOM removal, and the ratio between hydrophobic and hydrophilic peak areas can be used for quantification of NOM treatability.

With size exclusion column application, HPSEC separates molecules based on molecular weight (MW) size. An original chromatogram shows the signal collected by a detector during the solution process as a function of time. This profile can be standardised as a plot of signal against apparent molecular weights using molecular weight standards to analyse molecular distribution of a sample. Molecules that are larger than the pore size of the packing materials in columns are not absorbed strongly and elute first at the void volume, where smaller molecules can penetrate throughout the porous stationary phase and are attenuated, corresponding to a higher retention time. The average retention time depends generally on the effective size of the compounds (Her et al., 2002). This technique has proved to be useful in characterising organic compounds and determine the performances of water treatment processes for DOM removal (Chow et al., 1999; Chow et al., 2008; Korshin et al., 2009, Liu et al., 2010; Xing et al., 2012a). In addition, the application of HPSEC to determine the molecular size distribution of NOM has shown significant correlation with disinfectant demand (Chow et al., 2008).

A series of analytical detectors, including Fourier transform infrared (FTIR), online DOC, mass spectrometry (MS) and absorbance based optical analysers, UV-Vis, photobiode array detectors and excitation emission florescence have been well coupled with HPLC/HPSEC techniques for DOM characterisation (Her et al., 2002; Allpike et al., 2007; Wu et al., 2007; Her et al., 2008a,b; Liu et al., 2008, 2010; Chow et al., 2008; Korshin et al., 2009; Ho et al., 2012, Yan et al., 2012; Nebbioso and Piccolo, 2013).

Mass spectrometry (MS) as a detector has been traditionally coupled with LC for DOM analysis for a history. Nebbioso and Piccolo (2013) have concluded current status of DOM characterisation in sea waters and mentioned that this detector appears becoming a standard method for DOM determination, since dissolved DOM appears mainly ionized, especially in negative charged mode. Electrospray ionization (ESI) source, as being negative mode, has been frequently chosen for MS analysis of dissolved humic substances. Direct and reverse phase adsorption (Mawhinney et al., 2009) as well as SEC (Koch et al., 2008) have been found to interface well with negative-mode ESI-MS. A series of MS detectors, such as ion trap, quadrupole time-of flight (TOF) or triple-quadrupole are capable and have resulted in outstanding outcomes. These detectors selection depends normally on the type of information request. In fact, there is a general consensus that ion-trap mass spectrometers have superior sensitivity whereas quadrupole instruments, especially those with the triple configuration, have better mass accuracy, and triple quadrupoles are also generally capable of high-resolution analysis.

HPLC-MS applied for DOM transport investigation has conducted and resulted in indicating that lower MW compounds are predominantly found in hydrophilic fractions, whereas higher MW constituents are identified in hydrophobic fractions (Liu et al., 2011).

Another innovative introduction, Guo et al. (2009) has combined MS and pyrolysis to understand the biogeochemical cycling of estuarine and marine organic matter.

The main drawback of these detectors is that they do not enable to characterize non-ionized compounds. An additional limitation of MS is irreproducible result, owing to molecular interferences as a result of complex inhomogeneous and supramolecular associations. Consequently, despite of the above mentioned advantages, the disadvantages cause more attentions to prevent reliance on MS detection alone to achieve structural identification and quantification of DOM.

Substantial analytical improvement has recently achieved from the applications of UV absorption and fluorescence detection, owing to their qualitative and quantitative reliability and reproducibility. Either used as a single technique or with other advanced separations / detect techniques presents opportunity to deliver outstanding results on DOM characterisation. However, extract DOM properties information from these analyses is challenging. The responses (signals) can be univariate, such as a single wavelength, where the chromatogram is an array of number of vector, however, a full UV or EEM detection, where the chromatogram consists of a data table, with the rows containing the full spectra collected during the elution process and the columns are the elution profiles at each channel of the response detector.

HPSEC coupled with fluorescence EEM for DOM analysis have been reported by a couple of studies (Wu et al., 2007; Li et al., 2013). Comparison between UV-Vis absorbance and 3D EEM florescence detections, Wu et al. (2007) demonstrated that EEM profile gives more insight the characteristics of NOM, including chemical qualification, structural features and molecular size distribution. Li et al. (2013) indicated that HPLC/HPSEC with multi-excitation/emission scan provides informative insights into the EEM spectra and is a promising technique for research on fluorescent DOM, especially proteins and humic-like substances.

Comparison between UV-Vis absorbance and on-line organic carbon detectors (OCD), Her et al. (2002), Allpike et al. (2007) and Huber et al. (2011) demonstrated that OCD provides better quantitative measurements. A detection limit of 0.1 mg/L was achieved using HPSEC with a modified commercial DOC-analyser (Her et al., 2002). Allpike et al. (2007) also suggested an OCD based on UV absorption is sufficient enough for most natural waters. Three detectors, including organic carbon, organic nitrogen and UV-absorbance were coupled with SEC to characterise aquatic humic and non-humic substances (Huber et al., 2011). The pool of organic matter in a natural water sample was subdivided into six major sun-fractions, which could be assigned to specific classes of compounds: biopolymers, humic substances, building blocks, low MW acids, low MW neutrals and hydrophobic organic carbons. This study also improves the knowledge of aquatic humic substances characterisation.

UV-Vis detector and photodiode array detector (PAD) have, however, been the most widely coupled with HPSEC for DOM analysis. UV detector is a simple and sensitive technique and is frequently used for analysing the dynamics of DOM in aquatic systems. The concentration and chemical composition of DOM influence the absorbance intensity. Both absorbance based index interpretation and peak-fitting application have been used for data interpretation. They become useful when further breaking down the HPSEC-UV profile obtained by quantifying and characterising the components of DOM.

Based on fundamental organic chemistry principle, transitions of n or pi to the pi excited state is the basis for the detection of organic compounds using UV detector. The energies of different types of molecular orbits vary remarkably and allow the absorption peaks into the spectral region (200 – 700 nm) (Her et al., 2002; Yan et al., 2012). Aromatic compounds occupied the main portion in DOM is generally drawn the most attention. Electron-transfer (ET) band, benzenoid (Bz) band and local excitation (LE) band are the three bands in the UV region form pi – pi transitions, detected at 254 nm, 203 nm and 180 nm, respectively. However, functional groups associated with non-aromatic compounds are more sensitive to lower UV wavelengths. Carboxylic acid and esters are non-conjugated form display absorption maxima at 206 nm, and Nitrogen containing chemicals, amides is at 206 nm.

To extract information about DOM physiochemical properties from these HPSEC-UV profiles, apart from parameters developed at single wavelength detection, several indicators that proposed by more than one wavelength have been introduced and applied for current water research (Her et al., 2008; Korshin et al., 2009; Yan et al., 2012). These studies have also demonstrated that HPSEC coupled with multi-wavelength detection is more informative and reliable (Korshin et al., 20109; Yan et al., 2012). Not only does this combination provides more informative data, but also promotes additional and potential vital information concerning the properties of DOM with varying MW distribution.

The UV absorbance-based index is the ratio between these two wavelengths and provides information on the relative proportion between UV absorbance intensity of the functional groups and unsaturated compounds.

Kim and Yu (2007) suggested A_{253}/A_{203} correlates positively with DBP formation. A_{254}/A_{436}, A_{254}/A_{204} and A_{250}/A_{365} have also been indicated to be useful parameters for DOM analysis (Jaffé et al., 2004; Hur et al., 2006; Li et al., 2009). Jaffé et al. (2004) indicated the relative composition of autochthonous and A_{280} shows better correlation DOM can be esitmated using the abosorbance ratio of $A_{254}/A_{436.}$ Li et al. (2009) described that the aromaticity and A_{250}/A_{365} of fulvic acid fractions correlates well. Using absorbance slope index interpretation, Helm et al. (2008) proposed that $S_{275\text{–}295}$ appears to be a good indicator for DOM MW.

Focusing on interpreting absorbance-based indexes with HPSEC-UV data, the absorbance ratio of DOM measured at two wavelengths, A_{210}/A_{254} has been shown to be able to estimate relative contribution of fulvic acids and microbial biopolymers (Her et al., 2008). Since the wavelength 210 nm allows the detection of DOM functional groups (hydroxyl, carboxyl, carbonyl, ester and nitrogen-containing compounds) and the wavelength at 254 nm is the recognisable absorbance for the conjugated aromatic substituents (Her et al., 2008). The wavelength around 210 nm has also been considered to associate particularly with nitrate concentrations, which relates to nutrient content and microbial activities (Whitehead and Cole, 2006). An alternative absorbance ration, A_{280}/A_{350} has also been applied to evaluate the degree of DOM activation (Korshin et al., 2009). Yan et al (2012) has also indicated that slope at wavelengths above 365 nm, $S_{>365}$ is a good spectroscopic parameter used for varying AMW of DOM prediction. The absorbance slope index (ASI), also illustrated by Korshin et al. (2009) was conducted to evaluate DOM removal and DBP formation potential in different Australian water sources. Detection of DOM at 220 nm corresponds non-aromatic and aromatic compositions, nitrate absorption estimation as well as aliphatic substances, whereas the absorbance of active aromatic substances is dominantly at 254 nm, and NOM measured at 272 nm reflects DBP formation (Korshin et al 2009). These authors found that ASI plays similar role as $SUVA_{254}$ can be used for coagulant dose prediction and DOM reactivity in DBP formation.

A couple of respective molar mass averages, number-averaged molecular weight (Mn) and weight-averaged molecular weight (Mw) are calculated following the below equations (1) and (2) (Chin et al., 1994). The Mw/Mn ratio as termed the molar mass polydispersity (*p*) indicates homogeneity and heterogeneity (Chin et al., 1994).

Mn = sum of hi/ sum of (hi/Mi)

Mw = sum of (hi*Mi)/sum of hi

Where *hi* is the relative intensities at the Mw corresponding to the solution time (*i*), and *Mi* is the molecular weight of the analytes eluted at the retention time.

These parameters derived from HPSEC-UV profiles have been widely used as indications of both the effectiveness of an applied treatment process and also as measures of the ease of treatment of a particular water source (Hur et al., 2006; Fabris et al., 2008; Chow et al., 2008; Xing et al., 2012a; Drikas et al., 2011). Hur et al. (2006) suggested that Mw may be a useful approach for tracking DOM mixing processes, and Mn can be better for distinguishing different DOM compositions. Chow et al. (2008) have demonstrated HPSEC in conjunction with peak fitting can be used successfully to predict DOM removal in high SUVA water samples, and Xing et al. (2012a) confirmed this approach can also be sued for low SUVA water samples.

The relationship between characteristic molecular weight profile features and groups of chemical substances has been demonstrated according to the UV absorbance peaks in the variable MW distribution (Korth et al., 2004). Molecular weight regions from high to low describe as organic colloids, high MW humics, low MW humics, building blocks and low MW acids. The primary organic component found in natural source water is humic-like substances produced from the decay of natural biomass.

Location and intensity changes of peaks in the HPSEC-UV profiles before and after coagulation have been used to evaluate the performance of the treatment process. Recent studies have also demonstrated that the HPSEC technique is informative and reliable when used to assess water treatability by comparison between raw and treated water based on the HPSEC profiles after coagulation in drinking water treatment (Chow et al., 2008; Fabris et al., 2008; Liu et al., 2010; Xing et al., 2012a, 2012b) or applying a peak-fitting model to predict treatability (Chow et al., 2008).

Fabris et al. (2008) compared selected raw water and conventional treated drinking water sources from different countries. Results from the HPSEC-UV profiles showed that although Australian source waters have lower AMWs than Norwegian waters, a similarity in the MW distribution of recalcitrant DOM from both countries is received after coagulation treatment. These authors also found treated waters with residual higher MW organics require higher chlorine demand flowing coagulation. Jiao et al. (2014) reported, however, the absorbance intensity of middle molecular weight fraction, ranged from 1 kDa to 2 kDa is increased after coagulation at full-scale treatment facilities located in China.

Ho et al. (2012) conducted a comparative study of DOM removal with a range of treatment processes. The results of HPSEC-UV profiles showed that MIEX treatment is the most effective single process to remove a wide MW range of DOM. Similar result can also be observed when combined alum coagulation with PAC absorption, since the alum removes effectively higher MW compounds whereas PAC removes lower MW fractions.

The relationships between DOM MW size and DBP formation have been studied (Hua and Reckhow, 2007; Singer and Reckhow, 2011). Korshin et al. (2009) investigated the relationships between MW and DBP formation. Higher MW fraction displayed predominantly more hydrophobic, and enriched with aromatic rings appears to form DBP easily (Kristiana et al. 2010, 2013). Singer and Reckhow (2011) indicated lower MW substances are not liked to DBP formation, such as amino acids. Chow et al (2005) found that polysaccharides with MW above 10 kDa reacts insignificantly with chlorine to form DBPs. Current researches demonstrated lower MW distribution and less aromatic compounds are also linked to brominated DBP formations (Kristiana et al. 2010). These results in each sample being concentrated to a different degree depend on the actual behaviour of the NOM species present in the water samples. Moderate MW compounds (1 to 10 kDa) have been considered to be pressures to form DBP when bromine presents particularly (Kristiana et al., 2010). One of the similarities shared between high and low MW fraction is that both of them have been revealed involved in the DBP formation, although the former is obtained more attentions. Current studies reported the nitrogen atoms containing compounds are associated with DBP formations (Kristiana et al. 2010, 2013). Hydrophilic fractions contained nitrogen atoms have been demonstrated by Kristiana et al. (2013).

6. Conclusion

A wide array of DOM characterisation techniques have been developed which have provided considerable knowledge for understanding the impact of DOM on drinking water supply system. These techniques differ considerably in terms of analytical approach. The use of combinations of different methods is required for proper analysis of the fate of different fractions of DOM. A basic approach of tracking DOC UV_{254} and SUVA changes can be used to understand the changes of DOM. High performance size exclusion chromatography coupled with a full UV range (multi-wavelength HPSEC) can be used to obtain additional information on molecular UV absorbance, size distribution, molar mass and DOM reactivity. Effective data interpretation is hence required for the complex multi-wavelength HPSEC data processing, and to extents of exited approaches of easily looking at large amounts of data at once, new designs and displaying is also required to develop for visually checking the validity of apparent relationships.

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Chapter 3: **Chemometrics approach to enhance data interpretation of high performance size exclusion chromatography with multi-wavelength detection**

Introduction

In recent years, fluorescence excitation-emission matrix (EEM) and high performance size exclusion chromatography (HPSEC) have been widely and dominantly applied for water quality investigation (Matilainen et al., 2011; Yan et al., 2012; Wei et al., 2013). Since the outstanding outcomes generated from these advanced analytical techniques are associated with different physiochemical peripteries, which are informative dissolved organic matter (DOM) character indicators enabling to leap to deliver more insight into the quality and quantity of DOM to improve water supply management. In addition, along with substantial technical improvements, compared with history, current state of DOM measurements has been promoting shifted from general simple linear regression analysis to visualising analysis which required the support from the modern computing power. Instead of displaying as simple two-dimensional figures, such as bar and/or pie charts, box plots as well as scatterplots, raw data delivered from characterisation methods can be capably plotted as more informative contours filled with judicious colour and even three-dimensional (surface or contour) graphs (Liu et al., 2010; Yan et al., 2012; Wei et al., 2013).

HPSEC coupled with a multiple UV wavelength detector (multi-wavelength HPSEC) is not only a very powerful separation technique for multi-component mixture of DOM based on molecular weight distribution, but also provides additional DOM characterisation imparted by a multi-wavelength detection compared to traditional one wavelength detection. To capture a sample run, huge amount data is acquired and this requires an efficient exploration, an appropriate interpretation and an effective presentation for researcher to be able to utilise the analytical information. High-quality graphical work and statistical modelling are becoming a necessity tool. Useful analytical protocols are required to simplify data-rich multi-wavelength HPSEC profiles, yielding more insight into the key information that is of importance in water quality monitoring.

R software (version 3.1.0, R Development Core Team) is a free licencing arrangement and is a programming packing and it provides the flexibility of complex data interpretation. This well-developed programing package comes with an integrated suite for statistical computing and graphical displaying. Its application has been widely associated with medicine, business, scientific and engineering researches, since it provides a large, coherent, integrated collection of intermediate tools for data analysis, and also enables to produce well-designed publication-quality plots and graphs. The extensive set of built-in functions allows analysts to handle several datasets and build their own specific functions for their research needs. In this study, an open and free software program, R is introduced for multi-wavelength HPSEC analysis of chromatographic data.

1. Test case development

Several data sets were selected to build test cases to assess the potential benefit in applying chemometics approach on multi-wavelength HPSEC. All samples presented in this chapter have been fully detailed in the following two of chapters, **Chapter 4** and **Chapter 5**. Each chapter covered a water quality investigation case study of applying HPSEC coupled with a UV detector for determining changes in DOM in both concentration and character in water systems. Each case study was carefully selected based on their suitability for this work. **Chapter 5** describes an investigation of water quality change in an chloraminated drinking water distribution system, samples including prior to water treatment, during treatment processes and subsequent to distribution systems were selected to demonstrate the ability of using R software to extract additional analytical info to gain new knowledge in managing chloramianted distribution system. This similar approach was also applied to a couple of stormwater samples selected from **Chapter 4** which was a case study to understand the impact of DOM in stormwater on drinking water supply. Both case studies demonstrated the benefit of multi-wavelength HPSEC characterisation and obtained more information and knowledge of DOM.

2. Application of R software on EEM analysis

EEM which is a latter technique based on fluorophore analysis has emerged as a rapid characterisation tool for DOM determination. As reported earlier, this technique has been widely applied for DOM determination in a number of drinking water supply systems (Wei et al., 2013; Xing et al., 2015) and has been suggested as a potential choice of online tool for real-time water quality monitoring (Henderson et al., 2009). EEM can provide semi-quantitative characterization information of DOM (Her et al. 2004) and the data were normally shown as 3D contour (Wei et al., 2013).

Quantity results can be obtained by determining the area or peak of the fluorescence signal of a certain region of the contour plot, four components, humic-like, fulvic-like, microbial protein-like and aromatic protein-like substances have been identified from fluorophores described in the literature (Chen et al., 2003). In fig 3.1, a contour plot, EEM spectra, was acquired using a fluorescence spectrophotometer (PLS55, Perkin Elmer Instruments, UK) for a water treatment plant (WTP) water sample. Emission spectra were scanned from 280 to 540 nm at 5 nm increments and excitation spectra were scanned from 250 to 400 nm with 5 nm increment. The slits for excitation and emission were 5 nm.

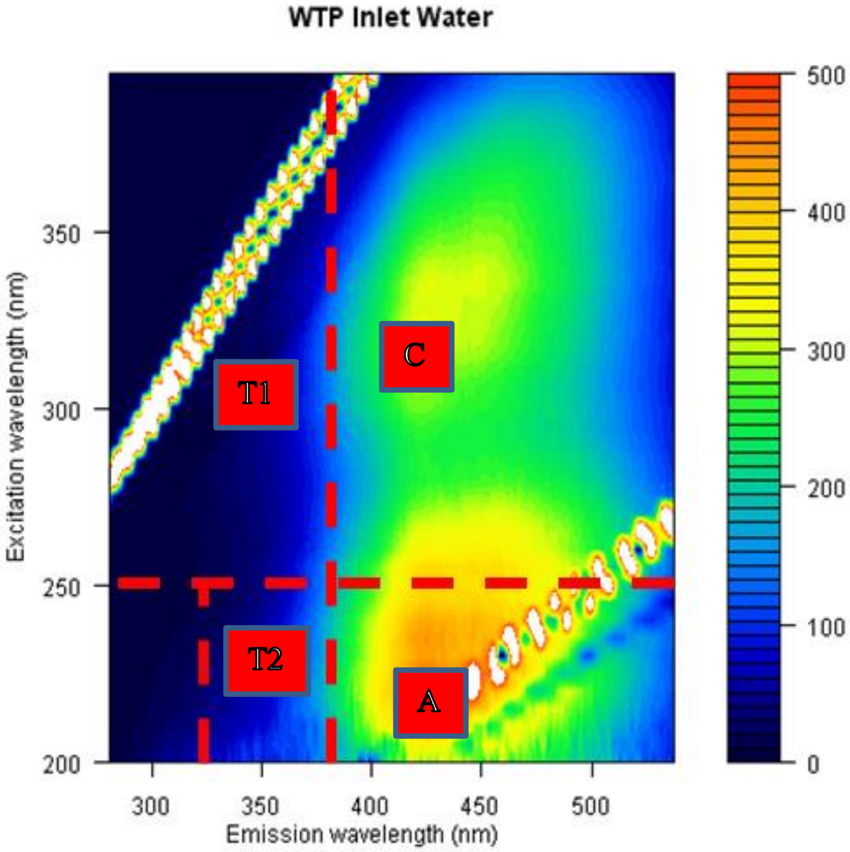
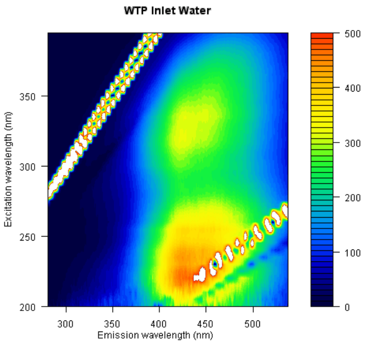


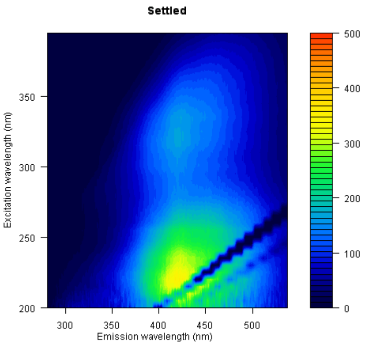
Fig. 1. 3D fluorescence EEM of raw inlet water sample.

Excitation and emission wavelength pairs for principle florescence intensities were analysed according to Chen et al. (2003).

