EVALUATION OF WATER QUALITY, SELECTED METALS AND ENDOCRINE-DISRUPTING COMPOUNDS IN THE RIVERS AND MUNICIPAL WASTEWATERS OF EASTERN CAPE PROVINCE, SOUTH AFRICA

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Where leaders learn

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ABSTRACT

South Africa is developing with increasing population, and so is the demand for use and access to water resources. Surface water is critical to the country because it provides about 77% of the needed water resources. Low rainfall (about 450 mm annually) with little runoffs to boost the rivers aggravates the problems of surface waters. Expansion of industrial and agricultural activities coupled with the population pressure had an impact on water quality, availability and the state of aquatic ecosystems in the country. Water management is a challenge in South Africa because of the socio-economic pressure and other factors such as mine wastewater, eutrophication, salinisation, and emerging contaminants. This research investigated some water quality parameters of Bloukrans, Buffalo, Swartkops and Tyhume Rivers in Eastern Cape Province of South Africa for three seasons. The parameters investigated include the physicochemical properties, functional groups of organic compounds, presence of endocrine-disrupting compounds and heavy metals in the rivers and wastewater effluents from wastewater treatment plants (WWTPs) released into these rivers. The aims were to determine the concentrations of these parameters in the rivers and wastewater effluents, compare the concentration levels with recommended values for aquatic lives, domestic and agricultural purposes, thereby contributing to the effective management of water in South Africa.

Water samples were collected for analyses at upstream, midstream and downstream reaches of the rivers. In contrast, wastewater influent and effluent samples were obtained from wastewater treatment plants releasing effluents to the rivers. Some physicochemical parameters were studied onsite with specialised meters while others analysed in the laboratory with ultraviolet (UV) spectroscopy. Chemical functional groups in the samples were determined with Fourier-transformed infrared (FT-IR) and nuclear magnetic resonance (NMR) spectroscopies. The Endocrine-disrupting compounds and heavy metals were determined with liquid chromatography coupled to a mass spectrometer (LC-MS/MS) and inductively coupled plasma with a mass spectrometer (ICP-MS) respectively. Method validation and calibration for all the spectrometry yielded good linearity ($r^2 > 0.99$). The results showed high oxygen demand above the concentration recommended by the South Africa Department of water affairs and forestry (DWAF) for Alice and Uitenhage wastewater effluents, midstream and downstream river samples. Phosphate concentrations were higher than the recommended level in wastewater effluents. Sulphate

concentration in the Bloukrans River was higher than the recommended value. The pH values of rivers at midstream and downstream reaches were higher than 8.0 except in Bloukrans River, where it was around 7.0. The functional group analyses show the presence of substituted aromatic compounds, alkyl halides, chlorobenzenes, vinylidenes, amides, amines, urethanes, cycloalkanes, acetonitriles, methenamine, imidazole and phenolic compounds among others, in the samples. The presence of these functional groups in the water samples is an indication of pollution by volatile organic compounds, persistent organic pollutants and pharmaceuticals. Results of LC-MS/MS analysis show that endocrine-disrupting compounds (EDCs) were present in the rivers and wastewaters samples. Descriptive statistics showed the mean concentrations of the EDCs in the samples as nonylphenol > dichlorophenol > bisphenol A > triclosan > octylphenol > imidazole > atrazine > triazole > estrone > estradiol. The results of the heavy metal analysis show that chromium had the highest mean concentration in the samples. The mean metals concentrations in the samples were in the order of Cr > Ni > Mn > Cu > As > Pb > Cd > Hg > Zn. The results showed an increase in the concentrations of metals in the rivers over the years. The chromium, manganese, nickel, copper, zinc, arsenic, cadmium, lead and mercury concentrations in the lower reaches of the rivers and wastewater effluents were higher than the values recommended by the United Nations Environmental Programme (UNEP) and DWAF. Improvement is necessary in wastewater treatment and adequate legislation on chemical usage. Some chemicals banned in developed countries, such as atrazine, were encountered in this study.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ADH	Anti-diuretic hormone
AE	Alice treated effluent samples
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
ASCII	America standard code for information interchange
AW	Alice wastewater samples
BD	Bloukrans River downstream samples
BM	Bloukrans River midstream samples
BPA	Bisphenol A
BU	Bloukrans River upstream samples
$CDCl_3$	Deuterated chloroform
CNS	Central nervous system
COD	Chemical oxygen demand
DCP	2,4-dichlorophenol
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DWAF	Department of water affairs and forestry
E1	Oestrone
E2	17β-oestradiol
EC	Electrical conductivity
EDCs	Endocrine-disrupting compounds
EU	European Union
FD	Buffalo River downstream samples
FM	Buffalo River midstream samples
FSH	Follicular-stimulating hormone
FT-IR	Fourier-transform infrared
FU	Buffalo River upstream samples