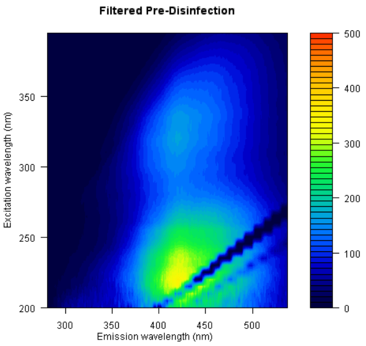
WTP Intet Water



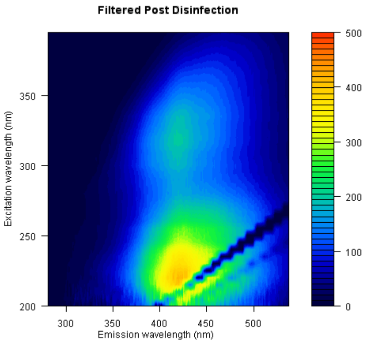
Setteled Water



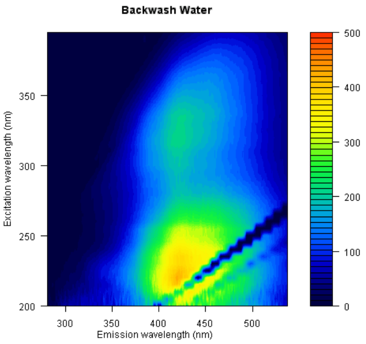
Filtered prior disinfection



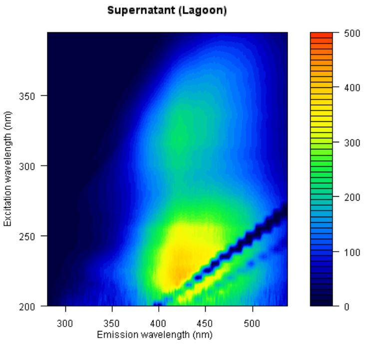
Filtered post disinfection



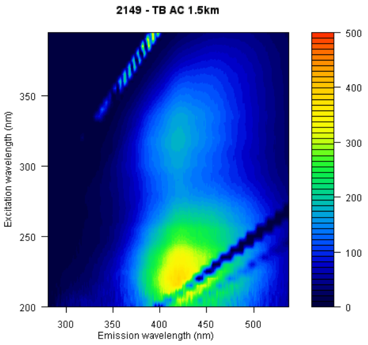
Backwash water



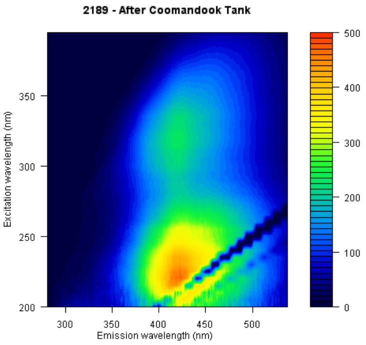
Supernatant (lagoon)



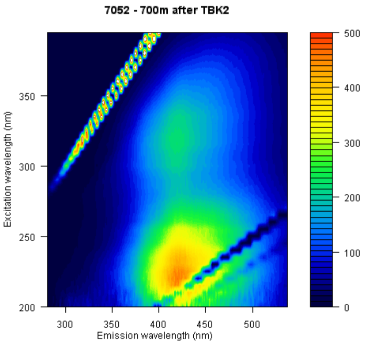
1.5km after chloramination

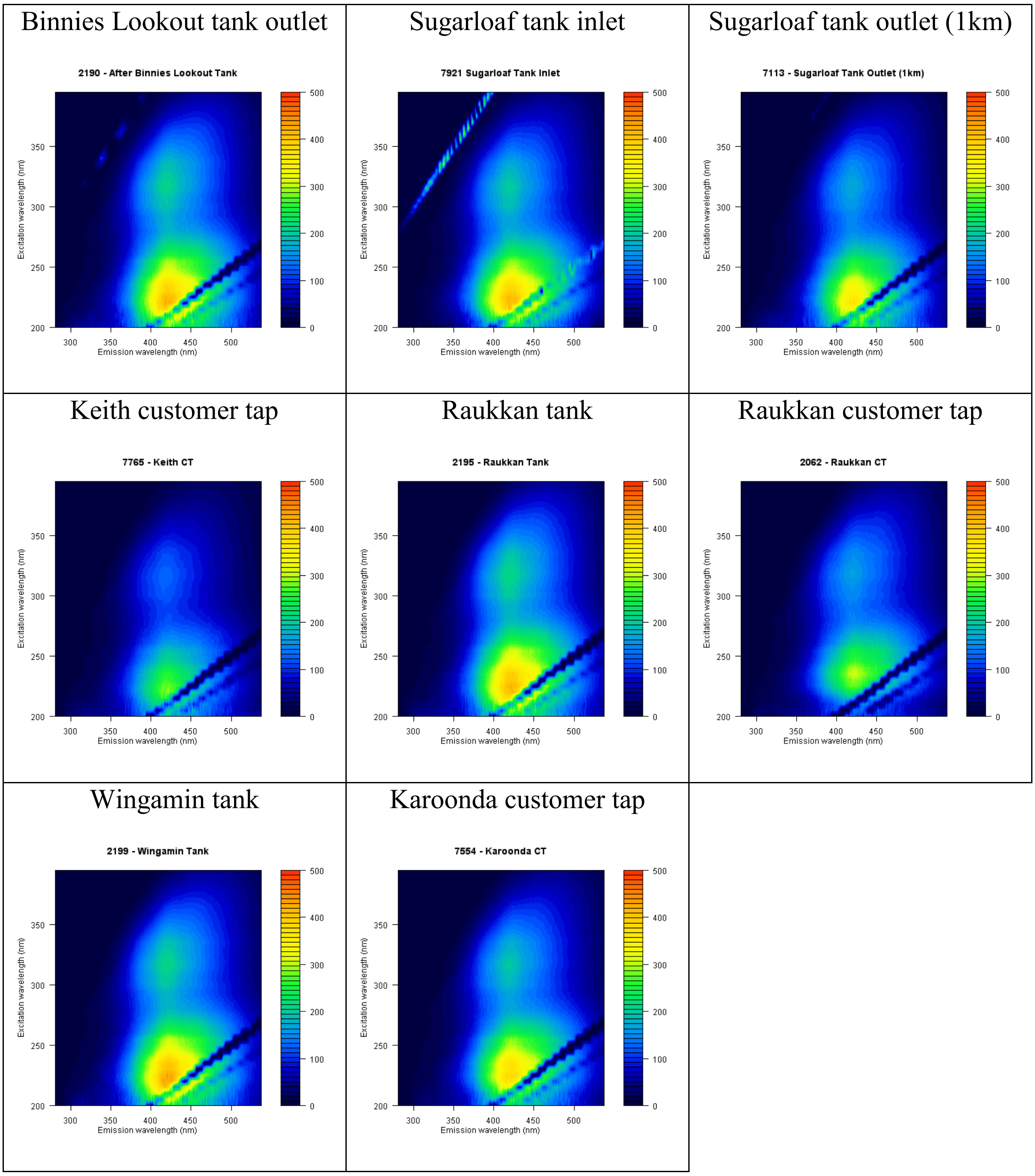


Coomandook tank outlet



700m after TB pump station





Binnies Lookout tank outlet

Sugarloaf tank inlet

Sugarloaf tank outlet (1km)

Keith customer tap

Raukkan tank

Raukkan customer tap

Wingamin tank

Karoonda customer tap

Fig. 2. 3D fluorescence EEM spectra from a series of samples obtained from different stages of the water treatment processes and subsequent distribution system.

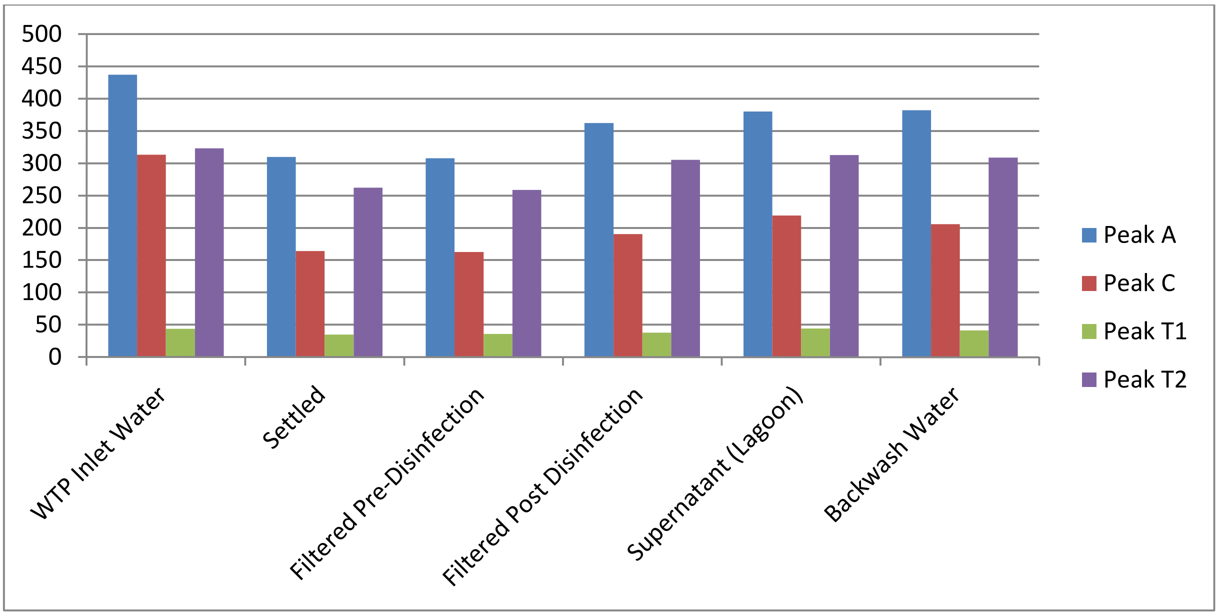


Fig. 3. EEM peak heights for characteristic regions of water treatment processes.

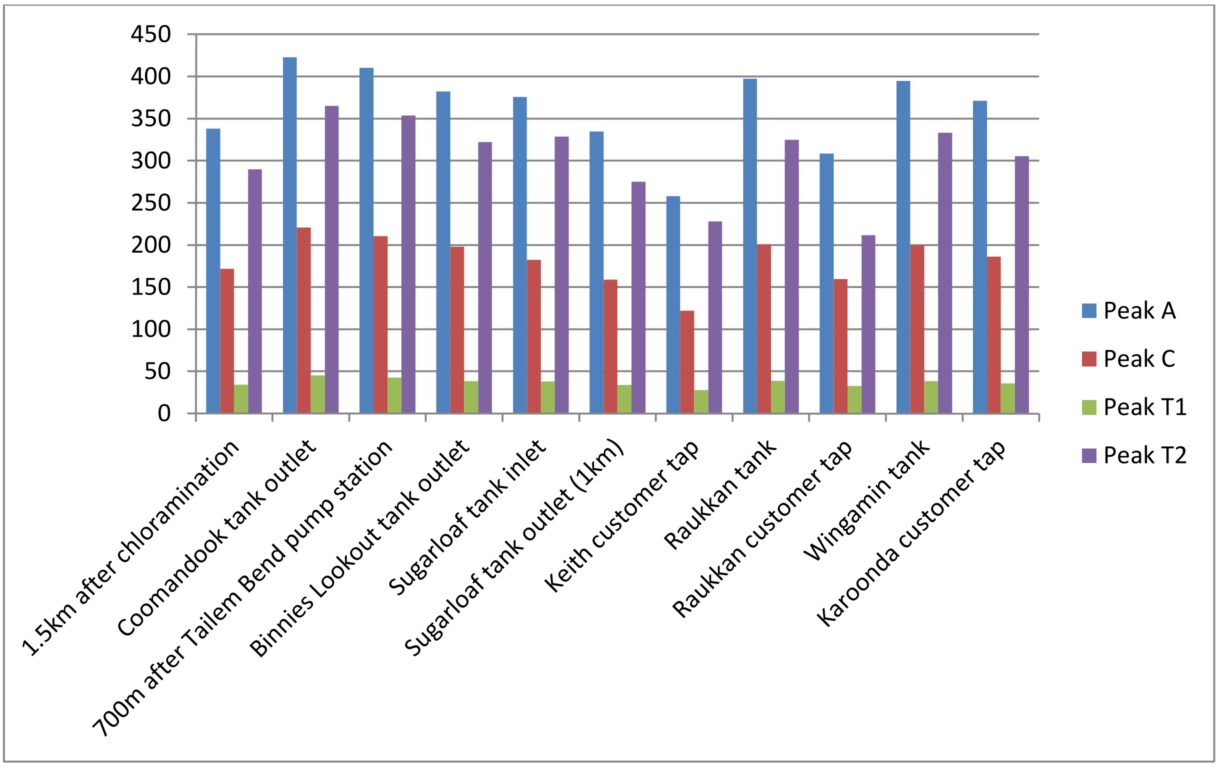


Fig. 4. EEM peak heights for four characteristic regions of distribution system samples.

With the use of the R software, R program codes have been developed to integrate EEM data (developed by AWQC team), not only did this software provide the colour scale contour plot (Fig. 1 and 2), but also it enabled to extract particular excitation-emission wavelength pairs in order to provide raw data exploration and improve data extraction efficiency. Fig. 3 and 4 presented the fluorescence intensity in the four characteristic excitation-emission regions.

Peak A: fulvic-like substances, emission at 426 nm and excitation at 235 nm

Peak C: humic-like substances, emission at 426 nm and excitation at 325 nm

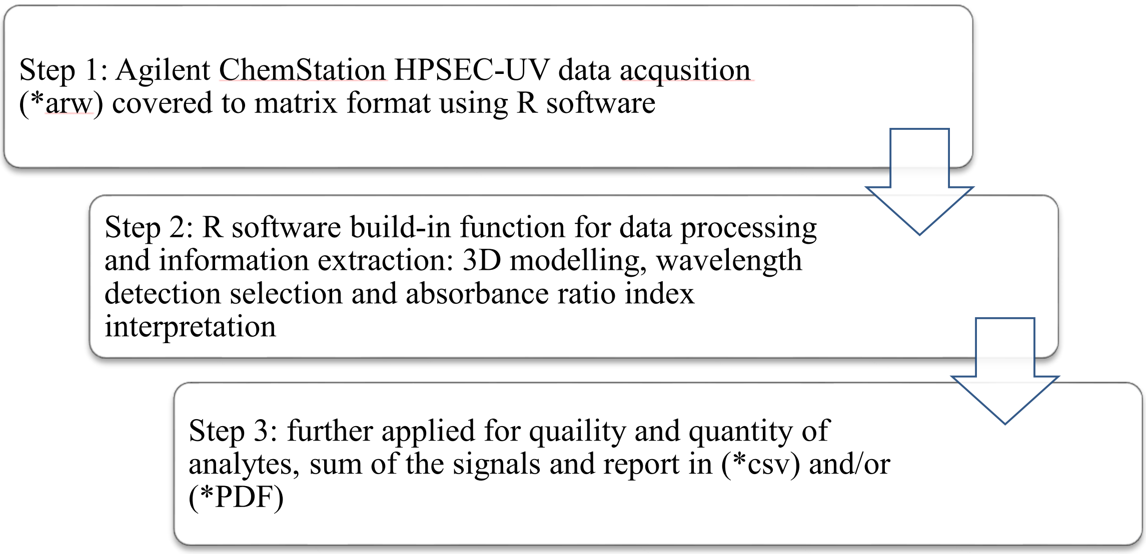
Peak T1: microbial protein-like substances, emission at 350 nm and excitation at 300 nm

Peak T2: aromatic protein-like substances, emission at 350 nm and excitation at 225 nm

The EEM spectra at different stages in full scale water treatment plants were analysed based on the variation of the intensity of the peaks shift in the location of their excitation-emission maxima, for samples from the water treatment plant to the subsequent distribution system. Most of fluorophore substances in the raw water were efficiently removed during the treatment processes, according to a comparison of inlet raw water and settled water samples. Both fulvic acid-like and humic-acid-like components had particularly low and consistent concentrations during the treatment processes. The EEM spectra at different locations and branches along the water distribution system were also obtained. These EEM spectra illustrated signal intensities (concentrations) of both fulvic acid-like and humic-acid-like components stay relatively stable passage through the distribution system. In each tank there was an increase for all the compounds and along the distribution system and after the tanks there was a decrease for all the regions. The comparison among four distribution system branches, Keith CT (TB-B1-CT), Raukkan CT (TB-B2-CT) and Karoonda CT (TB-B3-CT), showed some significant differences. This section described a simple R code can perform an automate data processing task for EEM spectra and facilitated result interpretation, this was especially useful in processing large number of samples required for this distribution system study. If this data processing task was done by using Excel or other similar software packages, the researcher may need to manually examining the full table of data, locate the 4 cells which represents Peak A, Peak C, Peak T1 and Peak T2 and obtained the readings manually for further data analysis.

3. Application of R software on multi-wavelength HPSEC analysis

When using a photodiode array (PDA) multi-wavelength UV detector, the HPSEC-UV prolife can provide a more comprehensive perspective on the relative absorbance intensity of various AMW compounds, compared with the traditional single wavelength detection (Liu et al., 2010; Korshin et al., 2009; Yan et al., 2012). An overview of the complete multi-wavelength HPSEC data analytics procedures from data acquisition to peak presentation and information extraction is shown in Fig. 5. Multi-wavelength HPSEC (a UV range of 200 – 450 nm) analysis results in an increase in the size of the data set with at least hundreds of variables in a *.arw format. This raw data set is able where it is converted and saved to *.csv format for further work in Excel. However, there are several limitations using Excel to process this data set, such as manual processing, time-consuming and difficulty in plotting and comparing several samples at the same time. This raw data can also be converted to matrices using R software program, which allows handle several datasets and build specific functions and models. The multi-wavelength HPSEC data was then arranged in a single 3-dimentional data structure in preparation for more complex interpretation. Prior to attempting a more complex chromatographic data analysis, the natural elution profile need to be optimise to be the best fit and the best chemically interpretable profile which will be adopted as the final resolution. Application of constraints is the starting point in the optimisation processes. Peak presentation is modified to fulfil a preselected property when a profile is constrained (de Juan and Tauler, 2007; Jalali-Heravi and Parastar, 2011). Non-negativity constraint was applied to multi-wavelength HPSEC profile in this study to fit the elution profile to a particular shape.



Step 1: Agilent ChemStation HPSEC-UV data acqusition (*arw) covered to matrix format using R software

Step 2: R software build-in function for data processing and information extraction: 3D modelling, wavelength detection selection and absorbance ratio index interpretation

Step 3: further applied for quaility and quantity of analytes, sum of the signals and report in (*csv) and/or (*PDF)

Fig. 5. A workflow for step-by-step procedure for qualifying and quantifying signals in chemometric models of multi-wavelength HPSEC spectra.

The spectral data from HPSEC would best be presented using 3D surface plot. R software includes an option to export raw data to 3-dimensional (3D) presentation. RGL is an R library and coupled with OpenGL to offer 3D visualisation. This library allows the user to generate conveniently and flexibly 3D graphics, which the user can rotate the graph for detail inspection. The function of 'persp3d' allows plot of surface over the axis x-y plane. The additional functions such as 'point3d' and 'lines3d' can be used to highlight the further interests. The multi-wavelength HPSEC profile of raw water sample was illustrated as 3D plot (Fig. 6), which was informative to illustrate the multi-wavelengths ranged from 200 to 300 nm, apparent molecular weight (AMW) distribution and the relative intensities of UV absorbance of various analytes. The attractive UV wavelengths, including 210, 230, 254 and 272 nm were also highlighted for comparison. The relative intensities of absorbance relevant on the concentration were illustrated with colour fill, which is a sequential colour function provided by library 'RcolorBrewer', which can create nicer looking colour patterns and provide a much clearer visualisation presentation. This way of presenting the entire absorbance data provided a holistic overview of the data with additional useful information compared with the presentation of traditional single UV wavelength detection (absorbance against molecular weight at a single wavelength). An additional perspective on the relative absorbance intensity of different AMW compounds at different wavelengths was also available for comparison at the same time. This 3D presentation also enabled to indicate clearly the most suitable detection wavelength in order to look for the analytes, which may not be observed in the typical HPSEC profile with single wavelength UV detection. Although plotting data in 3D can be considered as a simple task in modern day computing software package, however, in this case, the selection of a suitable code for this enhanced 3D presentation can be considered as an advancement of the development. More importantly it demonstrated with enhancing the display of data, additional analytical information can be obtained.

Evaluation the difference between before and after coagulation process was enhanced by the use of 3D plot combined with sequential colour fill. Comparison between raw water (Fig. 6) and settled water (Fig. 7) samples illustrated the efficiency of conventional treatment processes in the term of DOM removal. The DOM components with lower MW below 0.5 kDa were relatively stable and recalcitrant to the conventional treatment processes, whilst the high MW fractions were efficiently removal measured at the full wavelengths.

Further comparison of samples collected from different customer taps, the 3D plot presentation of both Keith (Fig. 8) and Raukkan (Fig 9) customer tap samples was presented. The differences in absorbance intensity of AMW below 0.5 kDa revealed the changes to product water quality within the distribution system. These changes were not captured based on the standard wavelengths 254 nm and 260 nm detections.

Another example to show the comparable differences, the 3D plot of stormwater-associated samples was illustrated in Fig.10 and Fig. 11. The 3D plot of Event-3 first sample (Fig.10) presented different AMW characteristics associated with different UV wavelengths. Figure 11 presented the average of total 23 stormwater samples captured in Event-3. Another attractive advantage of using R software is that it is able to subtract and/or average different samples without opening several HPSEC-UV files at the same time. The function to plot more than 20 samples on the same graph was also developed in current study. This process design only took a few seconds to generate a target graph and also can thus be compared with the alternatively manually opening all elution profiles into Excel (if Excel was the package to use), which depending on the complexity of the data may take up to several minutes to average and/or subtract different samples, including checking that the data has been selected correctly and applying the constraint to each selected sample profile. Therefore, it benefited time cost and improved accuracy of data processing.

R software program includes another option to export various customised graphs to several formats, including *.pdf, *.jpeg, *.eps, *.png and so on. These formats additionally enable the user to import back for further interpretation and editing.

To extract analytical information about DOM from these multi-wavelength HPSEC profiles, apart from parameters developed at original UV wavelength detection, several indicators that proposed by more than one wavelength have been introduced and applied on current water research (Her et al., 2008; Korshin et al., 2009; Yan et al., 2012). Focusing on interpreting absorbance-based indexes with HPSEC-UV data, the absorbance ratio of DOM measured at two wavelengths, A_{210}/A_{254}, A_{280}/A_{350} and absorbance slope index (ASI) were selected for further data interpretation.

A second method that proved useful in this study for resolving ambiguity of multi-wavelength HPSEC elution profile was to use above indicators and modelling to quality and quantify DOM.

The ‘par()’ is a function provided by R and is used to set up multiple plots in a single figure. Figures 12a and b illustrating by using the first sample of Event-1 and Event-3, respectively, were designed to place two plots in one figure using this function. It was more comparable and clearer to observe the absorbance intensities of different selected wavelengths in one plot and their associated absorbance ratio indexes in the other one. It was also comparable between different AMW fractions with their corresponding values. The usefulness of various absorbance-based indexes was also being able to be compared. These well designed plots indicated the importance of 210 nm, 254 nm and their ratio A_{210}/A_{254} and which have been employed to evaluate DOM characteristics in stormwater (**Chapter 4**) and correlation between DOM characteristics and disinfectant residual decay within distribution system (**Chapter 5**).

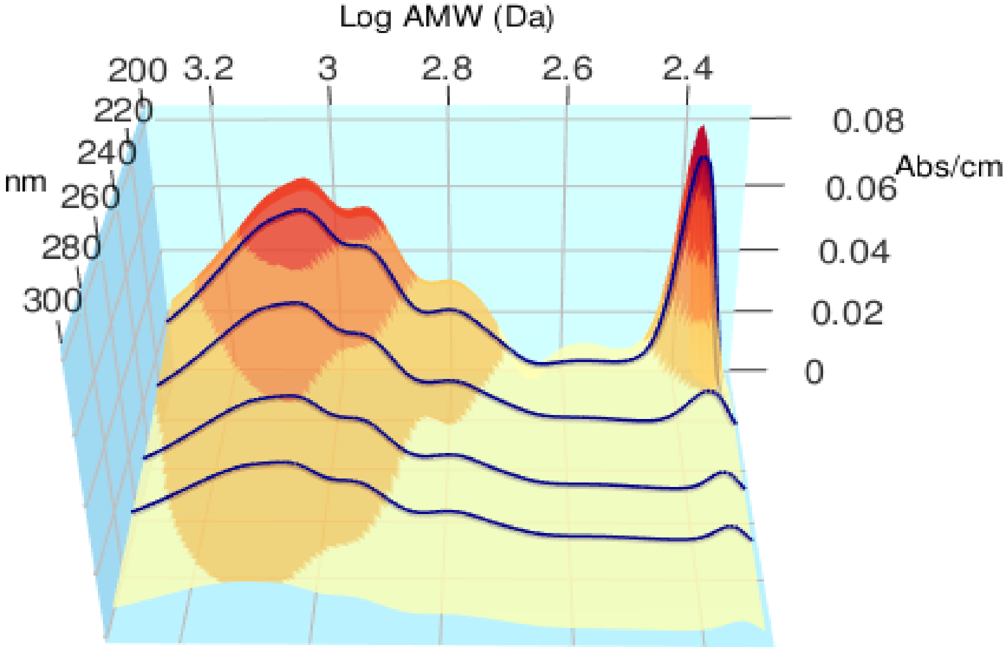


Fig. 6. 3D multi-wavelength HPSEC of a raw inlet water sample.

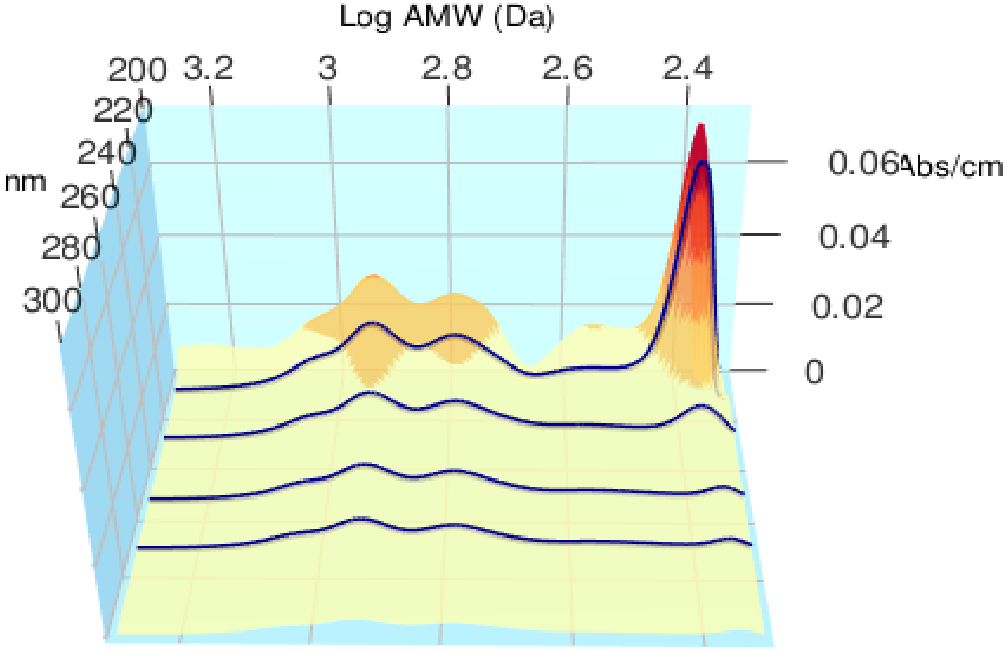


Fig. 7. 3D multi-wavelength HPSEC of settled water sample.

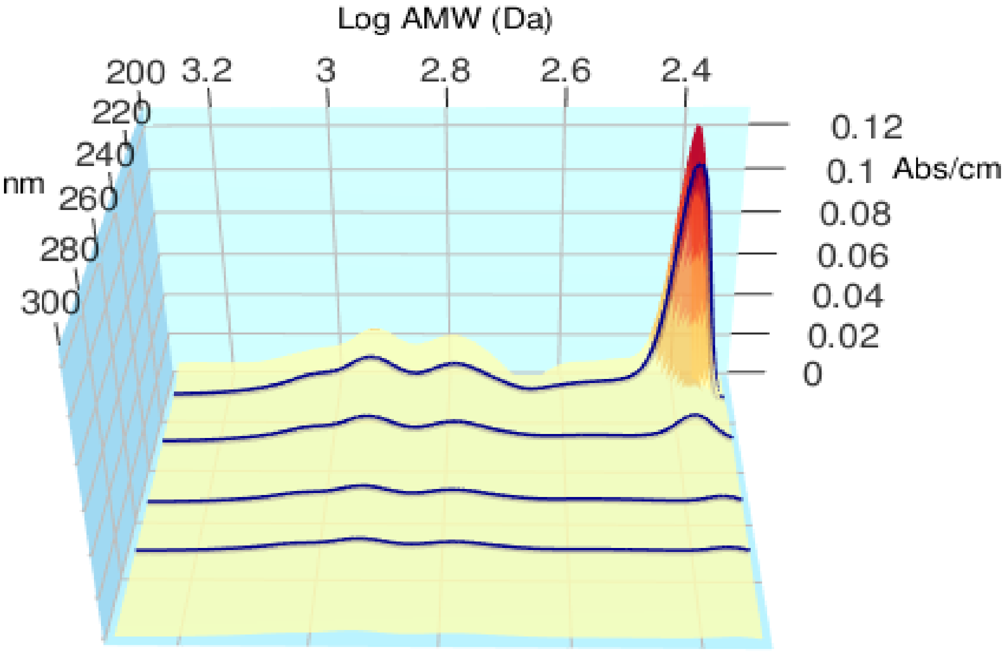


Fig. 8. 3D multi-wavelength HPSEC of keith customer tap sample.

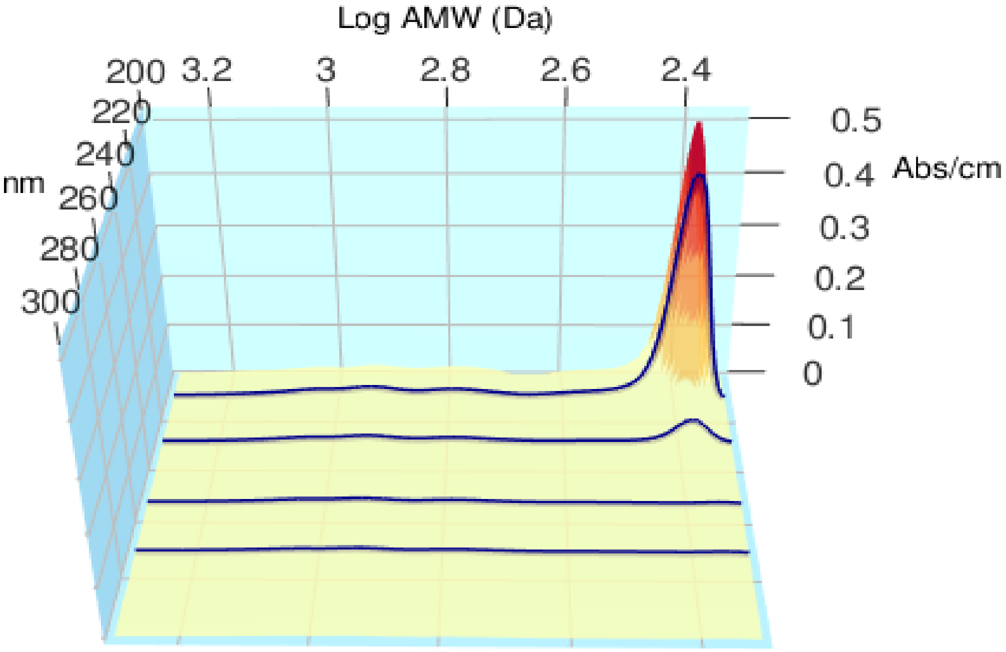


Fig. 9. 3D multi-wavelength HPSEC of Raukkan customer tap sample.

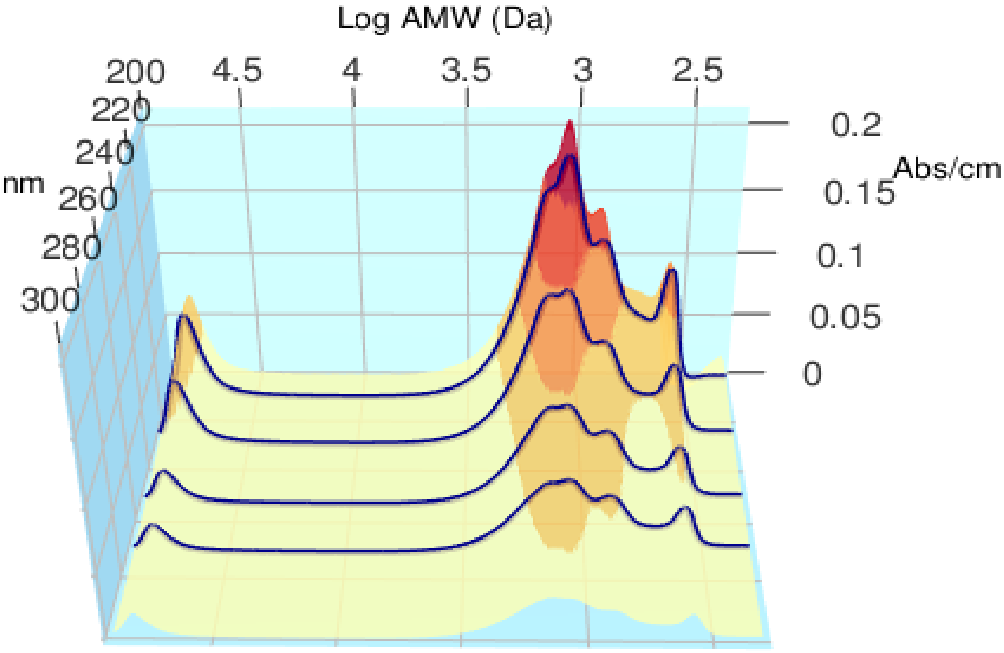


Fig. 10. 3D multi-wavelength HPSEC of Event-3 first sample.

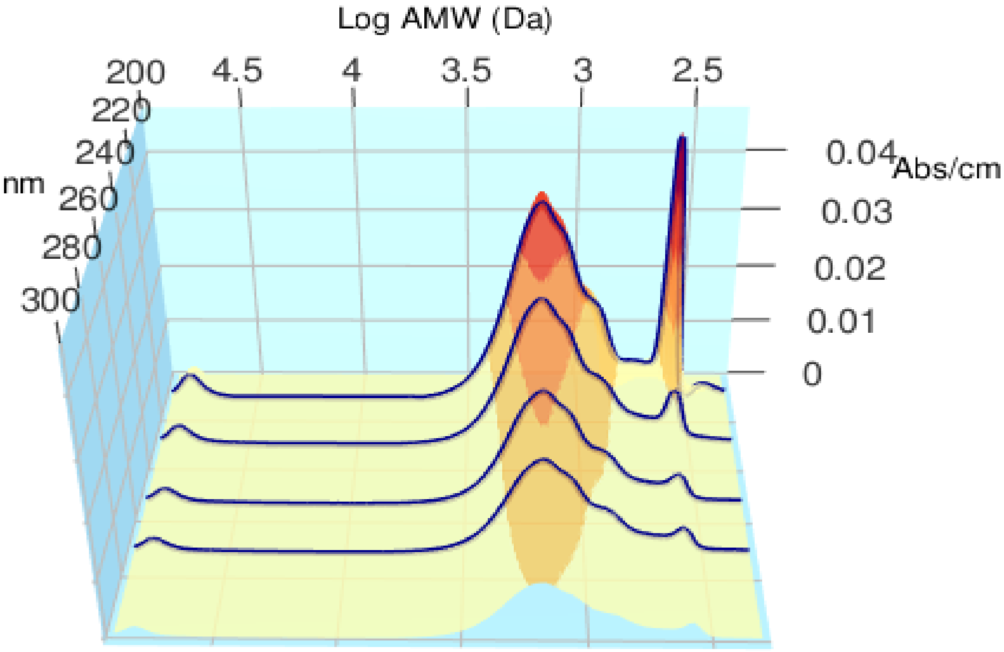


Fig. 11. 3D multi-wavelength HPSEC of averaged all Event-3 samples.

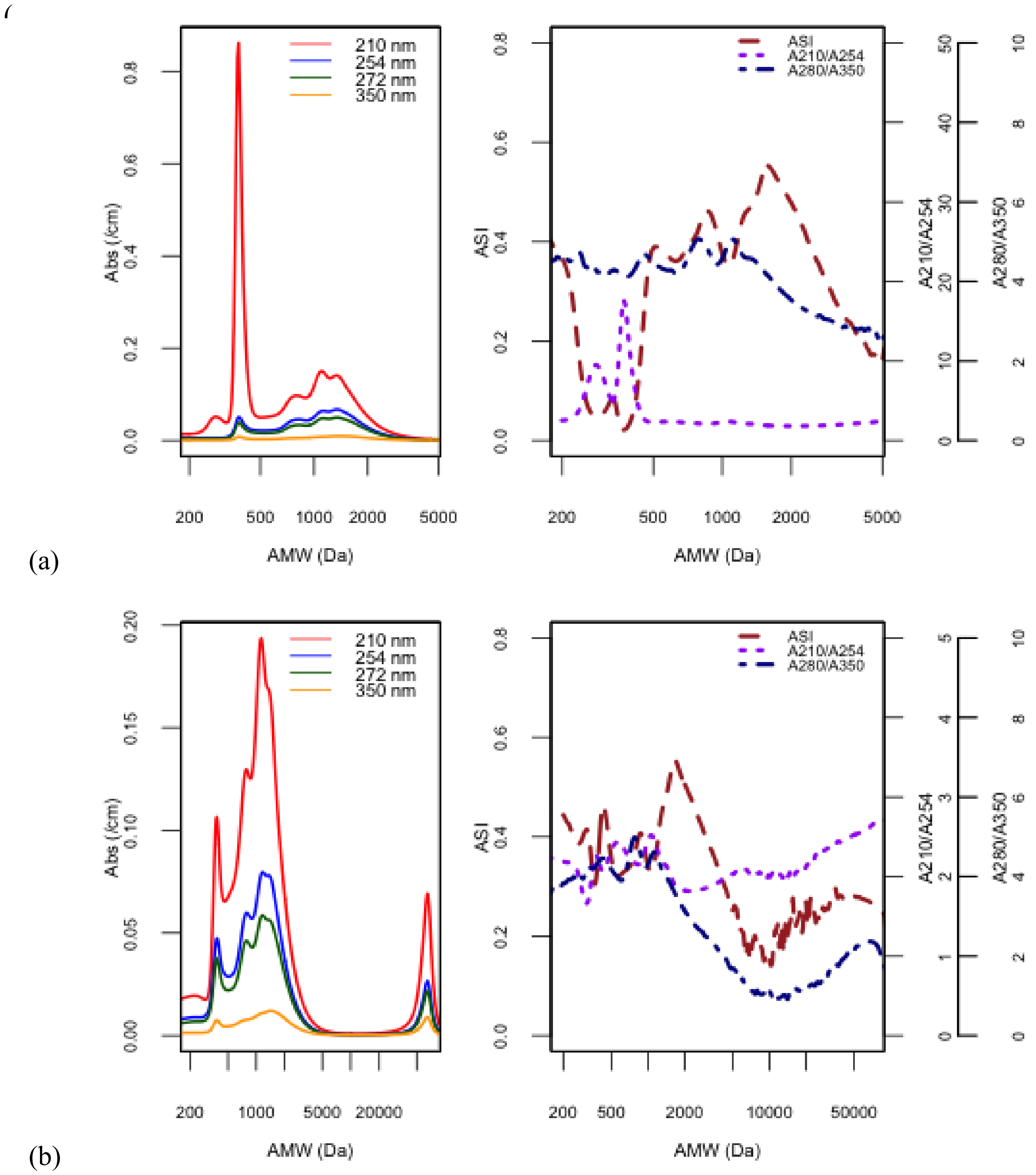


Fig. 12. Comparison of multi-wavelength HPSEC profiles with selected wavelengths and absorbance-based indexes, (a) Event-1 first sample and (b) Event-3 first sample.

4. Conclusions

This chapter is briefly to describe various functions provided by R software and which have been used to for chromatographic data processing. Considering a modern day office computer would have the software packages capable for data processing and presentation, such as using Excel. The work described may be seen as basic use of software, however, in our situation that large amount complex data were generated from each sample run together with a number of samples need to be processed and also better to be compared together the need of specific designed data processing package is essential. This R software allows analysts to build their own specific functions and research fields. Most figures presented in this study were generated based on 'built-in' function' and the codes developed during this study from the code library that can be used to process a routine sample with standardised graphs and to automate the processes. R is capable of importing, converting and determining peaks in a large number of data simultaneously; making it feasible to reduce the time spent optimising models by using the Excel as an external tool. This may be in the category of great research and development but this seems to be an essential component for us to enhance our data analytics capability by simultaneously comparing the data. This fosters efficient qualification and quantification of peaks in complex multi-wavelength HPSEC datasets, providing a useful new tool for the analyst tasked to qualify and quantify DOM in environmental and drinking water samples. This enhancement open up opportunity to explore additional analytical information to add value and new knowledge by re-visiting analytical data collected earlier from different research projects.

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Chapter 4: Characterisation of dissolved organic matter in stormwater using high-performance size exclusion chromatography

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Abstract

Understanding the complexity of dissolved organic matter (DOM) in stormwater has drawn a lot of interest, since DOM from stormwater causes not only environmental impacts, but also worsens downstream aquatic quality associated with water supply and treatability. This study introduced and employed high-performance size exclusion chromatography (HPSEC) coupled with an ultraviolet–visible (UV–vis) diode array detector to assess changes in stormwater-associated DOM characteristics. Stormwater DOM was also analysed in relation to storm event characteristics, water quality and spectroscopic analysis. Statistical tools were used to determine the correlations within DOM and water quality measurements. Results showed that dissolved organic carbon (DOC) and UV absorbance at 254 nm (UV_{254}) as conventional DOM parameters were found to be correlated well to the changes in stormwater quality during each of the three storm events studied. Both detector wavelengths (210 and 254 nm) and their ratio (A_{210}/A_{254}) were found to provide additional information on the physiochemical properties of stormwater-associated DOM. This study indicated that A_{210}/A_{254} is an important parameter which could be used to estimate the DOM proportions of functional groups and conjugated carbon species. This study provided also an understanding of stormwater quality constituents through assessing variability and sensitivity for various parameters, and the additional information of rainfall characteristics on runoff quality data for a better understanding of parameter correlations and influences.

Keywords:

Stormwater, Dissolved organic matter, HPSEC

Introduction

Stormwater brings various inorganic and organic substances into the environments (Göbel et al., 2007; Al-Reasi et al., 2013). These chemical discharges can worsen downstream water quality if the stormwater is used as water source, as well as impacts on the ecosystem. Among these chemical substances, dissolved organic matter (DOM) has drawn a great interest as it can enter aquatic matrixes, thus affecting the composition and quality of surface waters (Chong et al., 2013; McElmurry et al., 2013). DOM is also naturally present in the environment and has frequently been detected in source waters (Matilainen et al., 2011; Xing et al., 2012; Fabris et al., 2013). It can be responsible for the yellow-brownish colour, unpleasant taste and bad odour of natural waters. Hence the varying levels and compositions of DOM in stormwater sources need to be taken into account, since its chemical characteristics can be variable at any time depending on the local activities, climate conditions and rainfall influences. As a general concern in the course of drinking water treatment and/or wastewater recycling processes, DOM affects not only the performance of each treatment step, such as traditional coagulation–flocculation, adsorption and membrane filtration (Chow et al., 2004; Rosenberger et al., 2006; Fabris et al., 2008); but also more importantly, reacts with various disinfectants to produce harmful disinfection by-products (DBPs) (Richardson et al., 2007; Zhao et al., 2008).

Conventionally, pH, turbidity, colour and inorganics are the common parameters used to describe water quality, while dissolved organic carbon (DOC) determination, ultraviolet (UV) adsorption analysis, specific UV absorbance (SUVA) and specific colour are commonly used as parameters to measure DOM in water sources. They provide both quantitative and qualitative information. Along with substantial improvement in analytical techniques, compared to the earlier work in this field, current DOM analytical work has been shifted towards more advanced fractionation analysis. A series of advanced analytical techniques, including resin fractionation, fluorescence spectroscopy and size exclusion chromatography have been widely used in the water research field (Matilainen et al., 2011; Nebbioso and Piccolo, 2013). Hydrophobicity, molecular weight and aromaticity, provided by these techniques as indicators provide more insight into chemical qualitative and structural features of DOM and more informative outcomes, and either applied as a single technique or in combinations can generate additional values on DOM characterisation (Bazrafkan et al., 2012; Chong et al., 2013; Li et al., 2013; Wei et al., 2013).

Molecular weight distribution is an important physical property associated with DOM transport, reactivity and treatability. High-performance size exclusion chromatography (HPSEC) has been developed to characterise DOM predominantly for water treatment applications and also in various soil, aquatic and marine samples (Matilainen et al., 2011; Nebbioso and Piccolo, 2013). The principle of HPSEC is based on apparent molecular weight (AMW) separation. Additionally, it can couple with various detectors, such as DOC determination (Her et al., 2008), UV absorbance with a single or multiple wavelengths (Her et al., 2008; Korshin et al., 2009; Liu et al., 2010; Bazrafkan et al., 2012; Xing et al., 2012; Yan et al., 2012), excitation emission fluorescence detection (Li et al., 2013), and mass spectroscopy (Nebbioso and Piccolo, 2013).

An additional advantage of using HPSEC is the ability to separate inorganic constituents and minimise inorganic interferences, as these are generally in a range of molecular weights (MW) less than 0.25 kDa (Her et al., 2008). Several studies have also demonstrated that the HPSEC technique is informative and reliable when used to assess water treatability by comparison between raw and treated water based on the HPSEC profiles after coagulation in drinking water treatment (Chow et al., 2008; Fabris et al., 2008; Liu et al., 2010; Xing et al., 2012) or applying a peak-fitting model to predict treatability (Chow et al., 2008). Korshin et al. (2009) investigated the relationships between MW and DBP formation. HPSEC in conjunction with UV detector is particularly useful and informative. More than one wavelength and/or multi-wavelength absorbance detection have been introduced and applied by several researchers (Her et al., 2008; Korshin et al., 2009; Yan et al., 2012). The wavelengths at 210 nm and 254 nm have been used in previous work because the wavelength 210 nm allows the detection of DOM functional groups (hydroxyl, carboxyl, carbonyl, ester and nitrogen-containing compounds) and the wavelength at 254 nm is the recognisable absorbance for the conjugated aromatic substituents (Her et al., 2008). The wavelength around 210 nm has also been addressed to associate particularly with nitrate concentrations, which relates to nutrient content and microbial activities (Whitehead and Cole, 2006).

Elevated pollutant loadings, particularly of DOM, during a storm event can provide early notice of potential impacts of stormwater discharge on surface waters. Water quality and the potential risks of stormwater need to be assessed and controlled in order to improve watershed management. The aim of this study was to characterise DOM present in stormwater through extensive sampling of three representative storm events and develop some useful tools to understand stormwater DOM properties. The objectives were (1) to determine stormwater quality using a series of conventional measurement techniques and to describe their sensitivity and potential relationships, (2) to extend HPSEC with UV absorbance detection as a monitoring technique to characterise stormwater-associated DOM based on molecular weight distribution, (3) to determine DOM compositions using two UV wavelengths (210 and 254 nm) of the HPSEC and their ratio for further analysis, and (4) to estimate pollutant loadings using simple statistical methods, combining measured flow data with various water quality parameters.

1. Material and methods

1.1. Sampling strategy

A semi-urban catchment, located at Mannum, South Australia, was selected to determine the impact of stormwater quality on surface water quality, since the stormwater in this area (study) could enter directly into the river and can impact on surface water quality. A sampling point located in the underground stormwater pipe was selected to capture stormwater down-stream the stormwater drains. Fig. 1 shows the monitoring setup, including a pressure sensor as well as an automatic 24 bottle carousel sampler and their installation. The pressure sensor was used to measure water level in the stormwater drain continuously at 5 min intervals and to control the automatic sampling system. The automatic sampling system was triggered when water level was above a threshold (25 mm). The sampling strategy applied was based on flow condition and employed sequential (multi-bottle) sampling. Water level was also recorded corresponding to the sample (bottle) collection. As soon as the first sample was taken, a signal (SMS) was sent to the operator to initiate event control. Depending on the triggering time; usually a site visit was made the following morning to ensure a good capture of the event. However, if the trigger was in the early morning, the site visit would be in the afternoon. Samples were collected and transported back to our laboratory within 24 hr of the triggering time. The triggers of these three events all came at midnight, so all of the samples were collected the following morning and transported back to the laboratory for analysis.

1.2. Instrumental analysis

Turbidity was determined using a 2100AN Laboratory Turbidimeter (Hach, USA) with results given in nephelometric turbidity units (NTU). Samples for DOC, colour (456 nm) and UV absorbance at 254 nm (UV_{254}) were filtered through a 0.45 µm membrane. A 1 cm quartz cell and 5 cm cell were used for UV_{254} and true colour at 456 nm, respectively. Colour is expressed in Hazen Unit (HU) after calibration using a 50 HU cobalt platinum standard and UV_{254} is expressed in Abs/cm. DOC was measured using a Sievers 900 Total Organic Carbon Analyser (GE Analytical Instruments, USA). Specific UV absorbance ($SUVA_{254}$) was calculated as UV_{254} divided by DOC multiplied by 100, and expressed in L/(mg·m). Similarly, specific colour at 456 nm was calculated as colour divided by DOC and expressed in HU.L/mg.