GE	Grahamstown treated effluent samples
GW	Grahamstown wastewater samples
HPLC	High-performance liquid chromatography
ICP-MS	Inductively coupled plasma with mass spectrometry
KE	King Williams Town treated effluent samples
KW	King Williams Town wastewater samples
LC-MS	Liquid chromatography with mass spectrometry
LH	Luteinizing hormone
LLE	Liquid-liquid extraction
LoD	Limit of detection
NIST	National Institute of Standard and Technology
NMR	Nuclear magnetic resonance
ORP	Oxidation-reduction potentials
PCA	Principal Component analysis
pH	Hydrogen ion concentration
PLS-DA	Partial least square discriminant analysis
POP	Persistent organic pollutant
PPCPs	Pharmaceutical and personal care products
ppm	Part per million
SD	Swartkops River downstream samples
SDG	Sustainable development goal
SM	Swartkops River midstream samples
SPE	Solid-phase extraction
SU	Swartkops River upstream samples
TD	Tyhume River downstream samples
TDS	Total dissolved solids
TH	Thyroid hormones
TM	Tyhume River midstream samples
TMS	Tetramethylsilane
TSH	Thyroid-stimulating hormone
TU	Tyhume River upstream samples

UE	Uitenhage treated effluent samples
UNDP	United Nations development program
UNEP	United Nations Environmental Program
UNO	United Nations Organization
UPLC	Ultra-high-performance liquid chromatography
USEPA	United States environmental protection agency
USGS	United States geological survey
VIP	Variable importance in projection
VOC	Volatile organic compounds
WHO	World health organisation
WWAP	World water assessment program
WWTPs	Wastewater treatment plants

PUBLICATIONS RESULTING FROM THIS WORK

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Adebayo I. Farounbi

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DEDICATION

Dedicated to

The Lord, God Almighty

Who can do exceeding abundantly above all that we ask or think *Eph. 3:20.*

CHAPTER 1

INTRODUCTION

This chapter introduces the work embarked upon in this study and the rationale for the research. It highlights the importance of water and the reasons why it should be free of pollutants. It also contains the backgrounds to some water pollutants. This chapter also discussed some modern analytical instruments in water analyses. The importance of water pollution as it relates to health, biodiversity loss and national development were discussed in this chapter. The menace of emerging contaminants was also discussed in this chapter. This chapter also explains the rationale, aims and objectives of this research work.

1.1 Background and Rationale

Pollution is the introduction of substances, energy, noise, heat or light into the natural environment in quantities and durations that may cause adverse effects on living things and their environment. Pollutants are the materials introduced into the environment from natural and human-made sources. Woodford (2017) explained that pollution started with the industrial revolution and that before then, people live in harmony with their environment. The burden of population increase on earth has put more pressure on the environment because of over-exploitation of resources. The problem of pollution extends to all the media of the environment: air, land and water. The earth's surface is made up of about 71% water (USGS, 2016). Less than 2% of this water is available as freshwater (rivers and lakes). Freshwater is the primary source of drinking water for humans and animals. It also serves other human domestic purposes, such as recreation and inland transport. The United Nations reported that over two billion people lack access to good drinking water (WHO, 2018b).