Molecular weight profiles were determined using a Waters 2690 Alliance system (Waters Corporation, USA) with a Shodex KW802.5 glycol functionalized silica gel column, which was equilibrated at 30 °C. Samples were filtered through a 0.45 µm membrane filter prior to analysis and 100 µL samples were injected. The mobile phase was 0.02 mol/L phosphate buffer at pH 6.8 adjusted to an ionic strength of 0.1 mol/L with sodium chloride. The system was operated at isocratic conditions with an eluent flow rate of 1.0 mL/min. Polystyrene sulfonate standards (Polysciences, USA) with MW 4.6, 8, 18 and 35 kDa were used to calibrate the retention time response to AMW.

1.3. Statistical analysis

All statistical analysis was applied using R (version 3.1.0, R Development Core Team). R is a free and relatively well- developed programming language and provides an effective environment to implement statistical techniques. The standard analysis of variance (ANOVA) was utilised to evaluate the significant influence of seasonal variation on DOM characteris-tics. Pearson's Product Moment Correlation (PPMC) was used to evaluate if correlations of various general and spectroscopic parameters existed. The correlations between colour evaluation and other parameters were the main purpose of PPMC analysis in the current study. Both correlation factor (R^2) and probability (p) values were used to determine significance.

2. Results and discussion

2.1. Storm event characteristics

This stormwater study was conducted in 2010, and three storm events spread over the year were agreed by the project team during the planning phase of the case study. For each event, the auto-sampler was triggered by the flow condition, and samples were taken for an approximately 25 mm change in the water level. According to a previous study provided by Leecaster et al. (2002), 12 samples in one event would be sufficient for efficient characterisation of a single storm event. Thus, event less than 12 samples were disregarded in this current study. All three storm events presented provided more than 12 samples per event. The first event (Event 1) was conducted over 7 hr on 29 July, 2010. The period of July– September is considered as the wet season in South Australia (supported by rainfall data in 2010 provided by the Bureau of Meteorology). If the sampling plan was just based on following rain events, the second event would have actually been in the same month. However, it was decided that the second event (Event 2) would be that which occurred on 18 August, 2010 (over 12 hr). This allowed a longer period after Event 1 (the auto-sampler was physically turned off). Event 2 had similar rainfall values compared to Event 1 which happened to be useful for comparison as this could minimise the rainfall interference factor. Event 3 was conducted on 25 November 2010 (over 11 hr). This last event was planned to capture the stormwater quality after a period of the dry season in order to study the impact of seasonal change. The three events reported in this study were thus carefully selected to obtain the maximum amount of information.

A summary of the meteorological data of the 3 storm events is given in Table 1. Data obtained from the Bureau of Meteorology, including total rainfall, rainfall duration, antecedent dry period and runoff samples. Event 1 was captured after a longer antecedent dry period (14 days), while Event 2 was captured after a shorter antecedent dry period (7 days) following a heavy rainfall event. Event 3 shared a similar antecedent dry period (7 days) with Event 2 but was captured during a warmer season. It was notable that the number of samples captured across a storm event was proportional to the intensity of rainfall, and was linked to flow conditions but not rainfall duration. During Event 3, because of the highest rainfall (14 mm), 24 samples were collected, followed by Event −1 (10 mm) 18 samples and Event 2 (8 mm) 13 samples. Fig. 2 shows the relationship of water level and rainfall duration during each storm event when samples were collected. This duration graph illustrates that although Event 2 was in the longest rainfall period, it had relatively more stable and lower flow (low water level compared to the other two events) during the event, whereas Event 1 and Event 3 had larger dynamic changes of the flow condition during the runoff process. Based on the observed flow conditions, samples were collected more frequently at larger fluctuations of water level changes and less frequently at smaller fluctuations of water level changes, and as water level changed rapidly, the time between samples decreased. At the beginning of a heavy rain, 7 and 11 samples were collected within 100 min for Event 1 and Event 3, respectively, whereas only a couple of samples were triggered within a similar period time for Event 2. These observations imply the sampling method used in this case study could be sufficient to capture the characteristics of rainfall–runoff process in this catchment area. These sequential samples collected based on flow sampling were analysed to gain insight into the changes of stormwater quality and quantity during each storm event.

2.2. General stormwater quality analysis

Analytical data shown in Table 2 reveal that the characteristics of the dissolved components in the stormwater as determined by DOC and UV_{254} varied significantly among events. The average DOC concentration from the samples collected in Event 3 was 14.7 mg/L which was found to be higher than those in Event 1 and Event 2, which were 13.5 mg/L and 9.9 mg/L, respectively. Both UV_{254} and colour measurements showed similar trends as the DOC concentrations for all three events. The results of UV_{254} for Events 1, 2 and 3 were 0.432 Abs/cm, 0.301 Abs/cm and 0.501 Abs/cm, respectively. Colour for Event 3 stormwater samples was detected with an average of 99 HU, which was also higher than those in Event 1 and Event 2, which were 77 HU and 41 HU, respectively. These analytical data might suggest that stormwater samples in Event 3 had relatively higher amounts of humic substances. A strong correlation between DOC and UV_{254} was also observed ($R^2 = 0.99$, $p < 0.001$) from all samples based on statistical PPMC analysis.

These observations were predicted to indicate that the stormwater DOM from this site had aromatic structures in nature. Additionally, it was worth pointing out that Event 3 had the most scatted data of DOC, UV_{254} and colour, resulting in the highest standard deviation values, followed by Event 1 and Event 2. A possible explanation for this observation could be due to dynamic flow variations during the event. The stormwater quality would additionally depend on rainfall intensity and environmental conditions. The other two potential factors, rainfall duration and antecedent dry period might be expected to have less influence on stormwater quality. The chemical loads in Event 3 stormwater were higher than those in Event 2 although their antecedent dry periods were similar. This could be explained by environmental conditions, since temperature has impacts on physicochemical and biological reactions (Chong et al., 2013; Tang et al., 2013; McElmurry et al., 2013). Event 1 had higher rainfall intensity and was likely to lead to higher pollutant loadings in stormwater compared to Event 2. However, the DOM character and water quality parameters were not correlated well, since stormwater runoff volume could be a potential factor influencing stormwater monitoring.

2.3. HPSEC profile analysis

A new combined profile based upon use of two wavelengths coupled with size exclusion chromatography (SEC) was introduced. These HPSEC profiles revealed that DOM in all samples had mostly similar AMW ranges, from 0.3 to 2 kDa. Both the first and last samples collected from each storm event were chosen for analysis in Fig. 3.

Similar HPSEC profiles were observed for both Event 1 and Event 2 but there was a difference obviously in Event 3. In all the HPSEC chromatograms obtained from Event 1 and Event 2, aside from the differences of DOM absorbance intensities, insignificant changes of peak patterns were observed across of each storm event under various flow conditions or water levels. It was also worth noticing that the stronger absorbance intensities were measured at the lower wavelength of 210 nm. A maximum absorbance at approximately 0.3–0.5 kDa was followed by weaker absorbance intensities at approximately 1–2 kDa. These high levels of absorbance intensity measured at 210 nm could be an indication of DOM enriched with various non-aromatic functional groups (Her et al., 2008; Korshin et al., 2009). The absorbance intensity patterns at 254 nm, on the other hand, were likely to be stable for each sample. These observations support the hypothesis that stormwater DOM had a relatively high concentration of aromatic carbon and/or phenolic compounds, regardless of the levels of absorbance intensity (Xing et al., 2012). However, HPSEC profiles for Event 3 appeared much more complex and varied prominently through all samples. For instance, remarkable differences between the first and the last samples were exhibited in Fig. 3e and f. While the HPSEC profile for the last sample shared similar dominant peaks with those from Events 1 and 2, presenting totally different results. HPSEC profiles for the first sample demonstrated that the DOM was comprised of relatively higher absorbing compounds with adsorption maxima at higher AMW fractions, ranging from 1 kDa to 5 kDa. Another identified difference was due to larger AMW absorbance, at approximately 50 kDa. This could be associated with the contribution of a large amount of plant and/or microorganism cell deaths, and vegetation decay under dry-weather conditions or comprise organic colloidal material (organometallic complexes) (Chow et al., 2008).

DOM fractions with higher AMW values were likely to have a higher absorbance at higher wavelengths (O' Loughlin and Chin, 2001). AMW above 1 kDa, for instance, had a stronger absorbance at wavelengths above 254 nm than AMW below 1 kDa. This observation could be explained by the fact that unsaturated compounds are more sensitive to a higher UV wavelength (254 nm), while functional groups including hydroxyl, carboxyl, carbonyl, ester and nitrogen-containing compounds, may be associated with a lower wavelength (210 nm) (Her et al., 2008).

2.4. Interpretation of A_{210}/A_{254} on HPSEC profiles

The absorbance ratio index (ARI) as a spectroscopic parameter has been widely reported associated with DOM characterisations. The ARI of A_{210}/A_{254} introduced by Her et al. (2008) was found to be able to provide information on the relative proportion of UV absorbance between the non-aromatic and aromatic components (Yan et al., 2012). The A_{210}/A_{254} was applied for DOM analysis in the current study in order to gain further insight into the composition of DOM in stormwater. A_{210}/A_{254} data were plotted in corresponding graphs (Fig. 3) for comparison.

In accordance with data shown in Fig. 3, the dominant fraction at 0.3–0.5 kDa was likely to give a couple of sharp peaks for A_{210}/A_{254} values which were in a range of 10–40. These high readings could imply that the corresponding DOM sources contained a higher functional group proportions which could be related to protein-like materials and/or simple amino acids associated with nutrient organic matter. Her et al. (2008) stated that A_{210}/A_{254} increases with the increase in microbiologically derived components that have a high functional group proportion. The unexpected peak exhibited below 0.3 kDa was considered due to the presence of inorganics, such as nitrate, sulfate and phosphate, as these inorganic species have UV absorbance at less than 230 nm wavelengths. The two-wavelength approach on the basis, one being in a range of 200–220 nm and the other being selected above 250 nm was previously applied to estimate nitrate concentration in various water sources (Edwards et al., 2001). Therefore, these peaks could be thought as a result of the presence of nitrate containing compounds. However, A_{210}/A_{254} ranging from 1 to 3 was observed in some samples in Event 3, such as the first-sample (Fig. 3e). These low A_{210}/A_{254} values could indicate that these DOM sources could be comprised of higher aromatic content, including a larger amount of both humic acid and fulvic acids. Her et al. (2008) have also confirmed that humic acids and fulvic acids with higher and intermediate aromaticity have the lower A_{210}/A_{254} values at 1.59 and 1.88, respectively. The A_{210}/A_{254} value below 5 for the AMW located at approximately 50 kDa, also suggested these constituents could have high aromatic characters. In agreement with the previous literatures (Her et al., 2008), our study has also illustrated A_{210}/A_{254} as a phenomenological parameter that can help characterise DOM in stormwater samples.

2.5. Influence of stormwater runoff volume

Several researchers have attempted to model and under-stand rainfall–runoff processes, since it is a crucial factor to determine pollutant movement and to estimate contaminants' fate in environments. Many previous studies have emphasised stormwater rainfall–runoff transformation characterisation analysis, particularly of runoff process, since they act as a major pathway for transport of contaminants from urban areas into surface water bodies (Avellaneda et al., 2009). Pollutant wash-off load has generally been assumed proportional to the rainfall intensity or runoff volume in previous studies. The pollutant wash-off load was assumed as a function of runoff volume, which increases would result in increase in pollutant loads. Runoff volume as a useful parameter allows the analysis of the variation of the pollutant mass during storm events and determines the total pollutant mass in relation to the total runoff volume (Chen and Adams, 2007). Following the rainfall–runoff model provided by Chen and Adams (2007), the corresponding water level measured in the drain (Fig. 1) was assumed as runoff volume, since surface area was consistent in the current study. It appears that the action of combining water level data and water quality parameter results can be also developed and employed as an essential and simple tool for stormwater character analysis.

On the basis of the flow condition sampling process, simple multiplications of values of general parameters and corresponding water levels could be applied to estimate the pollutant loadings in stormwater at a specific time period during a storm event. For instance, the DOC loading could be obtained by multiplying measured DOC concentration by the corresponding water level as expressed in mg/m2 (Fig. 4a). Other general water quality parameters, such as UV254, colour and turbidity were also interpreted in conjunction with water level shown in Fig. 4b–d. This information could be used to evaluate the qualitative and quantitative removal of contaminants from the land surface across a runoff event (Avellaneda et al., 2009). An additional advantage of this multiplication appeared to minimise stormwater dilution factor and hence enabled to analyse pollutant mass distribution during storm events. Event 3 had the highest water levels across the storm event and these led to the highest DOM washed-off load compared to the other two events. This observation could be linked to the effects of rainfall intensity. It is also worth pointing out that higher pollutant loadings were observed at the beginning of each event.

Additionally, due to the conversion from concentration to mass-based values, the correlations between general DOM character parameters and general water quality parameters were improved. Strong statistical correlations ($p < 0.001$) using PPMC analysis were found between colour evaluation and other parameter determinations, as summarised in Table 3. Similar trends in stormwater quality were observed in most parameter analysis based on combined water level analysis as illustrated in Fig. 4 and PPMC analysis (Table 3). R2 values above 0.80 were revealed between colour evaluations and DOM measurements, DOC, UV254 and SUVA254. The highest R2 = 0.92 was obtained for the correlation between colour and UV254. A relatively weak correlation was given between colour and turbidity (R2 = 0.61). Turbidity, an indication of the concentration of colloids and suspended particulates, was measured in an extremely high range for each storm event from 1 to 3. These relatively high turbidity results are an indication of the stormwater in this area containing high and stable portions of solid particles.

The above findings for stormwater quality assessment indicated that, although water level could be the main contributor to these phenomena, the stormwater colour appeared to respond proportionally to DOM characteristics. The higher DOC results tended to be positively correlated with higher UV254 measurements and higher colour observations, indicating higher pollutant concentrations in the runoff process. The outstanding definitive correlation (R2 = 0.99, $p < 0.001$), between DOM and UV254 indicates that UV254 is also a good surrogate for DOM in the stormwater samples in this semi-urban catchment area. Moreover, as a result of this observation, stormwater in this area could be considered as naturally high in aromatic content, regardless of the impacts of rainfall intensities. The statistical results revealed that there was a strong relationship between rainfall intensity and the loads of pollutants across each storm event. Event 3 had the highest levels of rainfall intensity which led to the highest pollution. The stormwater quality of Event 2 was lower than that of Event 1 which followed also the rainfall intensity levels. The good distributions of samples throughout the flow variations proved that the protocol of an approximately 25 mm change in water level to trigger sample collection was valid and could represent effectively the character of stormwater flow events.

We also applied statistical analysis tools to evaluate the results generated from the HPSEC profiles and general water quality parameters. As a result of multiplying by the corresponding water level, the A210/A254 values averaged over a 1–2 kDa range were found to be correlated strongly with the SUVA254 (R2 > 0.91), and the A210/A254 values averaged over the 0.3–2 kDa range were correlated with the specific colour (R2 = 0.83) (Fig. 5). Compared to Fig. 5b, all linear regressions represented in Fig. 5a were stronger on the basis of R2 values. These observations imply that the value of A210/A254 aver-aged over 1–2 kDa range are affected by DOM aromaticity, whereas the specific colour values were not only dependent on aromatic contents associated with AMW 1–2 kDa range but were also a reflection of the non-aromatic content involved in AMW below 1 kDa. This finding indicates that A210/A254 could be used to simplify complex HPSEC profiles and effectively represent DOM character changes during a storm event.

2.6. Influence of seasonal variation

In order to statistically determine the influences of seasonal variations on stormwater characteristics, we used the statistical tools PPMC and ANOVA to assess the correlations between colour measurements and those water quality parameters. Table 3 indicates that turbidity was the only parameter found to be unrelated to season related variables ($p > 0.05$) when comparing the results of Event 3 and those of the combined Event 1 and Event 2. This could imply that the suspended substances entering into surface water bodies were independent of seasonal changes. Other general parameters and A_{210}/A_{254} averaged over the AMW range 1–2 kDa of DOM were found to be significant ($p < 0.05$). Due to the limited rainfall and surface runoff in the warmer season, microbial processes could explain the associated increases in aromaticity and higher results of DOC, UV_{254}, $SUVA_{254}$, colour and specific colour.

Considering seasonal change influences as discussed above, both Event 1 and Event 2 occurred during rainy seasons, in which the stormwater samples may have had similar DOM characterisations, while Event 3 under hot summer conditions showed distinctively different DOM. Sharp et al. (2006) investigated the seasonal variation in surface water DOM in England and found that there was a significant change in DOM composition throughout the year. There was agreement between these observations and a similar study reported by Chong et al. (2013). These authors also found that the dry-weather storm event differed from another three wet-weather events. In addition, fulvic-like and humic acid-like compounds were mainly attributed to the dry-weather event. All wet-weather event samples had higher concentration of soluble microbial by-product-like substances than other regions.

3. Conclusions

DOC and UV_{254}, as conventional DOM parameters, were found to be strongly correlated to the changes in stormwater quality during each storm event. Colour measurements of stormwater were indicative for both non-aromatic and aromatic compounds of DOM. The profile of HPSEC–UV could provide additional physiochemical characteristics of stormwater-associated DOM, molecular weight and size distribution, and also provide some interesting information on the influence of DOM character on UV absorbance measurements at 254 and 210 nm. A_{210}/A_{254} is an important parameter which could also be used to estimate the DOM proportions of functional groups and conjugated carbon species. The water quality results combined with the flow data could provide further insight on pollutant loadings and their characteristics during storm events. This implies that flow condition indeed plays an important role in affecting pollutant load in storm events. The correlation among various parameters associated with DOM properties and water qualities were explored using simple statistical methods. This study only provides limited data and did not fully indicate various factors influencing pollutant runoff and accumulation in stormwater, such as land use, seasonal changes and urban activities. The results from this study suggest, moreover, that specific treatment may be required to reduce contaminants from urban stormwater.

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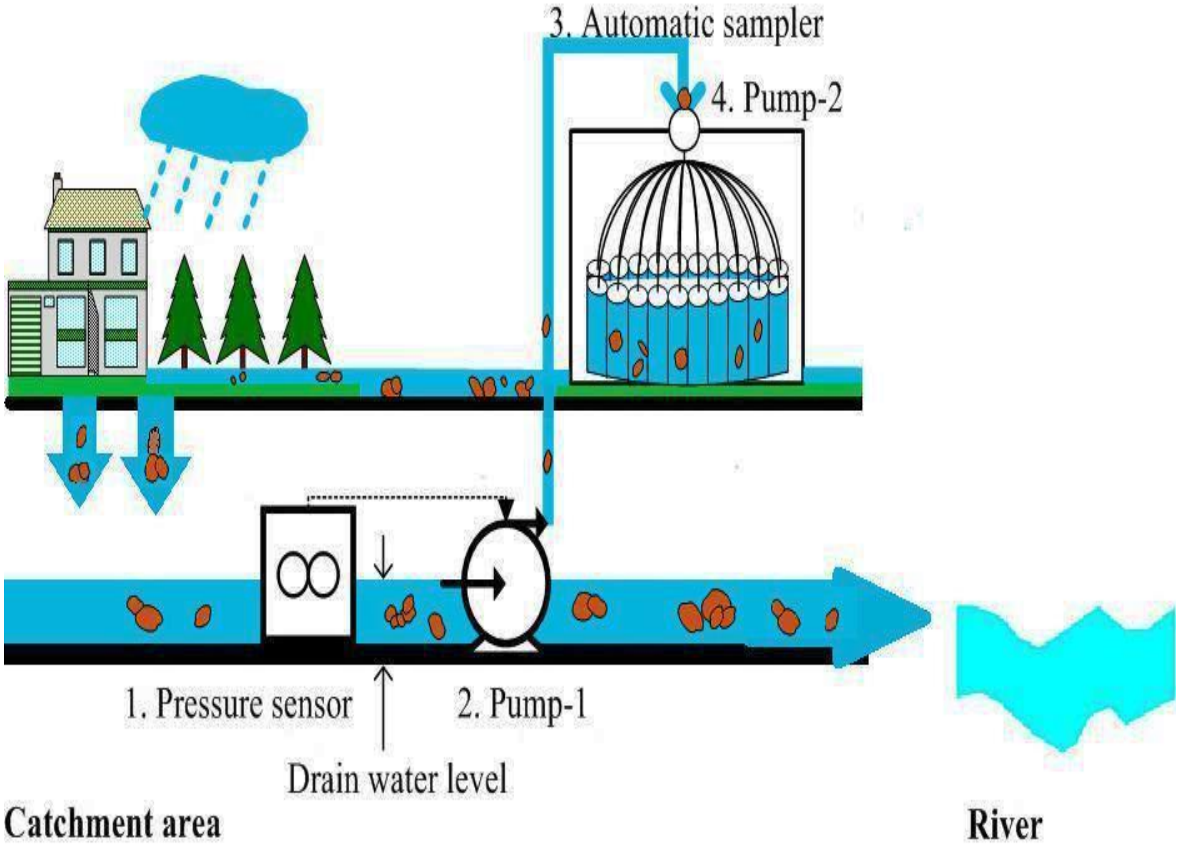

Fig. 13. Schematic of the stormwater capturing system used for sequential sampling.

(1) Pressure sensor: Placed in the drain to measure water level (m) in 5 min intervals and also send signal when drain water level changed (up and down) by 25 mm;

(2) Pump-1: Installed in the drain to capture stormwater after active by the signal from pressure sensor;

(3) Automatic sampler (24-bottle carousel): Installed and housed in the cabinet;

(4) Pump-2: Part of the automatic sampler assembly for pumping stormwater into the sampling bottles.

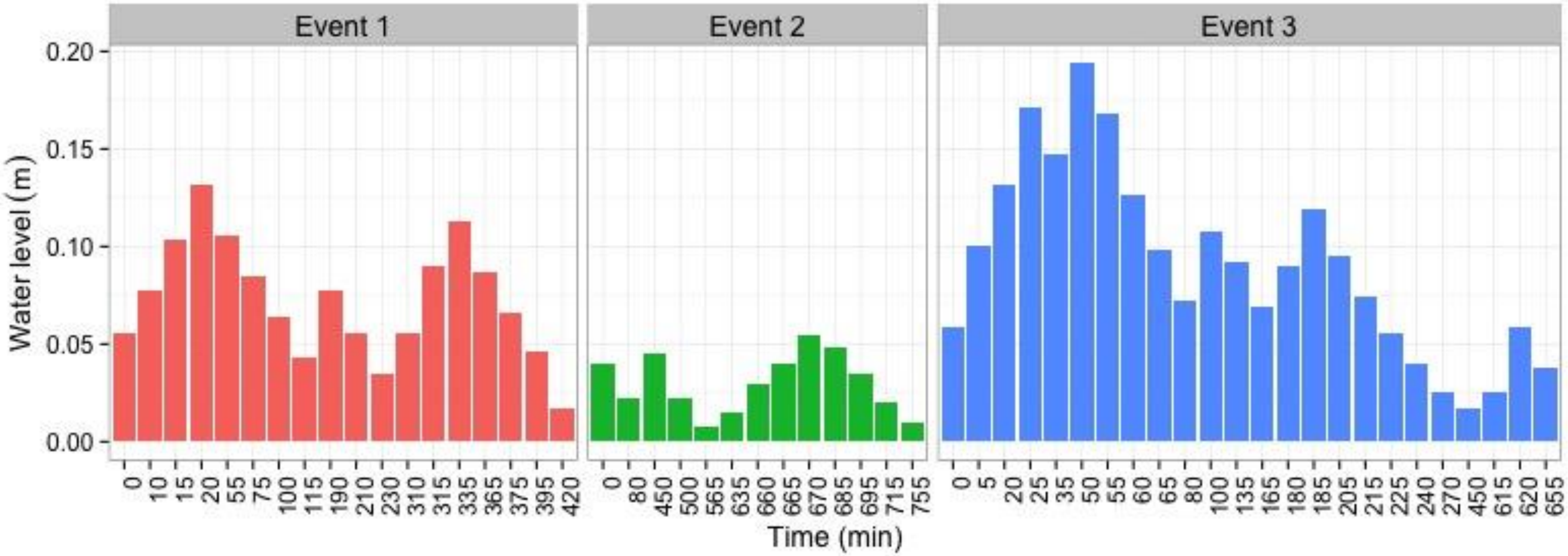

Fig. 14. The relationship of water level and the corresponding storm event duration.