Pollution of freshwater may be point or non-point source. Point source pollution is traceable to the source where the pollutants emanated, but the non-point source may not be easily traceable. Such non-point source pollution may be because of runoff from farmlands or airborne pollutants, washed off into rivers and lakes. Freshwater may also receive contaminated water from the rain with dissolved airborne pollutants emanating from industries (Khan, 2014; Rueda-Holgado et al., 2014) and natural sources such as volcanic activities (George, 2014; Stewart et al., 2006). River water may also carry dissolved minerals from the weathered rocks and soil through which it

flows. Human settlements, agriculture and industries are the major sources of water pollution. About 80% of the global municipal wastewater is released directly into rivers and streams with little or no treatment (World Water Assessment Programme, 2017). Millions of tons of industrial wastes are dumped into the environment yearly through industrial activities. Such wastes contain toxic compounds and metals (Bassem, 2020). Other sources of pressure on water resources include increasing population growth, economic activities and climate change, all of which participate in spoilage of natural water resources, threatening aquatic and other ecosystems as well. Water pollution has become a global problem that requires adequate monitoring and derivation of solutions for proper management (Geissen et al., 2015).

Humans did not understand the importance of clean water until the late nineteenth century. Ancient civilisation used sewers to carry human wastes into the river (Markham, 1994). Romans dumped their sewage into the Tiber River; by 312 BC, the river was so polluted that it was not clean enough for drinking (Havlíček and Morcinek, 2016). The inability of humans, in ancient times, to link pollution with diseases led to the outbreak of water-borne diseases such as typhoid and cholera in many parts of the world (Mehlhorn and Klimpel, 2019). Many developing nations with inadequate water supply and sanitary facilities still experience the outbreak of these communicable diseases (Das, 2016). A part of London experienced a cholera outbreak in 1854, resulting from the contamination of a public well with wastewater from a sick baby's diapers (Caplan et al., 2020). It took the authority a long time to understand and heed the suggestion of the physician, John Snow, who suggested that the washing of babies' diapers in a cesspool near the well led to the outbreak of the disease (Markham, 1994; Caplan et al., 2020). The practice of dumping wastes (industrial and domestic) in rivers and seas happened in many nations for centuries. Some Asian countries spread human wastes on their farms as fertiliser, a practice that encouraged the contamination of crops with communicable diseases from helminths, bacteria and fungi (Lam et al., 2017). The early known pollutants of water were mainly from sewage, leather tanning, and butchering waste before the industrial revolution (Markham, 1994). The industrial revolution brought a major crisis to the environment as factories found it convenient to dispose of their wastes into rivers. Chemicals from industries were dumped directly into water bodies. This trend continued into the twentieth century before the various government came up with environmental protection legislation (Markham, 1994). Industries and technology increased significantly after

World War II with advances made in engineering and medicine. The discovery of antibiotics and pesticides such as DDT saved millions of lives worldwide during and after the war, but also became pollutants because their wastes and by-products found their way into the environment (Hong et al., 1994). These compounds can enter into the environment through direct dumping by companies, leaching into groundwater from dumping sites or household wastewater from the end-users of the products. These by-products were linked to massive wildlife die-off and elevated levels of non-communicable diseases such as cancer, congenital disabilities, and a lower IQ in people who subsisted on water polluted by heavy industries (Brender and Weye, 2016; Landrigan et al., 2019).

1.2 Sources of Water Pollution

Mining activities generate acids, expose heavy metals to the surface, and release many chemical contaminants into water resources, thereby making them acidic. Mine wastewaters also increase the levels of suspended solids in the receiving water bodies and mobilise metals because of low pH. The mobilised metals had been causing environmental problems in some parts of the world (Kibria, 2014). Mine wastewaters from coal and metal mining sites frequently contain high levels of sulphuric acid and heavy metals, which may enter rivers and streams through runoffs eventually found their ways to agricultural lands in the downstream through irrigation. Flooding may also wash mine wastewaters to farmlands. Plant uptake of metals from the soil is facilitated at acidic pH, posing a high health risk to the consumers of the contaminated agricultural products (Boularbah et al., 2006). South Africa is one of the countries with the problems of acid mine drainage in surface waters (du Plessis, 2017). The overall effect of mine water is the deterioration in the quality of many surface water sources and the attendant adverse effects on the domestic, industrial, and agricultural users (Ochieng et al., 2017). Mine wastewater pollution is a difficult problem because it can extend its effects beyond 10 km from its source (Naicker et al., 2003).