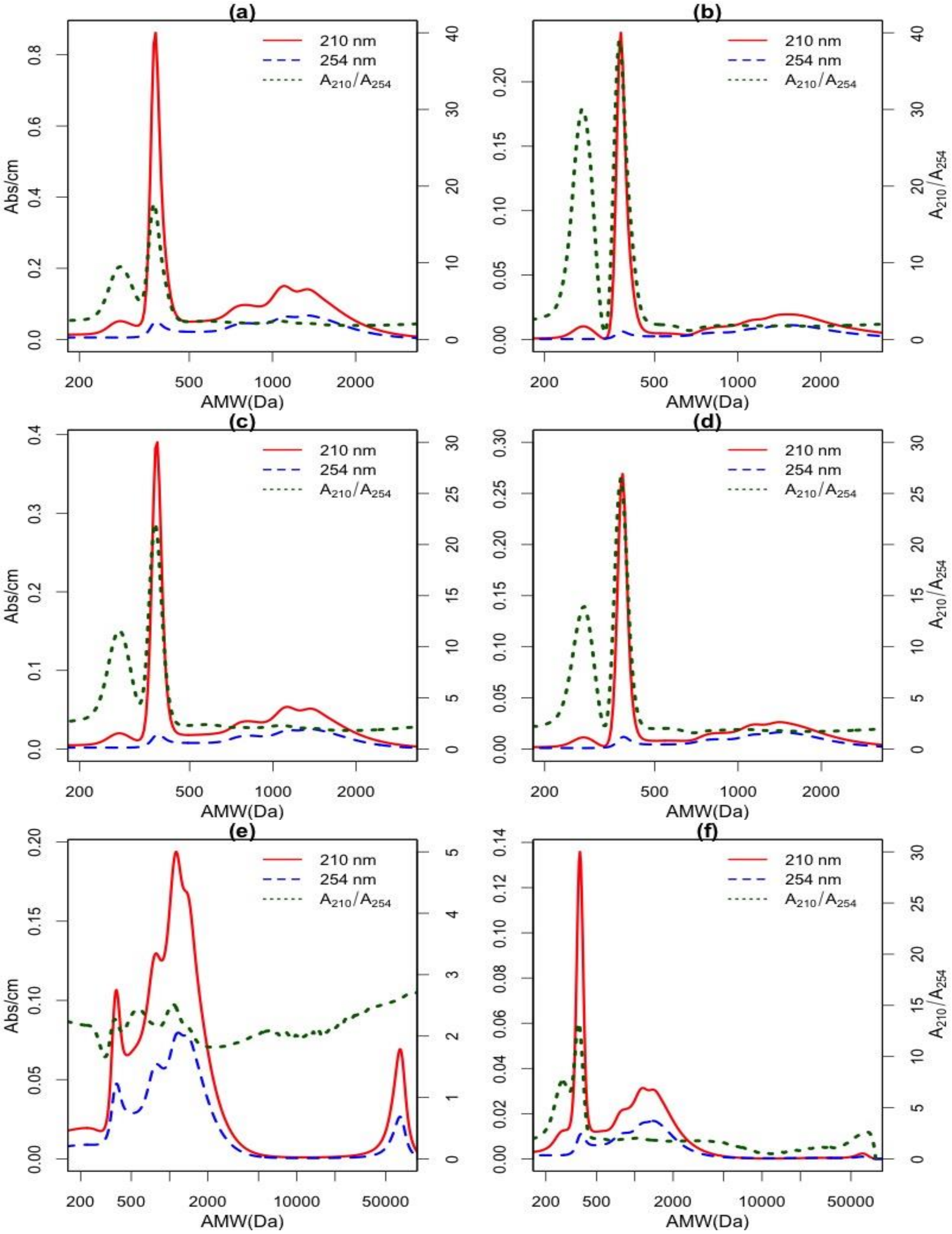

Fig. 15. Comparison of A_{210}/A_{254} values and HPSEC-UV chromatograms obtained at 210 nm and 254 nm: (a) Event-1 First-sample, (b) Event-1 Last-sample, (c) Event-2 First-sample, (d) Event-2 Last-sample, (e) Event-3 First-sample, and (f) Event-3 Last-sample.

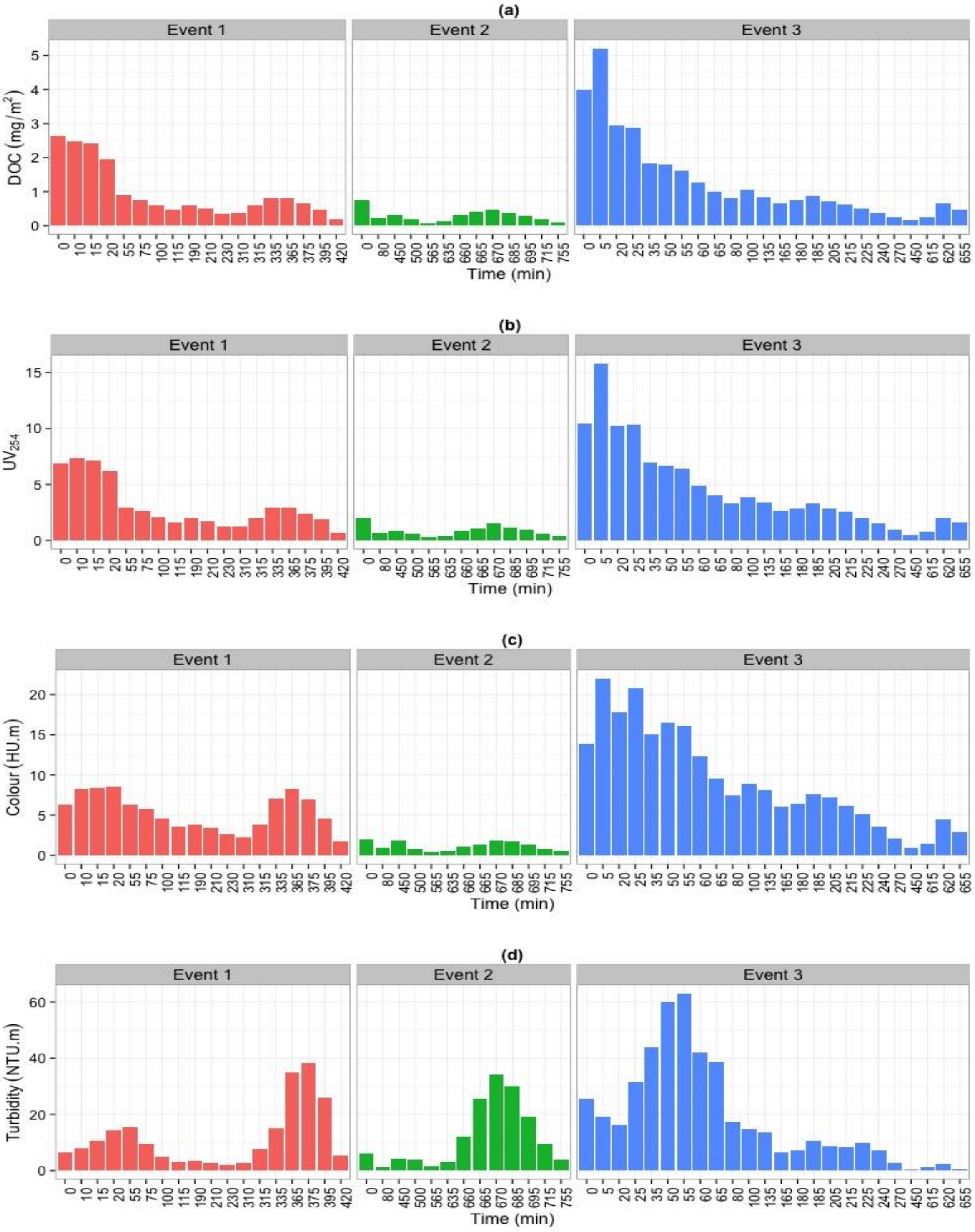

Fig. 16. The relationship between the results of general parameter values multiplied by water levels and the corresponding storm event duration, (a) DOC, (b) UV254, (c) colour, and (d) turbidity.

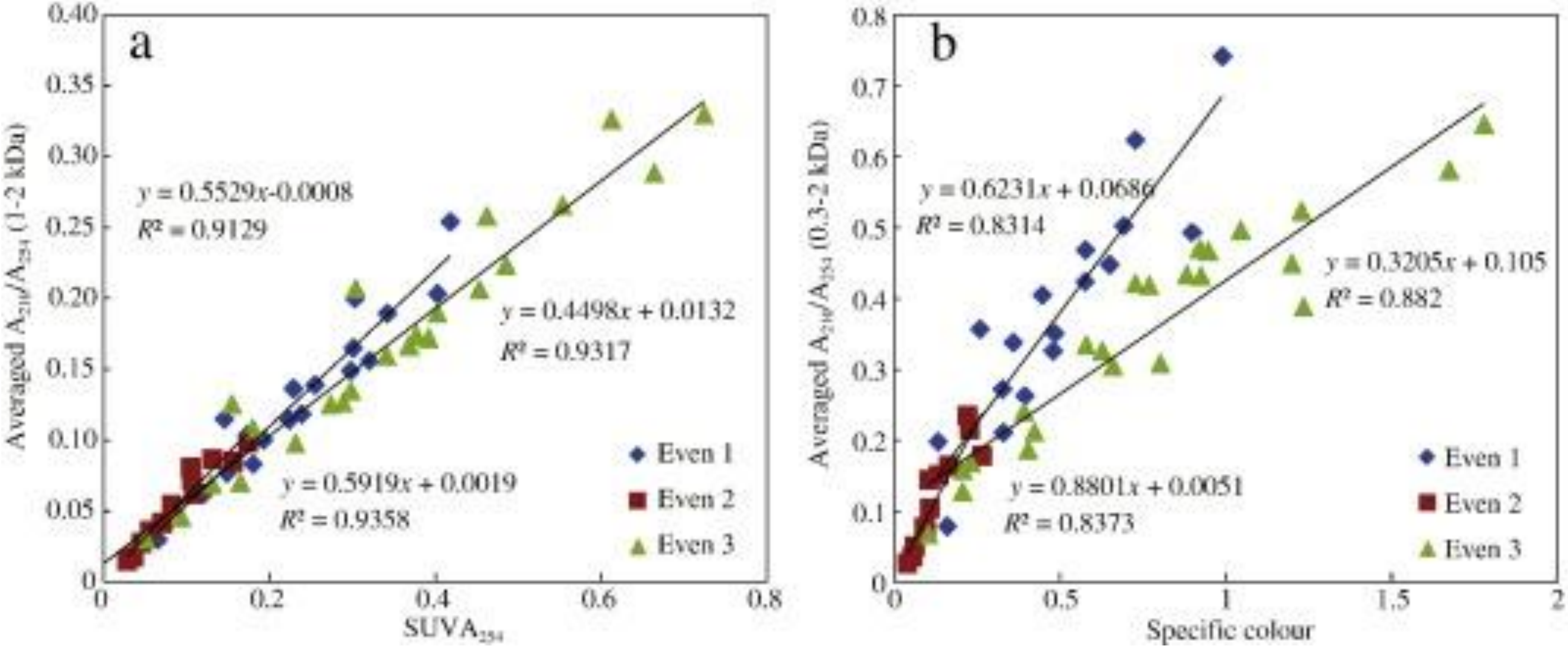

Fig. 17. Correlation improved by runoff volume integration (a) between $SUVA_{254}$ and A_{210}/A_{254} averaged over AMW 1–2 kDa, (b) between specific colour and A_{210}/A_{254} averaged over AMW 0.3–2 kDa.

Table 1. Summary of monitored storm events.

Rainfall event (m/day)	Rainfall (mm)	Rainfall duration (min)	Summary Antecedent dry period (days)	Number of samples (n)	Season
Event-1 (29/07)	10	420	14	18	Winter
Event-2 (19/08)	8	755	7	13	Winter
Event-3 (25/11)	14	655	7	24	Summer

Table 2. Results of DOM characterization of the stormwater samples (n= number of samples).

	Event-1		Event-2		Event-3	
	Range	Mean ± SD $^{n=18}$	Range	Mean ± SD $_{n=13}$	Range	Mean ± SD $_{n=24}$
Turbidity (NTU)	47 - 580	172 ± 172	59 - 638	352 ± 214	14 - 431	175 ± 122
Colour (HU)	41 - 112	77 ± 22	34 - 57	41 ± 7	57 - 236	99 ± 44
UVA_{254} (Abs/cm)	0.217 - 1.227	0.432 ± 0.267	0.198 - 0.497	0.301 ± 0.074	0.278 - 1.768	0.501 ± 0.378
DOC (mg/L)	6.7 - 47.2	13.5 ± 10.6	6.8 - 18.9	9.9 ± 2.9	7.3 - 67.5	14.7 ± 14.3
$SUVA_{254}$ (L/(mg.m))	2.6 - 3.9	3.4 ± 0.3	2.6 - 3.7	3.1 ± 0.3	2.6 - 4.1	3.7 ± 0.4
Specific colour (L.HU/mg)	0.13 – 0.99	0.49 ± 0.24	0.04 – 0.23	0.13 ± 0.04	0.10 – 1.23	0.75 ± 0.46

Table 3. Correlations between colour measurements, other parameters and the influences of seasonal variation.

	Colour	Seasonal variation
	PPMC (R^2, $p<0.001$)	ANOVA (p)
Colour		< 0.001
Turbidity	0.61	> 0.05
UVA_{254}	0.92	< 0.05
DOC	0.85	< 0.05
$SUVA_{254}$	0.81	< 0.001
Specific colour	0.74	< 0.001
Averaged A_{210}/A_{254} (1 – 2 kDa)	0.87	< 0.001

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Chapter 5: **High-performance size exclusion chromatography with a multi-wavelength absorbance detector study on dissolved organic matter characterisation along a water distribution system**

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Abstract

This study examined the associations between dissolved organic matter (DOM) characteristics and potential nitrification occurrence in the presence of chloramine along a drinking water distribution system. High-performance size exclusion chromatography (HPSEC) coupled with a multiple wavelength detector (200–280 nm) was employed to characterise DOM by molecular weight distribution, bacterial activity was analysed using flow cytometry, and a package of simple analytical tools, such as dissolved organic carbon, absorbance at 254 nm, nitrate, nitrite, ammonia and total disinfectant residual were also applied and their applicability to indicate water quality changes in distribution systems were also evaluated.

Results showed that multi-wavelength HPSEC analysis was useful to provide information about DOM character while changes in molecule weight profiles at wavelengths less than 230 nm were also able to be related to other water quality parameters. Correct selection of the UV wavelengths can be an important factor for providing appropriate indicators associated with different DOM compositions. DOM molecular weight in the range of 0.2–0.5 kDa measured at 210 nm correlated positively with oxidised nitrogen concentration ($r = 0.99$), and the concentrations of active bacterial cells in the distribution system ($r = 0.85$). Our study also showed that the changes of DOM character and bacterial cells were significant in those sampling points that had decreases in total disinfectant residual. HPSEC-UV measured at 210 nm and flow cytometry can detect the changes of low molecular weight of DOM and bacterial levels, respectively, when nitrification occurred within the chloraminated distribution system.

Key words:

HPSEC-UV, distribution system, DOM, sum of nitrate and nitrite concentration

Introduction

The character of dissolved organic matter (DOM) is a key index used in drinking water quality management (Chow et al., 2008; Korshin et al., 2009). DOM is a complex molecular mixture variable in composition and functionality which depends on its source. The presence of DOM in waters can be one of the principal causes of microbes' growth. A decrease in disinfectant residual has also been suggested to be associated with interaction between disinfectant and DOM (Wilczak et al., 2003; Kristiana et al., 2013). The properties of DOM, particularly that of molecular weight, have been demonstrated to strongly affect natural organic matter reactivity in natural systems as well as different stages of the treatment process (Chow et al., 2008; Korshin et al., 2009; Kristiana et al., 2010). Hence, an effective determination of the molecular weight distribution of DOM is critical not only for improving the water treatment process in terms of removal but also for understanding the disinfection process downstream in the distribution system.

Although promoting various benefits and improvements for drinking water treatment processes and distribution system, the application of chloramine as secondary disinfectant is associated with a number of adverse effects on water quality (Lipponen et al., 2002; Motzko et al., 2009; Zhang et al., 2009; Krishna et al., 2010; Bai et al., 2015). Nitrification management has drawn the most attention. A decrease in disinfectant residual and an increase in oxidised nitrogen concentrations (either nitrite or nitrate) as well as an increase in microbial risks are the common adverse effects owing to the occurrence of nitrification. Microorganism growth and regrowth and nitrite concentration increase have serious potential health risks (Lipponen et al., 2002; Krishna et al., 2013; Bai et al., 2015). Management of chloramine decay, maintenance of adequate disinfectant residual and determination of several operational, chemical and microbiological parameters throughout the water treatment process and distribution system are essential to ensure the supply of safe and high quality potable water to all consumers.

Nitrification is a two-step process. Ammonia is initially oxidised to nitrite, and nitrite is further oxidised to nitrate (Lipponen et al., 2002). These processes are associated with nitrifying microorganism occurrences and activity. An increased amount of ammonia in chloraminated waters is linked to nitrification in the drinking water distribution system. Increase in either nitrate or nitrite concentration is normally considered to be positively correlated with nitrification (Odell et al., 1996). Many researchers have also indicated that the level of microbes in pipe water is inversely related to the content of total disinfectant (Lipponen et al., 2002; Zhang et al., 2009). Monitoring and understanding treated water quality in the distribution system is hence required to effectively manage distribution system performance.

High performance size exclusion chromatography coupled with UV detection (HPSEC-UV) has been successfully and widely used for determining the performance of drinking water treatment processes, such as DOM removal evaluation, potential disinfection by-product (DBP) formation prediction and treated drinking water distribution management (Fabris, et al., 2008; Chow et al., 2008; Xing et al 2012; Kristiana et al., 2013). These previous studies have demonstrated the low molecular weight DOM compounds are recalcitrant to removal by conventional coagulation treatment. Some studies have also indicated these low molecular weight fractions remaining in treated water are particularly associated with water quality degradation in distribution systems (Kristiana et al., 2010, 2013). In most cases, such measurements have generally focused on aromatic components by detection at a single wavelength in the UV range of 250–280 nm. The non-aromatic groups of DOM associated with low molecular weight fractions do, however, not absorb strongly at this range of wavelengths but can potentially impact water quality. HPSEC coupled with a multiple wavelength detector may further characterise DOM molecular weight distribution and yield more insight into the key information that is of importance in water quality investigations.

Additionally, flow cytometry in combination with a fluorescence staining method has been utilised to characterise microbes present in distribution system. Due to the ability to provide rapid, accurate and high throughput, this advanced analytical method has been frequently used to characterise bacterial removal throughout water treatment processes and distribution systems (Hoefel et al., 2005; Hammes et al., 2008; Ho et al., 2012).

In this paper, HPSEC coupled with a multiple UV wavelength detector, from 200 nm to 280 nm, was employed to characterise DOM as an indirect assessment tool for potential nitrification occurrence along an operating distribution system. This multi-wavelength HPSEC approach would be useful to investigate the impact of different fractions of DOM on nitrification, including those fractions of relatively lower molecular weight, less aromatic character and with a weak absorbance response in the 250–280 nm wavelength range. A key focus was on developing an organic characterisation tool for understanding how DOM impacts on water quality in chloraminated distribution systems. This study covers both aspects of general water quality parameter changes associated with nitrification occurrence in the distribution system and their associations with changes in the DOM molecular weight profile. Due to the nature of an operating system with possible changes in both environmental and operational conditions along the distribution system, the study was designed to compare the interrelationship between samples in different sections of a distribution system supplied from the same water treatment plant.

1. Materials and methods

1.1. Sample site description

For this study, the Tailem Bend (TB) water treatment plant (WTP) and drinking water distribution system (DS) in South Australia were selected. This is a country water supply distribution system consisting of a single long trunk main with branches to several remote communities. Chloramine as secondary disinfectant to provide protection against microbial contamination is most suitable for this type of long distribution system to ensure disinfectant residuals reach the end of the system. The TB system sources its water from the River Murray and the raw water has variable colour (18 ± 20 HU January 2007–April 2015, n = 429 analyses) and DOC concentration (5.4 ± 2.8 mg/L January 2007–April 2015, n = 432 analyses). Conventional treatment, including coagulation, flocculation, sedimentation and filtration followed by UV disinfection and chloramination are the main steps used at the TB WTP to produce good quality water to enter into the DS. The main focus of this study was on TB WTP and subsequently through the DS with different branches and different locations, including inlet and outlet of storage tanks and customer taps to provide a study of water quality changes within the distribution system using an operating water supply system.

1.2. Sampling procedure

Seventeen sampling points across the WTP and DS of the TB system were chosen based on the size and layout (position of tanks and customer taps (CTs) at the end of the major branches) with a full survey completed in May 2012 (**Fig. 1**). Six samples were collected from the WTP, including the raw water sample (TB-Raw), samples throughout the treatment processes (settled water, TB-WTP-1, post filtration prior to disinfection, TB-WTP-2, post disinfection prior to filtered water storage tank, TB-WTP-3, filter backwash water, TB-WTP-4, and supernatant, TB-WTP-5). The distribution system consists of multiple branches, three of them were selected based on available data of previous disinfectant decay study, including the main branch TB-Keith (7 sampling points including customer site, from TB-treated (located 1.5 km after WTP), and TB-B1-1 to TB-B1-CT) as well as branches TB-Lower Lakes (2 sampling points, TB-B2-1 and TB-B2-CT) and TB-Karoonda (2 sampling points, TB-B3-1 and TB-B3-CT). Extended sampling points focusing on Branch 2 (TB-Lower Lakes) was further completed in September 2014, since the analytical results of the first survey indicated that the Lower Lakes (LL) had the most degradable water quality compared with the other two branches. Ten sampling points around many main communities were hence selected for further analysis, designated TB-LL-1 to TB-LL-10, as shown in **Fig. 1**. TB-B2-CT and TB-LL-6 were the samples collected at the same customer tap (CT) location.

1.3. Instrumental analysis

Samples for DOC and UV_{254} determinations were filtered through a 0.45 μm membrane. A 1 cm quartz cell was used for UV_{254} analysis. It is expressed in Abs/cm. DOC was measured using a Sievers 900 Total Organic Carbon Analyser (GE Analytical Instruments, USA). Specific UV absorbance ($SUVA_{254}$) expressed in L/(mg· m) was calculated as UV_{254} divided by DOC multiplied by 100.

Total chlorine residual was determined using N,N-diethyl-p-phenylenediamine (DPD) – ferrous ammonium sulphate (FAS) titrimetric procedure (Standard Method 4500-Cl (F), APHA, 1998). Free ammonia concentrations were analysed using an ammonia-selective electrode (Standard Method 4500-NH_3 (D), APHA, 1998). Analysis of nitrate and nitrite were conducted through Standard Method 4500-NO_3^- (I) and 4500-NO_2^- (F), respectively (APHA, 1998).

Molecular weight profiles were determined using a Waters Acquity H-Class system with a photodiode array detector (Waters Corporation, USA) acquiring between 200-280 nm. The Shodex KW802.5 packed silica column (Showa Denko, Japan) was equilibrated at 30 °C. Samples were filtered through a 0.2 µm membrane filter prior to analysis and 100 µL samples were injected. The mobile phase was 0.02 mol/L phosphate buffer at pH 6.8 adjusted to an ionic strength of 0.1 mol/L with sodium chloride. The system was operated at isocratic conditions with an eluent flow rate of 1.0 mL/min. Polystyrene sulfonate standards (Polysciences, USA) with molecular weights 4.6, 8, 18 and 35 kDa were used to calibrate the retention time response to apparent molecular weight (AMW).

Flow cytometry (FCM) analyses were conducted using a FACSCalibur flow cytometer (Becton Dickinson, USA) equipped with an air-cooled 15 mW argon ion laser, emitting at a fixed wavelength of 488 nm. Data were analysed using CellQuest software (Becton Dickinson, USA). Total numbers of bacteria were enumerated following staining of the bacteria with SYTO-9 and the BacLight bacterial viability kit (Molecular Probes, USA) as described previously (Hoefel et al., 2005). Results for FCM were presented as cells/mL.

1.4. Statistical analysis

Statistical and graphical analyses were made with R programming language (version 3.1.0, R Development Core Team). The relationship between HPSEC-UV profiles, chemical and microbial parameters were analysed by Pearson's Product Moment Correlation (PPMC). Both correlation factor (r) and probability (p) values were applied to determine the significance of correlation. Chromatographic data obtained from multi-wavelength HPSEC analysis were also explored and interpreted using *R*.

2. Results and discussion

2.1. General water quality analysis

Results of general water quality analyses for all samples are combined and illustrated in Fig. 2. Water samples taken through the WTP, DS and CT at the terminal sites of the system were included in order to understand both aspects of the system; treatment processes at the WTP and water quality change in the distribution system with an overall view of integrating the two together as well as determine potential factors associated with nitrification occurrence. UV_{254}, DOC and $SUVA_{254}$ as conventional DOM parameters were used to indicate DOM quality and quantity. As standard indicators for nitrification assessment, concentrations of free ammonia and both nitrate and nitrite were also analysed. Analytical results shown in Fig. 2a reveal that DOM removal was significant in the TB WTP process based on a comparison of raw (TB-Raw) and treated (TB-Treated) water samples, from 12.6 to 5.2 mg/L. DOC concentrations in both the first survey and the extended sampling points around the LL branch were relatively stable, in a range of 4.1–5.9 and 2.3–3.0 mg/L, respectively. The differences in treated water DOC concentrations between the first survey and the extended sampling points was due to the change in source water quality from the River Murray during the study period; approximately three magnitude reduction of DOC concentration was observed between 2012 and 2014. Braun et al. (2014) have illustrated drought followed by floods resulted in River Murray source water with high levels of natural organics and turbidity during 2010 - 2012. The river water quality between the period of September 2010–August 2012 was referred as floodwater with extreme organic intrusion, with DOC in a wide range of 4.6–19.1 mg/L and UV_{254} in a range of 0.139–0.799 Abs/cm. Higher DOC in source water challenges the conventional water treatment process and usually results in slightly higher DOC concentration in treated water (Fabris et al., 2008; Braun et al., 2014). Hence, the DS samples collected in 2012 had higher DOC concentrations than samples captured in 2014. The values of UV_{254} and $SUVA_{254}$ of the first survey were also higher than those collected in 2014. These observations illustrate that the DOM from the first survey had more aromatic structures, since both UV_{254} and $SUVA_{254}$ are more representative of aromatic DOC groups (Her et al., 2008; Korshin et al., 2009). $SUVA_{254}$ values of both the first survey and extended samples were stable with minor variations within the distribution system (Fig. 2a). These observations show that water quality in DS was stable and indicate no measurable difference in DOC concentration passage through the distribution system.

The sums of nitrate and nitrite concentration and free ammonia concentration are presented in order to understand potential nitrification occurrence (Fig. 2b). It was notable to observe that the sums of nitrate and nitrite and free ammonia concentrations were relatively stable with a slight increase along the TB main branch 1 (TB-B1), from TB-Treated to the terminal customer tap site (TB-B1-CT). The changes of inorganic nitrogen in TB main branch 2 (TB-B2) and TB main branch 3 (TB-B3) were, however, obvious from TB-Treated to the terminal sites of TB-B2-CT and TB-B3-CT. Ammonia concentrations decreased dramatically from 0.52 mg/L at the sampling point (TM-B2-CT) to <0.01 mg/L at the terminal site customer tap (TB-B2-CT), where the sum of nitrate and nitrite concentration increased twofold. A similar trend was also observed in the extended DS samples focusing on the LL branch. The sums of nitrate and nitrite concentration increased sharply from <0.2 to >0.8 mg/L, while free ammonia disappeared below <0.01 mg/L at the last 5 sampling points along the DS. The various changes of inorganic nitrogen concentration, an obvious decrease in free ammonia and the increase in either nitrate or nitrite, could indicate that nitrification occurred in this TB system, Branch 2 Lower Lakes in particular, since nitrification is a microbial process in which ammonia is oxidised, forming nitrate and nitrite. Increases in nitrate and nitrite concentration are the standard parameters that are used as indicators of potential nitrification occurrence. These observations suggest that nitrification occurred in some sampling sites regardless of the initial treated water quality, and that the general DOM parameters, UV_{254}, DOC and $SUVA_{254}$, may not be sensitive enough to indicate water quality degradation linked to potential nitrification occurrence.

2.2. Application of HPSEC-UV on distribution systems

With the availability of multi-wavelength UV absorbance detection, the analytical information obtained from the HPSEC-UV profile has been improved compared with the traditional single wavelength detection. This multi-wavelength detector can give a more comprehensive perspective on the relative absorbance intensity of various molecular weight compounds at different wavelengths (Her et al., 2008; Liu et al., 2010; Yan et al., 2012). In this study, HPSEC coupled with a multiple UV wavelength (200–280 nm) detector analysis was used to characterise DOM for both the water treatment process and subsequently through the distribution system. AMW data were explored and plotted against the entire response range (200–280 nm) of the UV detector using R. These three-dimensional surface plots of the multi-wavelength HPSEC data revealed there were significant differences in low AMW fraction (less than 0.5 kDa) of DOM in the distribution system samples and an increase in absorbance in the wavelength region less than 230 nm while the UV absorbance at the traditional range of 250–260 nm did not show any changes. This finding is supported by several previous studies suggesting the importance of using other wavelengths, such as less than 230 nm, for analysis different to the traditional 254 nm. Edwards et al. (2001) have reported the application of UV wavelengths in the range of 200–230 nm to estimate nitrate concentration in various water sources. Whitehead and Cole (2006) also addressed the fact that the wavelength around 210 nm correlated well with nitrate concentration, and also indicated microbial activity and nutrient content. Previous research studies have shown that when applying HPSEC-UV as DOM characterisation tool, UV absorbance spectra can be affected by the non-aromatic fraction of DOM with additional bands appeared at wavelength below 250 nm (Her et al., 2008; Korshin et al., 2009). Her et al. (2008) indicated the application of two wavelengths, 210 and 254 nm, is useful to estimate the contributions of functional groups (hydroxyl, carboxyl, carbonyl, ester), nitrogen containing compounds and the conjugated aromatic substituents. Korshin et al. (2009) also used the absorbance measured at two wavelength regions, 220-230 nm and 254-272 nm, to evaluate non-aromatic and aromatic compositions associated with DOM removal and DBP formation potential.

Based on a systematic multi-wavelength HPSEC data exploration with the combination of visual inspection and the findings from the above studies, 210, 230 and 254 nm were selected for this study to characterise various molecular weight fractions of DOM. The HPSEC-UV profiles at these wavelengths revealed that although DOM in all samples was mostly similar in the AMW ranges between 0.2 and 2 kDa, changes in HPSEC profiles in the higher AMW ranges between 1 and 2 kDa were observed across the WTP process and changes in the lower AMW ranges between 0.2-0.3 kDa were observed along the distribution system. Eight representative HPSEC profiles, including raw water, treated water, DS and CT waters, were chosen to illustrate this (Fig. 3).

Figs. 3a and 3b show the HPSEC-UV profiles of raw water and treated water after TB treatment processes, respectively. Fig. 3a shows there were two broad peaks located at AMW approximately 0.2-0.3 and 1-1.3 kDa. Fig. 3b shows that compounds with higher AMW (>1 kDa) were effectively removed as indicated by the reduction of UV absorbance at all three wavelengths, and a slight increase in the absorbance was also observed at lower AMW of 0.2-0.3 kDa measured at 210 nm. The removal of high AMW fraction was consistent with the DOC and $SUVA_{254}$ results suggesting that the aromatic fraction was mostly removed and lower AMW fraction of DOM was still present and entered into distribution system. In addition, $SUVA_{254}$ is generally well used to describe and evaluate DOM reactivity and treatability in waters (Chow et al., 2008; Korshin et al., 2009; Xing et al., 2010), all DS samples had low $SUVA_{254}$ values and were in the range of 1-3 L/(mg· m) indicating that the remained fractions of DOM may have similar characteristics, relatively less aromatic in nature and possessing lower molecular weight components.

Figs. 3c-3f present the HPSEC-UV profiles of the first survey, two main branches 1 and 2 samples from DS and subsequent CT waters (end of distribution system). The peak located at lower AMW approximately 0.2-0.3 kDa, was so called the recalcitrant DOM and was observed in all these samples. This sharp peak was especially sensitive with higher signals at lower wavelengths, 210 and 230 nm, detection compared with the measurement at 254 nm. Applying a simple subtraction of absorbance intensities measured at 230 and 254 nm resulted in similar profile as measured at 210 nm. It is also worthwhile to point out that this salient feature was detected in all DS samples. Compared with the HPSEC-UV profile of TB-Treated water (Fig. 3b), both Figs. 3c and 3d show the absorbance signals of different AMW fractions were relatively consistent in TB-B1 DS at the beginning and end of the system. However the levels of absorbance response in the AMW range of 0.2–0.3 kDa were increased in the TB-B2 DS samples (Fig. 3e and 3f), with approximately 3 times increase in absorbance signal at 210 nm at the terminal site CT water sample (Fig. 3f) .

Similar changes in the level of absorbance response in the lower AMW range were also obtained from the extended DS samples around the LL branch collected in 2014. Although the DS samples around the LL branch has a stable water quality in terms of DOC and $SUVA_{254}$ values, the increases in the level of absorbance response in the lower AMW range of 0.3-0.5 kDa were also observed in the DS samples further away from the treatment plant. The samples obtained from TB-LL-1 to TB-LL-5 had similar absorbance in this AMW region, whilst increased absorbance was observed in the downstream samples collected from TB-LL-6 to TB-LL-10. Figs. 3g and 3h showing the multi-wavelength HPSEC-UV profiles of TB-LL-5 and TB-LL-6, respectively, were used to illustrate the changes of absorbance signals and hence the organic character.

Liu et al. (2010) have demonstrated more information can be extracted over a wider wavelength range measurement. These authors applied multi-wavelength HPSEC technique to investigate the differences between HPSEC-UV profiles, before and after water treatment, plotting AMW data against the entire response range (205-285 nm) of the UV detector instead of single wavelength detection at 254 nm. These authors also found low molecular acid and neutral compounds remained after treatment and these molecules with low AMW of 0.3 and 0.4 kDa absorb strongly at wavelength between 205 and 230 nm. The benefits of this multi-wavelength HPSEC method was also demonstrated in the current study with the increase of the peaks located at the low AMW region of the HPSEC-UV profile using the lower wavelength detection. This peak could be considered an indication of changes in organic compounds produced as a result of nitrification, and may be useful to indicate nitrification occurrence, since the absorbance signal changes in this AMW region coincided with the changes in sum of nitrate and nitrite concentration shown in Fig. 2b.

2.3. Association between DOM characteristics and nitrification

In order to ensure all samples can be standardised and further investigate the association between DOM molecular weight distribution and potential nitrification occurrence, a rapid and simple method of extracting HPSEC-UV information is needed to be developed for the rapid simultaneous analysis of DOM in various samples, raw, during treatment processes and treated water. The sum of absorbance intensities measured at 210 nm (A_{210}) in the range of AMW 0.2-0.5 kDa was hypothesised to be an indicator of potential nitrification occurrence.

Correlation analysis between the sum of absorbance intensities provided by HPSEC-UV profiles and conventional water quality parameters were further studied using simple linear regression. The sum of total area measured at 254 nm (A_{254}) could be applied to estimate DOC concentration ($R^2 = 0.96$, $p < 0.05$) (Fig. 4a). The active aromatic substances are generally the dominant proportion in DOM and are more sensitive to higher UV wavelengths (in the range of 250-280 nm) (Korshin et al., 2009). The sum of area measured at 210 nm in the lower AMW range (0.2-0.5 kDa) could also be used to estimate the sum of nitrate and nitrite concentration ($R^2 = 0.99$, $p < 0.05$) (Fig. 4b).

These findings indicate that lower AMW DOM was the main component associated with potential nitrification occurrence. There was agreement between these observations and similar research finding reported by Wetzel (1995) who also observed the importance of smaller and non-humic molecules as a source of energy for aquatic organisms. Our study also points out the advantage of using HPSEC to separate DOM based on molecular weight distribution and highlights the absorbance of the compounds of interest. HPSEC-UV analysis also showed the change of DOM molecular weight distribution in DS samples by the increase in absorbance signal at wavelength less than 230 nm. An earlier DOM study conducted by Chow et al. (2009) aimed to combine organic characterisation tools and biological analysis for distribution system managements and identified HPSEC with single wavelength detection at 260 nm can capture the changes in AMW distribution of DOM in distribution systems. These authors also indicated that the chloramination process (mild oxidation) has little impact on DOM characteristics. In agreement with this earlier study, our study has also illustrated there was no significant change in DOM characteristics along the chloraminated DS when measured at 254 nm, within the similar wavelength range of 250–280 nm. However, with the capability of the multi wavelength detector, our study confirmed the benefit of selecting wavelengths less than 230 nm can provide additional information of DOM characteristics and can also be used as an indicator of nitrification occurrence by detecting further changes in AMW distribution.

2.4. Microbiological analysis

In this study, the changes in microbial levels of waters in TB WTP and DS were also evaluated using FCM analysis. Concentrations of bacterial cell (total and active) in combination with total disinfectant residuals data are shown in Fig. 5. Changes in the bacterial cell concentrations were observed throughout the TB WTP processes. The active bacterial cell concentration of raw water (TB-Raw) was 1×10^7 cells/mL which was reduced significantly following treatment and disinfection. The conventional treatment removed physically 1 log reduction, leaving 1×10^6 cells/mL in the TB-WTP-2 sample and the disinfection process achieved 2 log removal, leaving 1×10^4 cells/mL in the TB-Treated sample (total of 3 log removal).

Although the total disinfectant residuals were lower in all TB DS water samples in comparison to the treated water sample (TB-Treated), the disinfectant concentrations were relatively stable with a slight fluctuation in TB-B1 DS. This resulted in stable bacterial cell concentrations throughout this branch. The total disinfectant residuals decreased continuously along both TB-B2 and TB-B3 samples. An approximately twofold decrease in disinfectant residuals was observed between TB-Treated and TB-B2-1 samples, and disinfectant was absent at the terminal site TB-B2-CT. The concentrations of total and active bacterial cells in TB-B2-CT sample increased to 1×10^6 cells/mL and were similar to the result prior to disinfection (TB-WTP-2). The order of the total bacterial cell concentrations at the three terminal customer tap sites was TB-B2-CT > TB-B3-CT > TB-B1-CT. This order coincided with the orders of sum of nitrate and nitrite and sum A_{210} of AMW (0.2–0.5 kDa) whilst the total disinfectant residual was in reverse order. Although originating from the same WTP process, the quality of customer tap water could degrade differently during passage through the DS.

The results for the extended DS samples around the LL branch indicated that concentrations of active bacterial cells increased whilst total disinfectant decreased along the DS. The last five sampling points sharing similar concentrations of active bacterial cells could be a result of total disinfectant loss. A decrease in concentrations of total disinfectant residuals (< 0.1 mg/L) and free ammonia (< 0.1 mg/L) and an at least twofold increase in concentration of oxidised nitrogen (sum of nitrate and nitrite) occurred in TB-Branch 2 customer tap site (TB-B2-CT) and the last five sampling sites in the LL system. Our results agreed with previous research and indicate that the level of microbials in DS areas increases when total disinfectant decreases (Lipponen et al., 2002; Bai et al., 2015).

The relationships between the HPSEC-UV profile and chemical and microbiological water quality parameters were studied by statistical analysis. Table 1 shows the significance based on PPMC results. The sum A_{210} of AMW (0.2–0.5 kDa) in both WTP process and DS correlated positively with the sum of nitrate and nitrite concentration ($r = 0.99$, $p < 0.001$, $n = 27$). The sum A_{210} of AMW (0.2–0.5 kDa) in the DS also correlated positively with the concentrations of active bacterial cells ($r = 0.85$, $p < 0.001$, $n = 21$). This HPSEC-UV indicator correlated negatively with the concentration of ammonia and total disinfectant residuals, r being -0.74 and -0.78 ($p < 0.001$, $n = 21$), respectively.

All the above statistical results obtained high values of correlation factor and probability ($p < 0.001$) therefore imply that the low AMW fraction detected at this wavelength is illustrating a relationship with nitrification occurrence. These high levels of absorbance intensity measured at 210 nm could be an indication of the increase in microbiologically derived components that have a high proportion of non-aromatic groups. The sum of area measured at 210 nm in the lower AMW range (0.2–0.3 kDa) could, hence, be used to simplify complex HPSEC profiles and effectively represent DOM character changes within the DS.

3. Conclusions

The well designed case study has demonstrated an operating chloraminated distribution system, with careful planning and selection of sampling points was appropriate to be used to compare water quality of different branches. The findings also showed the package analytical techniques applied can provide early notice of potential water quality degradation. Our results showed linkages between decrease in total disinfectant residual and degradation of water quality within the distribution system, such as higher concentration of nitrate and nitrite and higher level of microbial risks. These changes also coincide with the changes of the molecular weight distribution of DOM. Multi-wavelength HPSEC analysis demonstrated the occurrence of absorbance change of the lower AMW compounds (0.2–0.5 kDa) of DOM within the distribution system detected at less than 230 nm, this was not observed when traditional single wavelength, 254 nm, was only used for DOM detection. Our studies have also shown a positive correlation between low AMW (0.2–0.5 kDa) of signal measured at 210 nm and both changes in oxidised nitrogen concentration and bacterial activity. This study suggests both detector wavelengths (210 and 254 nm) are important to provide complete information on the physiochemical properties of DOM, and HPSEC-UV provides a convenient and effective way for simultaneous analysis of changes in DOM characteristics and potential nitrification occurrence in drinking water distribution systems.

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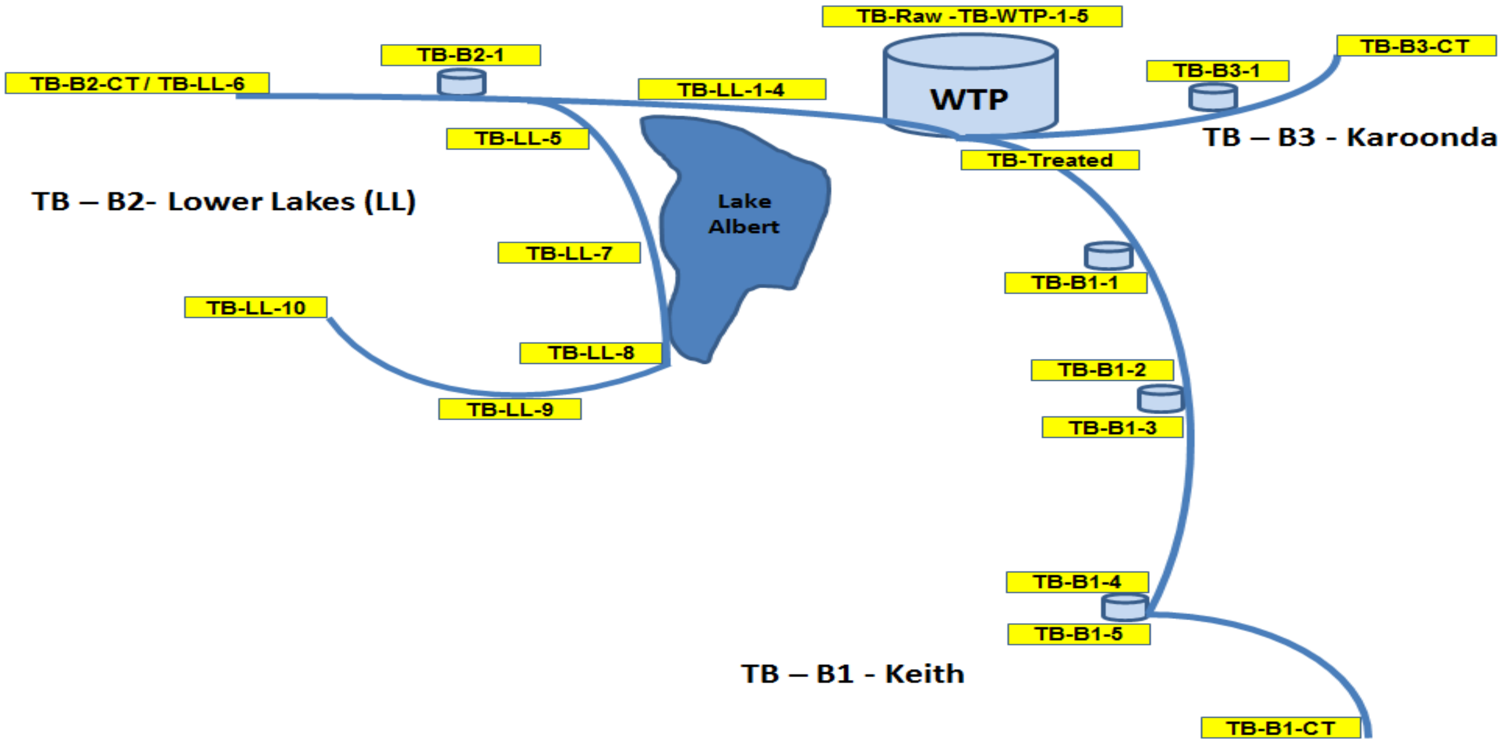


Fig. 18. Schematic illustration of sampling point location for the Tailem Bend (TB) system including the water treatment plant (WTP) and three main branches of the drinking water distribution system (DS). B and CT indicate branch and customer tap, respectively.