Wastewaters discharged from industries and household are other significant contributors to water pollution (Norah et al., 2015). The United Nations Organisation (UNO) estimated 2million tons of wastes discharged daily into the water around the world and 1500 km^3 of wastewater generated annually (UN-WWAP, 2003). Most of these wastewaters were poorly treated before release to the environment (UN-WWAP, 2003). The wastewaters, from homes, industries and farmlands are

composed of potentially dangerous compounds (Kuzmanovic et al., 2016). Industrial wastewaters contain various chemicals that are toxic to aquatic ecosystems (Soltan et al., 2018; Udebuani et al., 2016). Wastewaters are high in microbial load (Naidoo and Olaniran, 2014; Silva-Bedoya et al., 2016), pharmaceuticals and personal care products such as soap, cosmetics and residues of fragrances (Ebele et al., 2017; Kosma et al., 2014), which reduce their domestic utilities. Some of the pollutants have been escaping the WWTPs (Petrie et al., 2015) and end up in the receiving waters (Ebele et al., 2017). The pollutants escaping from WWTPs contribute to the reasons tap water does not meet its required health criteria (Jezierska et al., 2011).

Wastewaters from agricultural settlements are other sources of water pollution. Such wastewaters usually contain microorganisms, pesticides, antibiotics, hormones and chemicals employed in the control of pests for better farm yield. Pathogenic bacteria such as *Salmonella* species and *Escherichia coli* are present in the faeces of livestock, thereby making manure runoff and slaughterhouse wastewaters important sources of water contamination (Cao and Song, 2019). The most common, economical, effective, and convenient pest control in agriculture involves the use of chemicals in many countries (Collins et al., 2016). These chemicals were to control diseases, insects, weeds and rats. Pesticides are indispensable means of improving productivity in agriculture and had help agricultural production, but they have also caused harm to the environment (Baghapour et al., 2014). Agrochemicals pose threats to organisms in the freshwater systems. Runoffs from agricultural settlements containing herbicides from weed control pose a significant threat to aquatic organisms in many countries. The agrarian industries are growing globally since the second half of the 20^{th} century, steadily increasing the release of pesticides and solutes such as nitrates, phosphates, chlorides, sulphates, ammonia and other compounds into the environment (Hrachowitz et al., 2015). These chemicals will eventually end up in the rivers through erosions and runoffs. Chemical pollutants always influence the aquatic ecosystem by creating stress for the organisms inhabiting the water bodies (Kuzmanovic et al., 2016). Unprocessed livestock wastewaters are rich in ammonia (Chang et al., 2018). Elevated levels of ammonia in the water are toxic to fishes and can destroy their kidneys (Benli et al., 2008). The multiple stressor effects of these pollutants have made it difficult to establish the link between aquatic pollution and response of the biological community. However, studies have shown that chemical pollutants promote biodiversity loss in the aquatic environment (Feld et al., 2014). Over

the past years, the agrochemicals had led to a decline in the populations of fishes and mussel species (Lasier et al., 2016).

1.3 Water Pollution and Biodiversity

The major threats to global aquatic biodiversity are climate change, water pollution, over-exploitation, exotic species invasion, habitat loss and degradation, and changes in flow rate (Naeem et al., 2016; Mace, Norris and Fitter, 2012; Harrison et al., 2014; Tilman, Isbell and Cowles, 2014). Aquatic Pollution is the most significant problem facing aquatic ecosystems. The biodiversity of freshwater ecosystems is declining globally at a rate higher than that of terrestrial (Vaughn, 2010). The rate of extinction of freshwater animals in North America was estimated to be 4% per decade while it was 0.8% in the terrestrial ecosystems (Dudgeon et al. 2006). Aquatic ecosystems are known to be rich habitats by their diversity and number of species. The Millennium Ecosystem Assessment (MEA 5) observed that biodiversity degradation in freshwater systems occurs at a higher rate than other ecosystems due to the problem of pollution (Bassem, 2020). The ability of various aquatic ecosystems to function fully has decreased as a result of pollution, thereby causing negative impacts on plants, animals and human health (Bassem, 2020). Healthy and sustainable ecosystem are the targets of Sustainable Development Goals (SDGs) adopted in September 2015 (UNO, 2016). It has been estimated that aquatic species extinction, which is an indicator of biodiversity loss, has recently increased ten-fold than the level considered by scientists as the acceptable upper limit (Bassem, 2020).

Aquatic organisms are sensitive to variations in the environment. Different species respond in different ways to changes in the environment; some may migrate while others die. A change in water quality due to pollution may affect fishes' response to infections by lowering the effectiveness of the immune system, which results in increased mortality (Bassem, 2020). Other reactions may include suppression of vital metabolic enzymatic actions, neurological responses, and reduction in reproductive capacity (Liang et al., 2017). In events of water pollution, the populations of organisms are usually reduced and may disappear in highly polluted waters, leaving only tolerant species (Bassey, 2019). Zooplanktons and macrobenthic organisms are the worst hit in polluted water. These organisms play vital roles in the food chain as primary consumers and modulate the aquatic productivity by occupying the intermediate level in the food chain. A

reduction or disappearance of the population of primary consumers will affect other trophic levels above them (Manickam et al., 2018). The populations of these organisms are predictors of the environmental status of an aquatic ecosystem at a definite period. They may reduce or disappear as a result of pollution and eutrophication (Xie et al., 2008). Phytoplanktons are the primary producers in the aquatic ecosystems. The presence and population of some of the phytoplankton are indicators of deteriorating water quality resulted from pollution and eutrophication (El-Kassas and Gharib, 2016). Any disruption in the food chain that affects the primary producers and intermediate consumers will lead to a decrease in the populations of fish and other organisms at the top of the food chain. Biodiversity and species richness are the main determinants of ecosystem functions. Loss of species and biomass may affect ecosystem functions. Some species communities influence nutrient recycling in the aquatic ecosystem, and the efficiency of carrying out this depends on the overall abundance and species composition in relation to the prevailing environmental conditions (Vaughn, 2010).

Some chemical pollutants can disrupt the formation of gonads in fishes and other aquatic animals. Various researches document the effects of such compounds on wildlife, especially in freshwater ecosystems, such as abnormal development and death of embryos (Arukwe et al., 2016; Oritz-Villanueva et al., 2018), changes in sexual behaviour (Kanda, 2019), the feminisation of male animals (Carnevali et al., 2018) and altered immune functions (Nowak et al., 2019). These effects also extend to birds, especially those feeding in polluted waters (Roman et al., 2019; Jessl et al., 2018) and may ultimately lead to loss of biodiversity.

1.4 Water Pollution and Health

The environmental and health effects of water pollution made it a subject of global attention and a significant common challenge for both developed and developing countries. The water crisis is aggravated all over the world because of the improper handling of wastes, both in developing and developed countries (FAO, 2017). The Sustainable Development Goals Agenda for 2030 placed a high priority on ensuring water quality through the control of water pollution (UNO, 2016). Incidence of some communicable diseases such as typhoid, dysentery, diarrhea, cholera, constipation, jaundice, and amoebiasis arise from lack of clean water for drinking and sanitation purposes (Parvez et al., 2019). The problems associated with inadequate water for domestic purposes primarily fall on the poor people. These problems, in turn, put severe burdens on health

services. The United Nations Children Educational Fund (UNICEF) and World Health Organisation (WHO) observed a higher rate of child deaths due to diarrhoea in ten countries with poor and polluted water supplies (UNICEF, 2018; Parvez et al., 2019). Approximately 3.1% of deaths worldwide were linked to unsafe water for drinking and personal hygiene (UNICEF, 2018).

Some non-communicable diseases are traceable to chemical pollutants in water sources. When humans and other organisms in the environment take some chemical pollutants with water, they trigger physiological changes, even at nanogram concentrations (Pal et al., 2014). Zinc in the polluted river has been linked to chronic heart disease (Chang et al., 2018), and arsenic in groundwater is associated with the same disease (Zheng et al., 2014). Since the kidney is involved with filtration functions, it is the most affected when a toxic environmental pollutant is swallowed with water, and occupational exposures to such pollutants remain the common causes of kidney disease (Xu et al., 2018). Arsenic in drinking water has been linked to impairment of the blood vessels in the lower limbs of patients, leading to gangrene (Chang et al., 2018). Studies in China showed that incidences of gastric and oesophageal cancer were more frequent in settlements close to polluted river basins (Liu, 2010; Zhang et al., 2014) and mortality rate from these diseases depends on the proximity to polluted rivers (Ghaffar et al., 2018). Research in Belarus linked the increased incidence of thyroid cancer to an increased level of nitrate runoffs, from fertilisers into the groundwater (Drozd et al., 2016). Chemical pollutants in the water contribute to a wide range of chronic diseases and disabilities, directly costing society billions of dollars (Trasande et al., 2015). The effects of endocrine disruptors in the environment are felt most in IQ loss and intellectual disability, childhood and adult obesity, autism, diabetes, cryptorchidism, male infertility among others (Street et al., 2018; Trasande et al., 2015). It was estimated that the combination of these diseases is gulping an estimated amount of €157 billion from European Union (EU) countries annually, translating to about 1.23% of EU gross domestic products (Trasande et al., 2015).