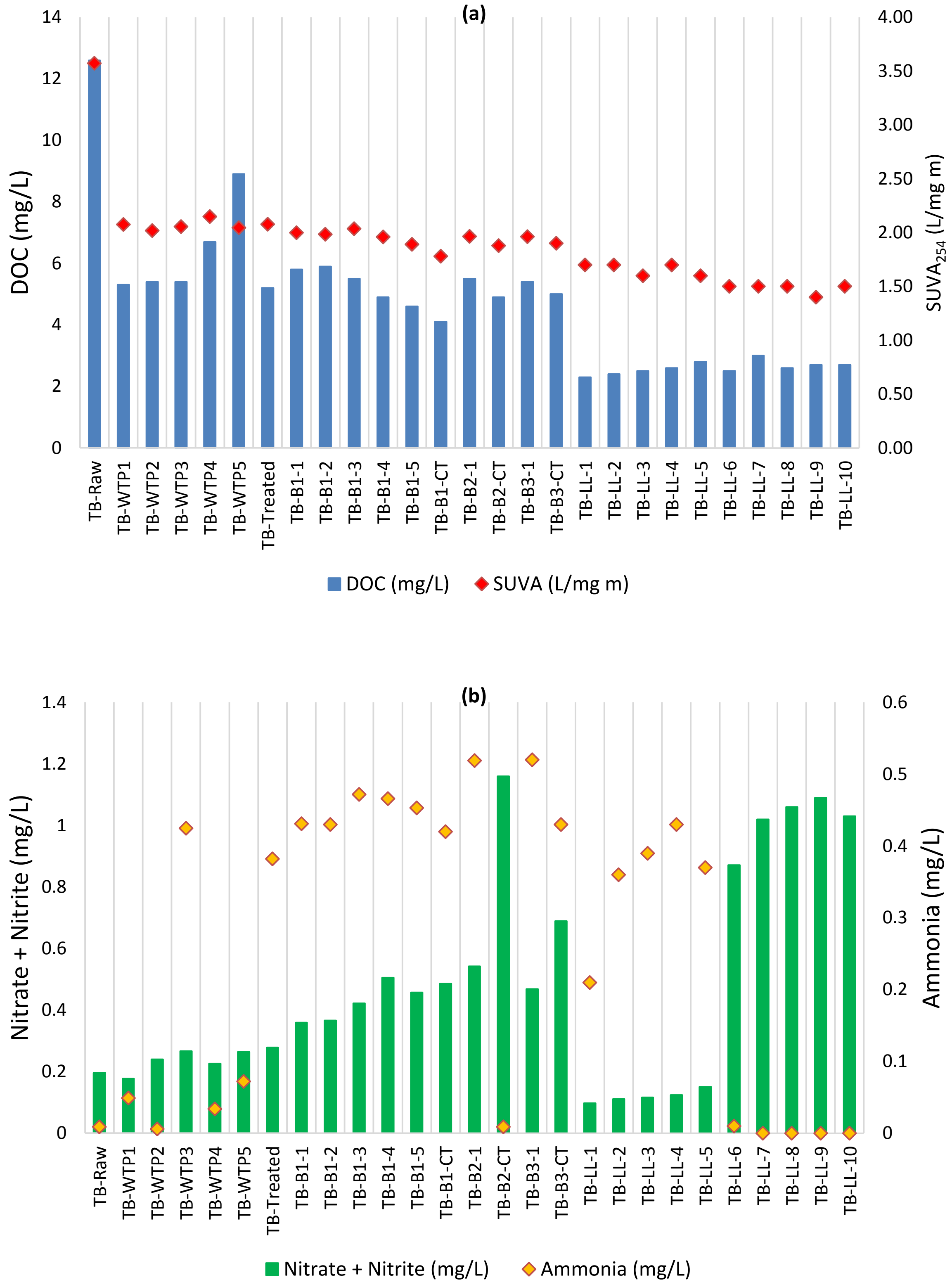
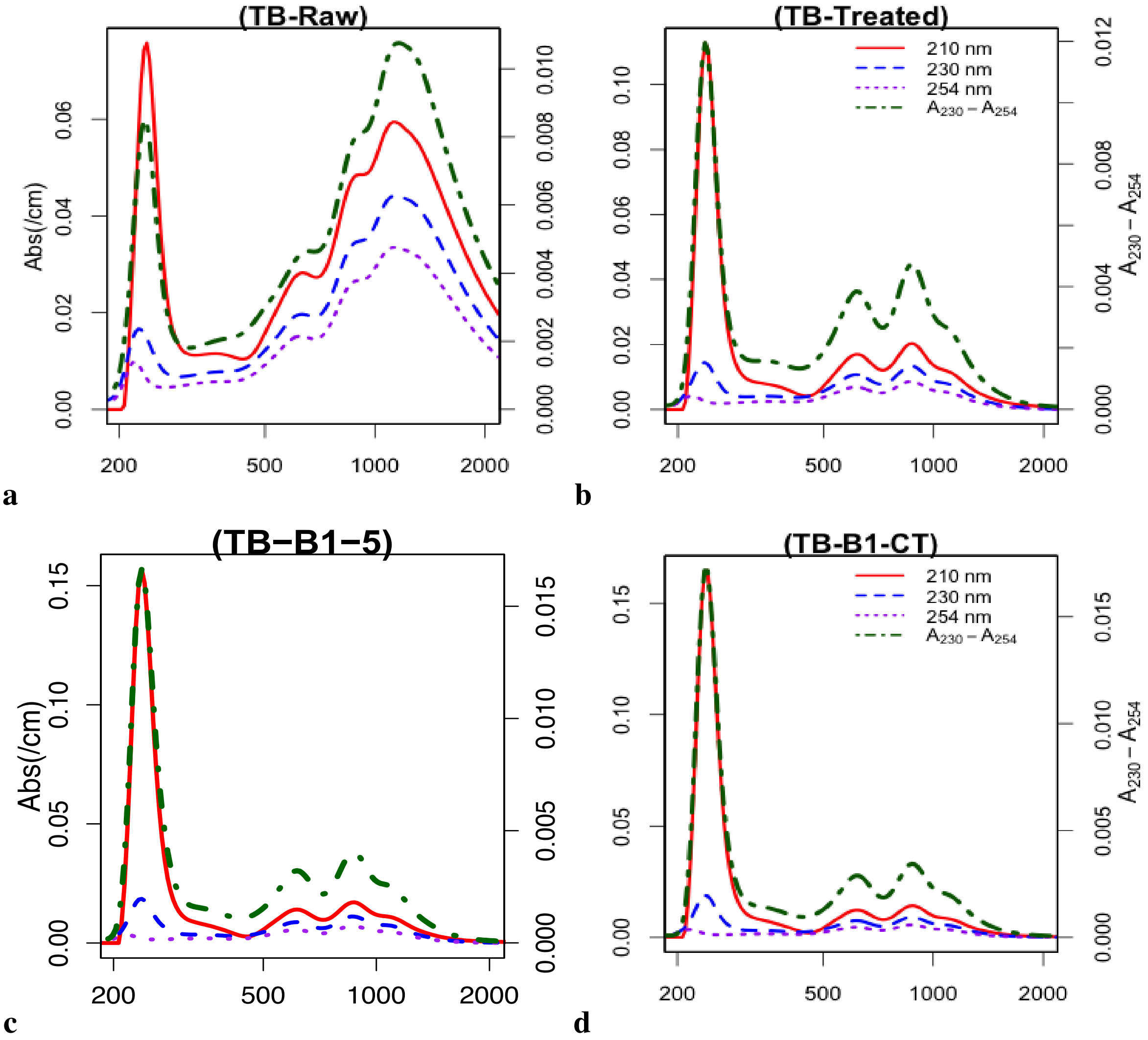


Fig. 19. General water quality results, (a) DOC and SUVA254 and (b) nitrate + nitrate and ammonia. DOC: dissolved organic carbon; SUVA: Specific ultraviolet absorbance.


(TB-Raw)
Abs(/cm)
0.00
0.02
0.04
0.06
0.000
0.002
0.004
0.006
0.008
0.010
200
500
1000
2000
a
(TB-Treated)
210 nm
230 nm
254 nm
$A_{230} - A_{254}$
0.00
0.02
0.04
0.06
0.08
0.10
0.000
0.004
0.008
0.012
$A_{230} - A_{254}$
200
500
1000
2000
b
(TB−B1−5)
Abs(/cm)
0.00
0.05
0.10
0.15
0.000
0.005
0.010
0.015
200
500
1000
2000
c
(TB-B1-CT)
210 nm
230 nm
254 nm
$A_{230} - A_{254}$
0.00
0.05
0.10
0.15
0.000
0.005
0.010
0.015
$A_{230} - A_{254}$
200
500
1000
2000
d

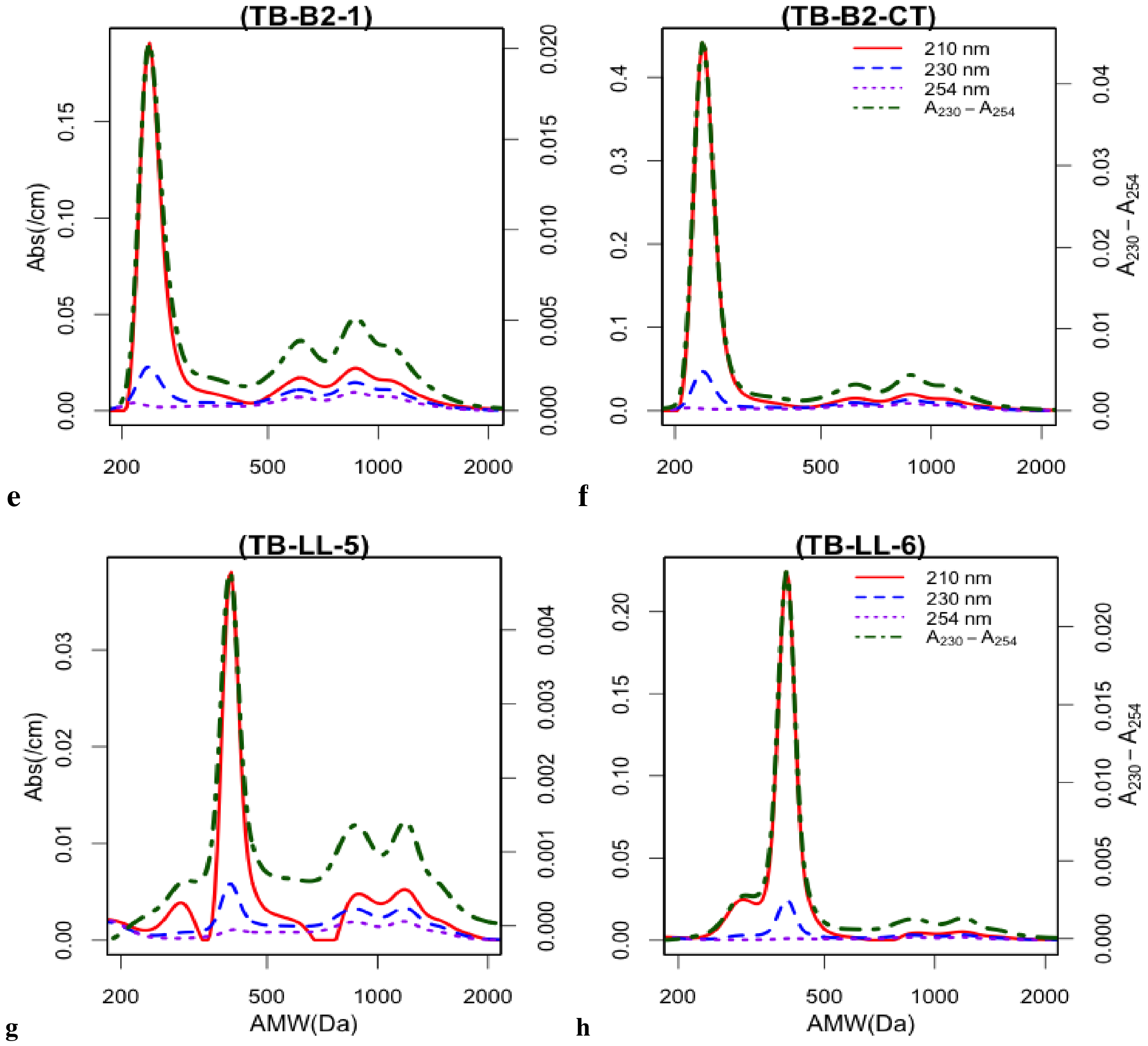


Fig. 20. Comparison of HPSEC-UV profiles meaured at different wavelengths (210 nm, 230 nm and 254 nm) and subtraction of A_{230} and A_{254} (a) TB-Raw (b) TB-Treated (c) TB-B1-5 (d) TB-B1-CT (e) TB-B2-1 (f) TB-B2-CT (g) TB-LL-5 and (h) TB-LL-6. HPSEC: high-performance size exclusion chromatography; UV: ultravilet; AMW: apparent molecular weight; TB, B and CT indicate, Tailem Bend, branch and customer tap.

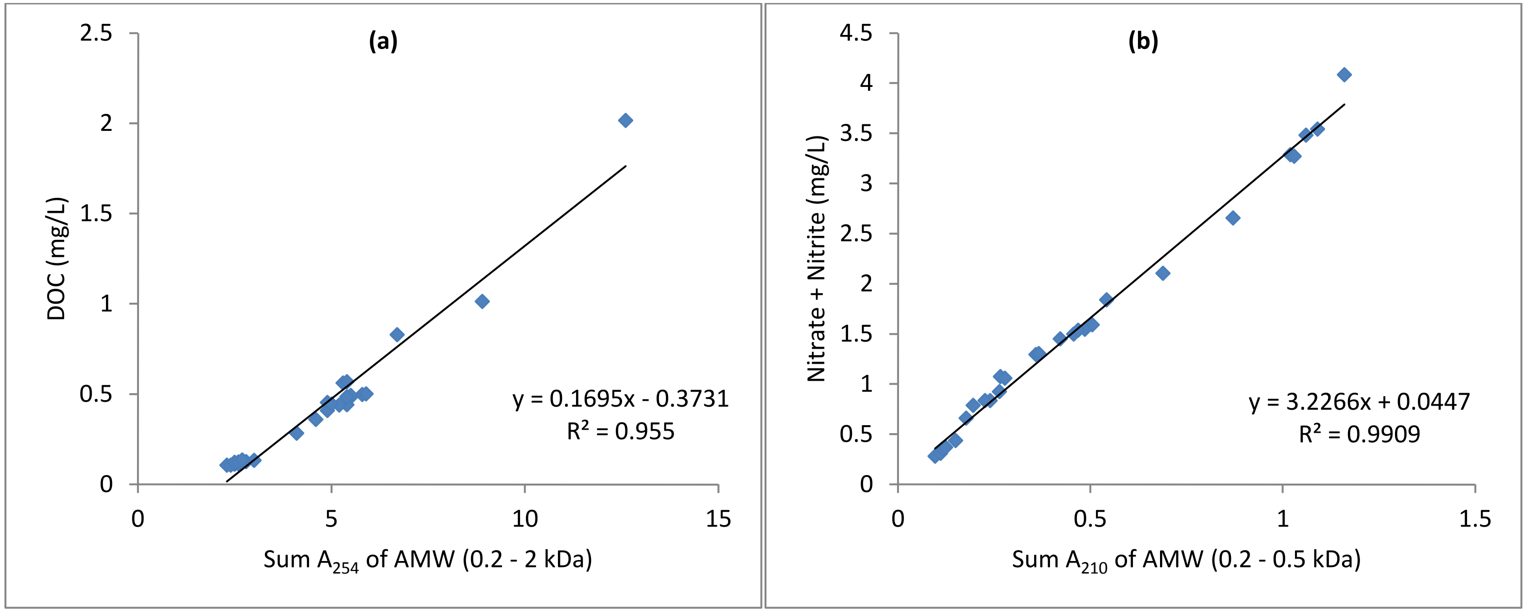


Fig. 21. Correlation between HPSEC-UV indicators and conventional parameters, (a) DOC concentration against the sum A254 of AMW in the range of 0.2–2 kDa, and (b) the sum of nitrate and nitrite concentration against the sum A210 of AMW in the range of 0.2–0.5 kDa. HPSEC: high-performance size exclusion chromatography; UV: ultravilet; AMW: apparent molecular weight; DOC: dissolved organic carbon.

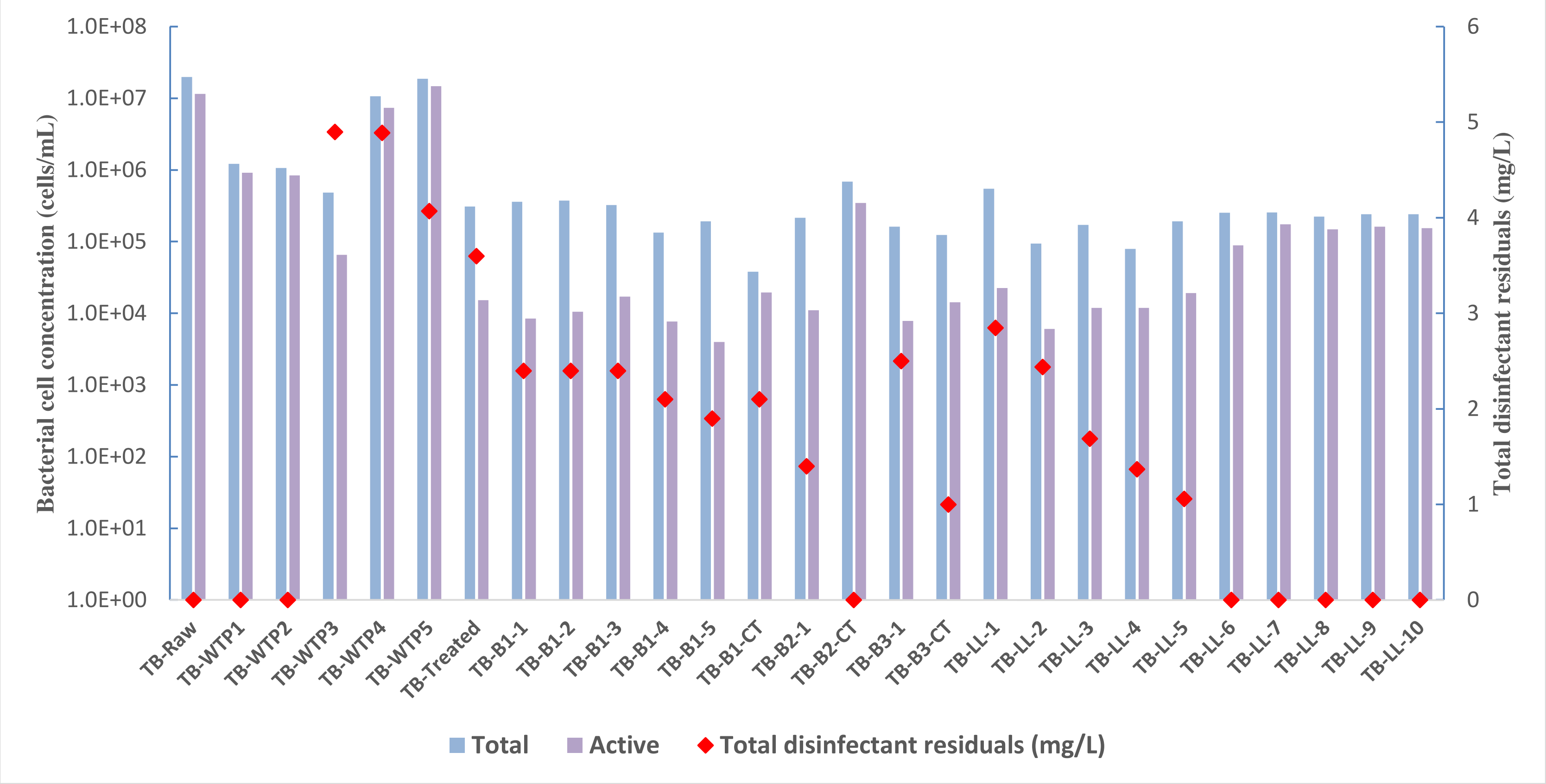


Fig. 22. Concentrations of bacterial cells (total and active) determined by flow cytometry and corresponding total disinfectant residuals results.

Table 4. Correlation coefficients between HPSEC-UV indicator and physiochemical and microbiological water parameters.

	Sum A_{210} of AMW (0.2–0.5 kDa)		
Water parameter	*n*	*r*	*p*
Nitrate + Nitrite (mg/L)	27	0.99	<0.001
Ammonia (mg/L)	21	-0.74	<0.001
Total disinfectant residuals (mg/L)	21	-0.78	<0.001
Total bacterial cells (cells/mL)	21	0.3	>0.05
Active bacterial cells (cells/mL)	21	0.85	<0.001

n = number of studied samples, TB WTP and DS samples (*n* = 27), DS samples (*n* = 21).

HPSEC: high-performance size exclusion chromatography; UV: ultravilet; AMW: apparent molecular weight;

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Chapter 6: **Conclusions and recommendation**

This study demonstrated the enhancement of data exploration, interpretation and information extraction by applying chemometrics to multi-wavelength HPSEC dataset. This task has been facilitated by the R software program with simple customised code the data interpretation and analytics purpose for the project. The benefits of the data analytics procedures and program codes developed in this study have also been demonstrated using a complex data sets consisting of natural dissolved organic matter (DOM) presence in environmental systems including drinking water supply system. The selection of HPSEC for further investigation in this project over other characterisation techniques clearly showed the use of this technique is very suitable for water studies and also that provides a good balance of the detail analytical information and the ease of conducting the analysis as well as the level of instrumentation requirement. Two case studies were carefully selected to bridge the knowledge gap by exploring the use of HPSEC as an environmental monitoring tool in a stormwater catchment and a drinking water distribution system. Application of HPSEC to study water supply system has been well documented with examples in transportation of NOM in source water, impact of NOM on water treatment process and limited studies in the chlorinated distribution system discussed in Chapter 2 but applying it for stormwater characterisation and interpreting the loss of chloramine residual in a distribution system were both new applications required investigation. These case studies have confirmed multi-wavelength HPSEC is capable of conducting more extensive DOM characterisation, which are valuable and provide new knowledge and better understanding of environments and drinking water supply systems.

Convention of HPSEC-UV data presentation is generally standardised as absorbance intensity (response) at one selected wavelength against retention time or apparent molecular weights because of limitations of software application and inefficiency of complex data processing. Extracting further information from multi-wavelength HPSEC is challenging, since this analytical technique results in a huge amount of data that requires efficient and careful interpretation. The vital purpose of this study was hence to introduce R software and determine its capability for multivariate data exploration, resolution, and interpretation and information extraction.

All aims and objectives of this thesis have been adequately achieved and the overall findings in this thesis have been summarised as follows:

The ability of the R software program is able to effectively process large quantities of data obtained from multi-wavelength HPSEC analysis. The data analytics procedure and program codes developed in this study made successfully the multi-wavelength HPSEC profile be more effectively integrated. And additional information of DOM characteristics is also obtained. This software now makes the data process routinely generate customised figures and tables easier. When visualised the data in the form of a 3D plot, the differences between samples are readily apparent and a very clarity of a figure makes the work seems to be simple and understandable, and yields more insight into the key information and knowledge of DOM composition and character that is of importance in water quality monitoring.

This work also reveals a variety of advantages of multi-wavelength HPSEC for characterisation of DOM associated with source water protection, drinking water treatability and subsequent distribution system management. This technique not only provides DOM physicochemical properties, in terms of molecular weight distribution, but also indicates the importance of correct UV wavelength selection. Both UV wavelengths 254 and 210 nm and their ratio (A_{210}/A_{254}) have been demonstrated enable to provide appropriate information associated with different DOM characteristics.

The performances of this combination technique extends as an environmental monitoring for stormwater determination and extends as early warning monitoring for product water quality analysis in the presence of chloramine along operating distribution system have been successfully demonstrated.

A series of general water quality parameters, such as DOC, UV_{254}, SUVA and colour, have also been found to be sensitive enough for stormwater quality assessment. The results of DOC and UV_{254} correlates well with the changes in water quality across storm events, and that of colour are indicative for aromaticity of DOM in stormwater.

A variety of chemical, physical and microbial analytical techniques applied for assessing the efficiency of water treatment and the quality changes of treated water in distribution within operating distribution system have also been found to be useful and correlated. The formation of lower AMW of DOM correlates positively with the sum of nitrate and nitrite concentration, and negatively with total disinfectant residuals and free ammonia concentration.

It is anticipated that the outcomes from this study will form an important basis for the future of software application for chromatographic data exploration and interpretation. Both case studies present the importance of full wavelength detection and suggest not only aromatic components but also non-humic substances need pay attention in future research. Lower molecular weight of dissolved organic matter is found to become more important and its composition and characteristics should drive more concerns in future water research.