1.5 Chemical Pollution of Freshwater

The sources of chemical pollutants in water are similar to the origins of water pollutants. Manufacturing industries contribute mainly to chemical compounds in water bodies with organic compounds from textile (Hossain et al., 2018) and agricultural industries (Cao and Song, 2019). Wastewaters from drug and chemical manufacturing industries also contain parts of their raw materials and products released to the environment through poor wastewater treatment. Other categories of industrial sectors contribute to the chemical pollution of water through their wastewaters. Some chemical substances are an essential part of life as they play crucial roles in life sustenance. These chemicals range from natural products to synthetic compounds. The increase in the production of these compounds to meet human demands also increases human and other organism's exposure to them (Weiss et al., 2016). These chemicals or their by-products, if not adequately managed, end up in the aquatic environment and move from one level of the food chain to the other. The combination of concentrations, toxicity, persistence, ability to bio-accumulate, coupled with environmental conditions, make these chemical pollutants of concern (Fischer et al., 2013). Chemical pollutants in the rivers and wastewaters are complex mixtures of many compounds, identifying them is a crucial issue for the protection of lives and the ecosystem (Vorosmarty et al., 2010). Aquatic environment monitoring mainly focuses on physicochemical status, hydromorphological quality and biological elements, based on some identified chemical pollutants and environmental parameters (Altenburger et al., 2015). However, the problem with proper management of chemical pollutants in the water is the anticipated contributory roles of different contaminants and the status of several chemical compounds in the water that are yet to be known (Malaj et al., 2014). Despite the work done on priority pollutants, this is not so with emerging pollutants in the water bodies. Accurate diagnoses of the combined effects of these chemicals in aquatic ecosystems are vital in addressing the problems they created. Several models were proposed to address the impact of chemical mixtures in environmental water quality assessment (Brack et al., 2015; Altenburger et al., 2015). A model calls for prioritisation of the chemical pollutants for effective monitoring and assessment (Dulio and Von der Ohe, 2013; Anna et al., 2016). Altenburger et al. (2015) proposed the separation and identification of mixtures whose compositions are representative for a particular site or typical for specific sources for a better chemical component-based assessment.

Some chemical pollutants not previously detected, or present in negligible amounts in the environment, are now raising global concern because of their impacts on humans and other organisms in the environment (Petrie et al., 2015). Some of the chemicals compounds in pharmaceutical and personal care products (PPCP), agricultural inputs, food preservatives, adhesives and paints that are present in trace amounts are in this category. They have been implicated in physiological disruptions in living things when taken up in small quantities from the environment (El Einin et al., 2019; Balbi et al., 2019). These chemicals form a group of emerging contaminants in the environment. The "Silent Spring", a book published by Rachel Carson was the first to raise the dangers associated with the use of pesticides and the need for preservation of the earth (Carson, 1962). Carson attributed the reduction in birds' population to the harmful effects of dichlorodiphenyltrichloroethane (DDT) insecticides. Her work was the foundation of research into the environmental impact of pesticides (Hong et al., 1994). DDT was introduced as an insecticide in the 1930s (Erckmann, 1986) but its toxicity, persistence, bioamplification and rapid transportation through the food chain, later made it an undesirable chemical compound (USEPA, 2019; Conis, 2010). Later researches into the effects of pesticides on the environment supported her observations, and DDT was banned (Sauvé and Desrosiers, 2014).

Before DDT invention, the world has recorded poisoning of plants, animals and humans from various metals and metal-containing substances released into the environment especially during mining and smelting (Thornton and Abrahams, 1984; Hong et al., 1994). The fall of the Roman Empire was attributed partly to the lead poison from lead-lined cooking-pots (Gilffilan, 1965). In this modern age, industrial emissions, soil and leaded pipes are the primary identified sources of lead in the environment. Corrosion of pipes carrying tap water leaks lead into drinking water conveyed to homes, thereby exposing households to lead (Lin et al., 2011; USEPA 2019). Children and foetuses are particularly vulnerable to lead pollution at low levels (USEPA, 2019). Lead pollution may lead to damages in the central nervous system, which may culminate in learning and hearing disabilities, growth retardation and malformation of the red blood cells (Lange and Candello, 2017; Mason et al., 2014). In adult humans, lead can cause miscarriage, cardiovascular problems, including hypertension, renal and reproductive failure (Zhang et al., 2015). Arsenic is another metal with a long history of poisoning. Records show that arsenic sulphides were used as pesticides in China around 900AD (Peryea, 1998). Later, copper acetoarsenite and lead acetate

were introduced into agriculture as pesticides (Sauvé and Desrosier, 2014). Arsenic is naturally present in groundwater and may be at high levels in some countries. Arsenic poison was implicated in cancer, cardiovascular diseases, diabetes, and impaired development in children (WHO, 2018; Tolins et al., 2014). The introduction of DDT reduced the usage of arsenates, but arsenic remains a contaminant of groundwater (Shankar et al., 2014; WHO 2019).

1.6 Endocrine-disrupting Compounds (EDCs)

Endocrine-disrupting compounds are groups of chemicals that can interfere with, and alter, the normal hormonal functions in animals. During development in animals, the endocrine glands started functioning before the nervous system and their chemical signals control growth and remain critical throughout life (Ahmed et al., 2008). In parts per billion or parts per trillion concentrations, hormones from the endocrine glands affect all aspects of development, maturation, reproduction and ageing. They affect metabolism, intellect and behaviour (Donovan, 1988; Fetene et al., 2017). Deficiency in the formation or shortfall in maternal production of the appropriate hormone during gestation can have life-long effects on the offspring up to adulthood and old age (Fetene et al., 2017; Moog et al., 2017). Among several chemicals in commerce and industries, many of them interfere with hormonal functions. Endocrine-disrupting compounds are exogenous substances, natural or synthetic, that can interfere with the normal functioning of the body's endocrine system, thereby causing adverse effects in an intact organism or its progeny or subpopulations (Lee et al., 2013; Zoeller et al., 2012). This definition covers human hormones and their metabolites excreted to the environment since they are exogenous compounds to other organisms. The World Health Organization (2012) estimated close to 800 chemicals with endocrine disruptive abilities out of which very few were investigated and documented. The effects of EDCs in the body may result in either under-function or over-function of the endocrine system, and any of these may result in the production of defective hormones, receptor or post-receptor signalling (Kiyama and Wada-Kiyama, 2015; Söder, 2016).

The disruption of endocrine functions will lead to a multitude of disorders, which may manifest immediately or have delayed onset (Söder, 2016; Maqbool et al., 2016). The effects of EDCs are not restricted to the localities where they were released because they can travel rapidly through the food chain, spread by running water, and transported by the wind far beyond the point of release

(WHO, 2012). These compounds may be present in the air, water, land, food and are prevalent in buildings and natural environments; human and animal bodies, and exist naturally as minerals such as heavy metals. Some of the EDCs, especially the sex hormone-related compounds, can alter sexual behaviour and promote feminisation in organisms. Dzieweczynski et al. (2014) observed a change in sexual behavioural pattern in male Siamese fighting fish exposed to a synthetic birth control hormone, 17α-ethinylestradiol. Orton and Tyler (2015) linked the decline in amphibian population to the same synthetic hormone, which has been finding its way to rivers through improperly treated wastewater from WWTPs. They can induce smaller testes in mammals (Jeng, 2014; Sweeney et al., 2015); sperm abnormalities (Gill et al., 1977; Rutkowska, 2016) and breast cancer (Rachon, 2015).