Appendix

- **Data analytics procedures and program codes build in R software program**

```
function(Y,X,file,wavelengthlimit,MWlimit,wavelength1,wavelength2,wavelength3,wavelength4,abs1,abs2,
abs3,abs4,abs5,abs6,abs7,abs8,abs9,abs10,abs11,abs12,abs13,abs14,abs15,abs16,ASI,A210A254,A280A350
){
  a=read.table(file,header=F,sep="\t",dec=".",fill=T)
  a=a[-2,-1]
  a=data.matrix(a)
  temps=seq(0,15,length=895)
  a=cbind(c(0,temps),a)
  neg=which(a<0)
  a[neg]=0

  acheck=a
  # for (j in seq (1:ncol(acheck))) {
  #   for (i in seq(1:nrow(acheck))){
  #     if (acheck[i,j]<0){
  #       acheck[i,j]=0
  #     }
  #   }
  # }

  atoreturn=acheck[2:nrow(acheck),]
  atoreturn[,1]=10^(Y*atoreturn[,1]+X)
  atoreturn=atoreturn[order(atoreturn[,1]),]
  avalue=atoreturn[,2:ncol(atoreturn)]
  aMW=atoreturn[,1]
  awavelength=acheck[1,2:ncol(atoreturn)]

  rgl.set(1)
  c=which(aMW>MWlimit[1])
  a=which(aMW<MWlimit[2])
  limit=c(0,0)

  if(wavelengthlimit[1]==200){limit[1]=1}
  # below formula was determined by linear regresion wavelenght against column number
```

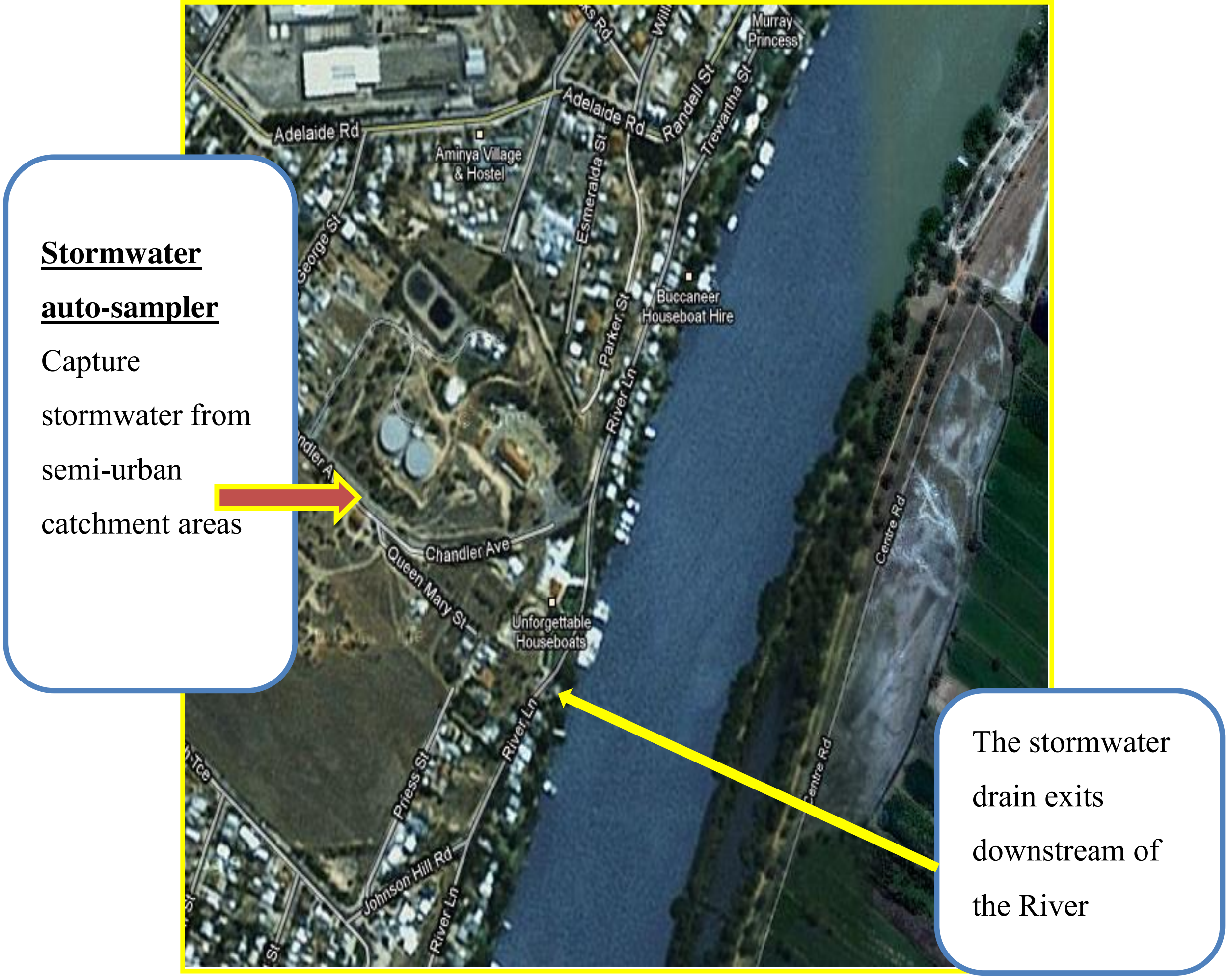
```
   else{limit[1]=floor((wavelengthlimit[1]-197.99)/1.1773)}
   limit[2]=floor((wavelengthlimit[2]-197.99)/1.1773)
   nbcol <- 5
   vertcol <- cut(avalue[c[1]:length(a),limit[1]:limit[2]], nbcol)
   cols <- get_colors(vertcol, brewer.pal(n=5, name="YlOrRd"))

   persp3d(log(aMW[c[1]:length(a)],10), awavelength[limit[1]:limit[2]],
avalue[c[1]:length(a),limit[1]:limit[2]],box=F, cex.axis=0.4,axes=F, lit=F, xlab="", ylab="", zlab="",
col=cols, lwd=0.8, alpha=0.8, smooth=T, pch=20, highlight.3d=T,angle=90)
   grid3d(c("z","y","x+-"),lwd=2)
   mtext3d("Log AMW (Da)","x-+",line=3.5,cex=1)
   mtext3d("nm","y--",line=2,cex=1)
   mtext3d("Abs(/cm)","z--",line=5,cex=0.8)

   if(wavelength1[1]==200){limit[1]=1}
   # below formula was determined by linear regresion wavelenght against column number
   else{limit[3]=floor((wavelength1[1]-197.99)/1.1773)}
   limit[4]=floor((wavelength1[2]-197.99)/1.1773)
   points3d(log(aMW[c[1]:length(a)],10), awavelength[limit[3]:limit[4]],
avalue[c[1]:length(a),limit[3]:limit[4]],box=F, axes=F, lit=T, xlab="", ylab="", zlab="", col="darkblue",
lwd=3, alpha=1, smooth=T, aspect=c(0.4,0.4,0.2),pch=20, highlight.3d=T,angle=90,add=T)

   if(wavelength2[1]==200){limit[1]=1}
   # below formula was determined by linear regresion wavelenght against column number
   else{limit[5]=floor((wavelength2[1]-197.99)/1.1773)}
   limit[6]=floor((wavelength2[2]-197.99)/1.1773)
   points3d(log(aMW[c[1]:length(a)],10), awavelength[limit[5]:limit[6]],
avalue[c[1]:length(a),limit[5]:limit[6]],box=F, axes=F, lit=T, xlab="", ylab="", zlab="",col="darkblue",
lwd=3, alpha=1, smooth=T, aspect=c(0.4,0.4,0.2),pch=20, highlight.3d=T,angle=90,add=T)

   if(wavelength3[1]==200){limit[1]=1}
   # below formula was determined by linear regresion wavelenght against column number
   else{limit[7]=floor((wavelength3[1]-197.99)/1.1773)}
   limit[8]=floor((wavelength3[2]-197.99)/1.1773)
   points3d(log(aMW[c[1]:length(a)],10), awavelength[limit[7]:limit[8]],
avalue[c[1]:length(a),limit[7]:limit[8]],box=F, axes=F, lit=T, xlab="", ylab="", zlab="",col="darkblue",
lwd=3, alpha=1, smooth=T, aspect=c(0.4,0.4,0.2),pch=20, highlight.3d=T,angle=90,add=T)

   if(wavelength4[1]==200){limit[1]=1}
   # below formula was determined by linear regresion wavelenght against column number
```

```
  else{limit[9]=floor((wavelength4[1]-197.99)/1.1773)}
  limit[10]=floor((wavelength4[2]-197.99)/1.1773)
  points3d(log(aMW[c[1]:length(a)],10), awavelength[limit[9]:limit[10]], avalue[c[1]:length(a),limit[9]:limit[10]],box=F, axes=F, lit=T, xlab="", ylab="", zlab="",col="darkblue", lwd=3, alpha=1, smooth=T, aspect=c(0.4,0.4,0.2),pch=20, highlight.3d=T,angle=90,add=T)
  par3d(windowRect=c(100,100,600,600),zoom=0.8)
  pl=par3d("userMatrix","zoom","FOV")
  rgl.viewpoint(zoom=pl$zoom,fov=pl$FOV,userMatrix=pl$userMatrix,interactive=F)
  bbox3d(color=c("#333377","black"), emission="skyblue",
      specular="#3333FF", shininess=5, alpha=0.8
  )
  aspect3d(c(0.5,0.5,0.5))
  rgl.snapshot(filename = "E_10_05_1.eps")

  dev.set(2)
  par(mar=c(5,4,4,5))

mylevels=seq(min(avalue[a[1]:length(a),limit[1]:limit[2]]),max(avalue[a[1]:length(a),limit[1]:limit[2]]),length=30)
  filled.contour(log(aMW[c[1]:length(a)],10), awavelength[limit[1]:limit[2]], avalue[c[1]:length(a),limit[1]:limit[2]],xlim=(c(min(log(aMW[c[1]:length(a)],10)),max(log(aMW[c[1]:length(a)],10)))),col=rainbow(length(mylevels)), levels=mylevels, main=file,
        plot.axes = {axis(2, seq(210, 390, by=20),cex=0.2)
              axis(1, seq(0, 6, by=0.5),cex=0.2)       },
        frame.plot = FALSE, ylab="Wavelengths (nm)",xlab="Log AMW (Da)", key.axes = axis(4, seq(0.0 , 0.06, by=0.01)), key.title = title(main="Abs (/cm)", line=0, cex.main=0.7))

  dev.set(3)
  colabs1=floor((abs1-197.99)/1.1773)
  colabs2=floor((abs2-197.99)/1.1773)
  colabs3=floor((abs3-197.99)/1.1773)
  colabs4=floor((abs4-197.99)/1.1773)
  colabs5=floor((abs5-197.99)/1.1773)
  colabs6=floor((abs6-197.99)/1.1773)
  colabs7=floor((abs7-197.99)/1.1773)
  colabs8=floor((abs8-197.99)/1.1773)
  colabs9=floor((abs9-197.99)/1.1773)
  colabs10=floor((abs10-197.99)/1.1773)
  colabs11=floor((abs11-197.99)/1.1773)
  colabs12=floor((abs12-197.99)/1.1773)
```

```
   colabs13=floor((abs13-197.99)/1.1773)
   colabs14=floor((abs14-197.99)/1.1773)
   colabs15=floor((abs15-197.99)/1.1773)
   colabs16=floor((abs16-197.99)/1.1773)

   colabs22=(avalue[,colabs1]+avalue[,colabs2]+avalue[,colabs3])/3
   colabs55=(avalue[,colabs5]+avalue[,colabs5]+avalue[,colabs6])/3
   colabs88=(avalue[,colabs7]+avalue[,colabs8]+avalue[,colabs9])/3
   colabs1111=(avalue[,colabs10]+avalue[,colabs11]+avalue[,colabs12])/3

   ASI=((colabs22-colabs55)/(abs2-abs5))/((colabs88-colabs1111)/(abs8-abs11))
   A210A254=avalue[,colabs13]/avalue[,colabs14]
   A280A350=avalue[,colabs15]/avalue[,colabs16]

limit=c(min(avalue[,colabs13],avalue[,colabs14],na.rm=T),max(avalue[,colabs13],avalue[,colabs14],na.rm=
T))
   par(mar=c(3,2.5,1,3))
   plot
(aMW,avalue[,colabs13],xlim=(c(min(aMW[c[1]:length(a)]),max(aMW[c[1]:length(a)]))),main="(f)",log="x
",type="l",ylim=c(limit[1],limit[2]),xlab="",ylab="",col="red",lwd=3,cex.axis=0.9)
   par(new=T)
   plot
(aMW,avalue[,colabs14],xlim=(c(min(aMW[c[1]:length(a)]),max(aMW[c[1]:length(a)]))),log="x",type="l",y
lim=c(limit[1],limit[2]),xlab="",ylab="",col="blue",xaxt="n",yaxt="n",lwd=3,cex=1,lty=8)
   par(new=T)
   plot
(aMW,A210A254,,xlim=(c(min(aMW[c[1]:length(a)]),max(aMW[c[1]:length(a)]))),ylim=c(0,30),log="x",ty
pe="l",xlab="",ylab="",xaxt="n",yaxt="n",col="darkgreen",lwd=4,lty=3,cex.axis=0.9)
   axis(side=4,line=0,cex.axis=0.9)
   mtext("AMW(Da)",side=1,line=2,cex=1)
   mtext(as.expression(bquote(~A[210]/A[254])),side=4,line=2,cex=1)
   legend("topright",
legend=c(as.expression(bquote(.(abs13)~nm)),as.expression(bquote(.(abs14)~nm)),as.expression(bquote(A[2
10]/A[254]))),  col=c(rep("red",1),rep( "blue",1),rep(
"darkgreen",1)),lwd=c(2,2,2),lty=c(1,2,3),bty="n",cex=0.9)

# save file

summaryframe=cbind(aMW,avalue[,colabs1],avalue[,colabs2],avalue[,colabs3],ASI,A210A254,A280A350)
```

```
  colnames(summaryframe)=c("MW",as.expression(bquote(~.(abs1)~nm)),
as.expression(bquote(~.(abs2)~nm)),as.expression(bquote(~.(abs3)~nm)),"ASI","A210/A254","A280/A350")
  write.csv(summaryframe,file="dataJ1.csv")}
```

Background of case studies



Stormwater auto-sampler
Capture stormwater from semi-urban catchment areas

The stormwater drain exits downstream of the River

Fig. 23. Map of the study area and sampling site in Mannum, South Australia.

A semi-urban catchment, located at Mannum, South Australia, was selected for the case one study to determine the potential impact of stormwater quality on surface water quality (Fig. 1). Mannum – Adelaide pipeline (MAP) is one of the major pipelines in South Australia, providing River Murray as a source of potable water for many main cities. Owing to urbanization growth, residential activities and agricultural developments, the downstream aquatic streams, Murray River quality has been detected to be degraded because of urban stormwater discharges. As a result, these raw waters quality can vary considerably and place challenges on water treatment processes. .

Mannum’s stormwater discharges have typically been assigned a high risk level because its discharges drain system is in close proximity to water off-takes (inlet of MAP) (Fig. 1). In addition, stormwater in the region is often highly turbid and nutrient, while treated wastewater is clearer but still with much higher nutrient concentrations. Evaluated pollutant loadings, particularly of DOM, during a storm event can provide early notice of potential impacts of stormwater discharge on surface waters (particularly used as drinking water source). Stormwater quality and risks are additionally required to be assessed and controlled in order to improve watershed management in this area.

The Tailem Bend – Keith (TBK) distribution system (Fig. 2) including the Lower Lakes running from TB to Meningie and also serving Narrung and Raukkan (Fig. 3) is chosen for case two study. Source water (River Murray) is treated by conventional treatment (coagulation / flocculation/ sedimentation / filtration (sand)) followed by disinfection by UV and chloramination (Fig. 4). The TBK system is chosen for the reasons include significant distribution system with a history of nitrification episodes, single source water input, linear design with distinct side braches and availability of historical water quality data and ease of sampling.

Seventeen sampling points across the water treatment plant and subsequent distribution system of the TB system were completed in May 2012. Extended sampling points focusing on Branch 2 (TB-Lower Lakes) was further completed in September 2014, since the analytical results of the previous survey indicates that the Lower Lakes (LL) has the most degradable water quality compared with the other two branches. Sample sites and description used in the TB study are described in Table 1.

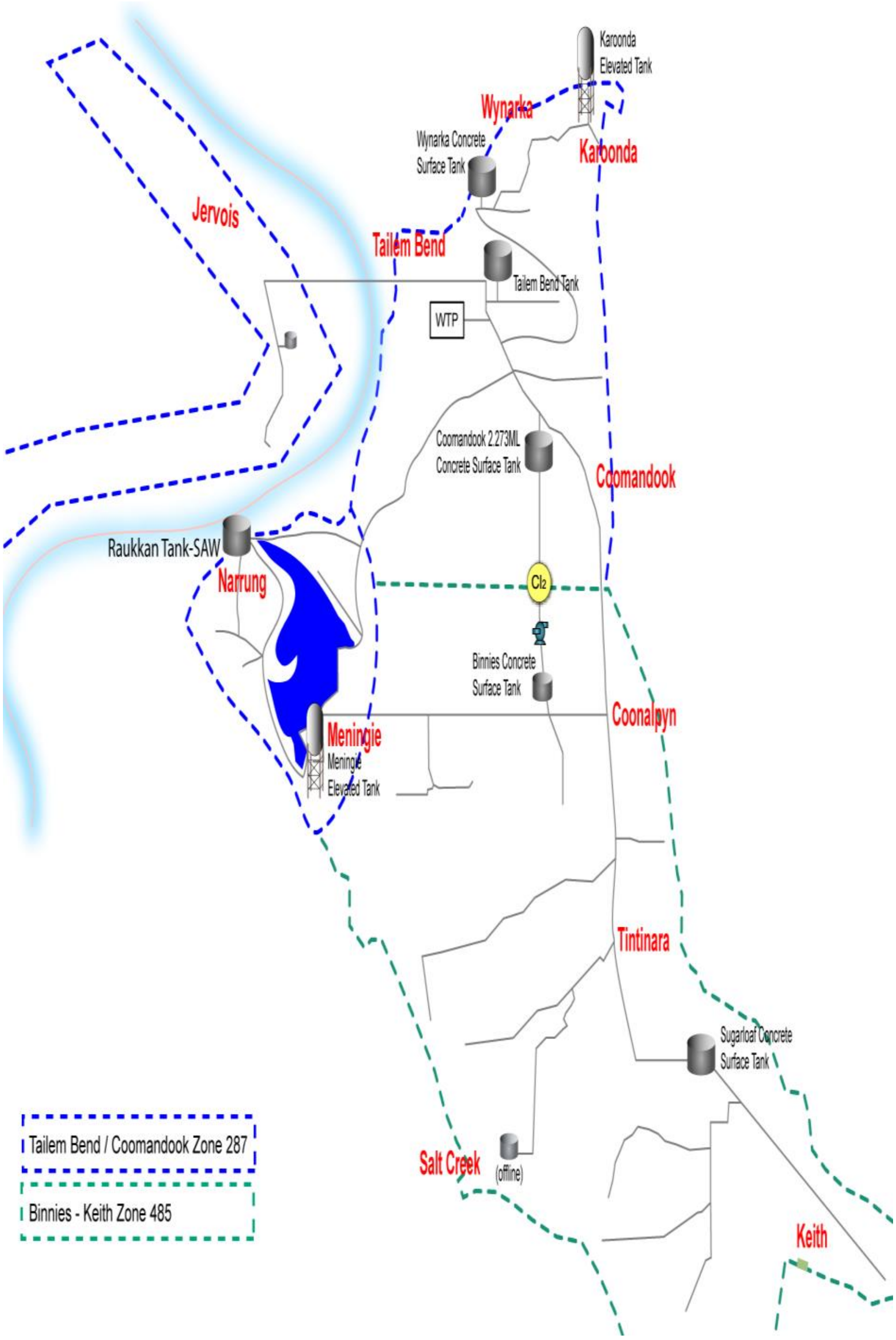


Fig. 24. TBK distribution system.

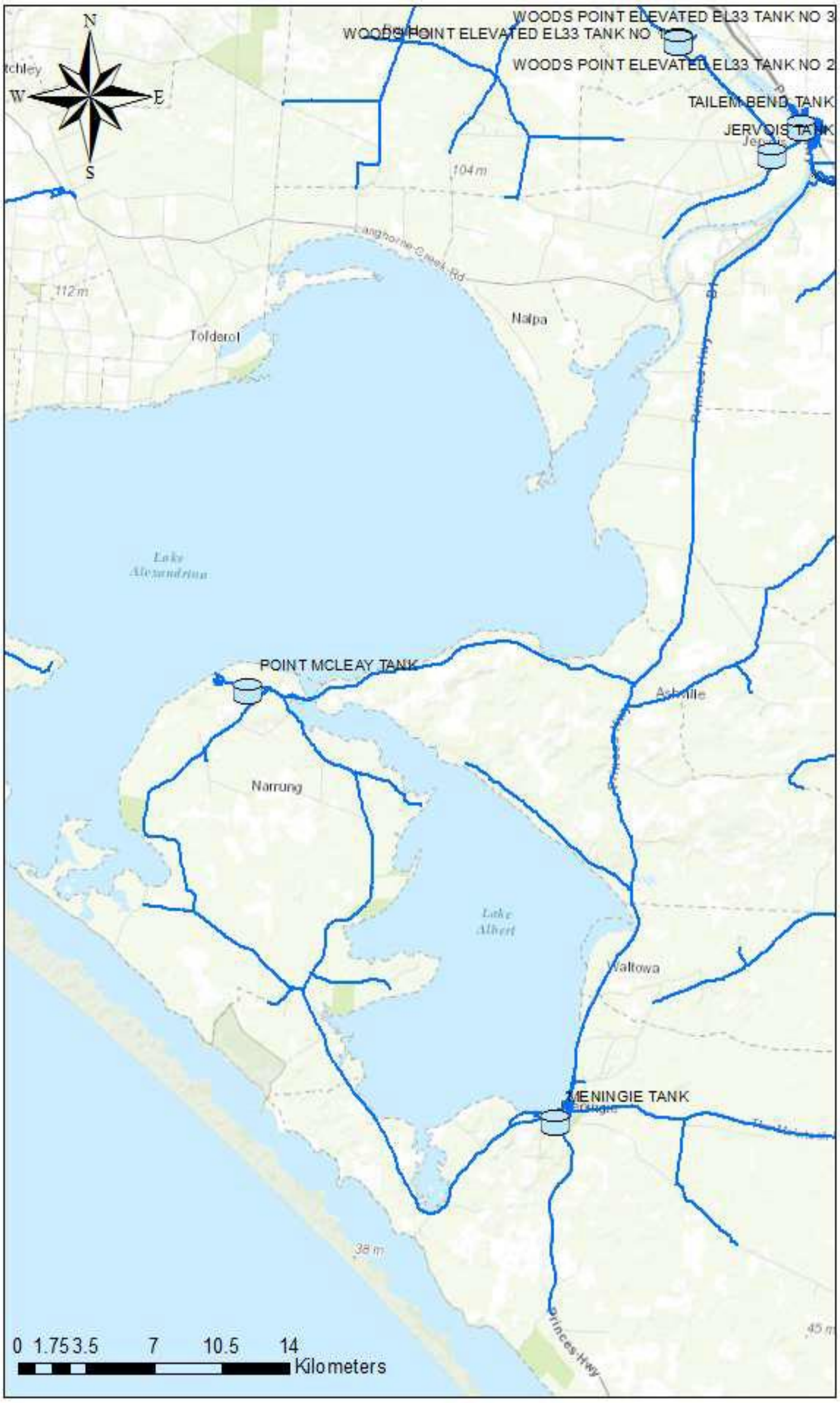


Fig. 25. The Lower Lakes branch distribution system.

Table 1. Description of sampling sites in both water treatment plant and distribution system.

Year	Location	Description of sampling sites
2012	water treatment plant	Raw - Inlet
		Settled water
		Filtered prior disinfection
		Post disinfection
		Backwash water
		Supernatant (lagoon)
	distribution system	1.5km after chloramination
		Coomandook tank outlet
		700m after Tailem Bend pump station
		Binnies Lookout tank outlet
		Sugarloaf tank inlet
		Sugarloaf tank outlet (1km)
		Keith customer tap
		Raukkan tank
		Raukkan customer tap
		Wingamin tank
		Karoonda customer tap
2014	distribution system	10km North Poltalloch
		Poltalloch/Princes Hwy
		7km down Poltalloch
		14km Poltalloch Road
		Narrung
		Raukkan customer tap
		11km Loveday Bay
		Loveday Bay intersection
		10km after Loveday/Narrung
		Narrung near intersection

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Signature		Date	30/10/2015

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