Endocrine disruptors enter the environment through various sources such as municipal and household wastewater, building materials, agricultural runoff, mining, and so on. They may not be effectively removed at WWTP and thus find their ways to the receiving water bodies where other organisms pick them up (Rogowska et al., 2019; Vega-Morales et al., 2013; Zhou et al., 2019). Consumer products such as cosmetics, lotions, fragrances and soaps contain EDCs such as phthalates and triclosan, which eventually find their ways to the environment through wastewater (Mageresse-Battistoni et al., 2017; Nicolopoulou-Stamati et al., 2015). Some packaging materials such as plastics contain bisphenol A, phthalates and phenols that have endocrine disruptive abilities (Benjamin et al., 2017; Hejmej et al., 2011). Children products contain materials such as phthalates and bisphenol A, known for their endocrine disruptive activities (Wong and Durrani, 2017). Food and water are the major routes of human exposure to EDCs (Wee and Aris, 2019; Scialabba, 2019; Russo et al., 2019). Some processed foods carry EDCs from manufacturing processes and some preservatives added to such foods have endocrine disruptive abilities (Maffini et al., 2016). Agrochemicals such as pesticides, livestock drugs, and hormones are contributing to the environmental EDCs' load. Products like atrazine and 2,4-Dichlorophenol are typical examples (Szekacs et al., 2015). Some plant products, like soy-based foods and clover feeds, contain phytoestrogen that can mimic oestrogenic activities (Lee et al., 2013; Dickerson and Gore, 2007). Certain air pollutants such as heavy metals, fragrance and pesticides spray were implicated in endocrine disruption (Darbre, 2018). Some EDCs such as phthalates, bisphenol A, triclosan, parabens and alkylphenols among others, may be present in the gas phase or attached to particulate

matter and released to the atmosphere from industrial processes, thereby present in ambient air (Teil et al., 2016; Darbre, 2018). Household consumer products such as pesticide sprays, air fresheners, paints, flame-retardants, plastic products in homes and offices are sources of indoor EDCs, and their concentrations may be higher indoor than outdoor (Oziol et al., 2017). Household woods coated with wood-finish may contaminate occupants with polychlorinated biphenyls (PCBs) (Rudel et al., 2008).

In the aquatic environments, the presence of some EDCs such as alkylphenols, phytoestrogens and oestrogens, in conjunction with hydrodynamic factors like temperature, promotes eutrophication in freshwater (Rocha et al., 2014). Jia et al. (2019) studied cyanobloom in freshwater, observed 29 EDCs (seven oestrogens, seven androgens, six progestogens, five adrenocortical hormones, and four industrial pollutants) promoting eutrophication. Sublethal doses of bisphenol A will have adverse effects on the metabolism of glycerophospholipids, purines, 2-oxocarboxylic acids and amino acids in the developing zebrafish embryos (Ortiz-Villanueva et al., 2018).

Endocrine disruptors pose a challenge to regulators because of their persistence in the environment, mobility within environmental media and ability to bio-accumulate (Kudlak et al., 2015). The persistence of some EDCs in the environment is related to their structural stability and hence, resistance to biodegradation (Dickerson and Gore, 2007). This quality makes it easy for them to pass from one level of the food chain to another. They are mostly organic compounds and fat-soluble, making it possible to accumulate in the adipose and other tissues in the body of animals. Lv et al. (2019) observed the accumulation of bisphenol A, 4-tert-octylphenol, 4-nonylphenol, oestrone and 17β-oestradiol in the tissues of wild fishes. Zhou et al. (2019) observed that fish livers accumulate more bisphenol A and oestrone than other tissues, implying that the livers play essential roles in the metabolism, excretion, and biotransformation of these compounds. EDCs pose a significant threat to water reuse; some of them were analysed in this research work.

1.7 Water Pollution and Economic Development

The economic importance of water as a natural resource cannot be over-emphasised, and if any nation does not take care of its water resources, such will eventually collapse. Water is the bedrock of nations' economic development, especially in agriculture, industry and transportation. The

inability of a government to sustain adequate water supply will lead to economic catastrophe. More than half a billion people around the world are facing severe water scarcity all-round the year (Hoekstra, 2016). Water pollution is a significant cause of global water scarcity (Guarino, 2017). The lack of proper sanitation is a significant contributor to water pollution in developing countries and severely hinder their economic development. A report linked weak Indonesia's sanitation system and water pollution to the country's low gross domestic product (GDP) (Chowdhury, 2008). It was reported that Africa is losing up to 5 per cent of its annual GDP due to illness and death resulting from poor water and lack of proper sanitation facilities (Guarino, 2017). Alleviating pollution is profitable for poor and developing nations because expenditure on water and sanitation yields between eight and ten-fold return in economic development (Tickner, 2016). Investment into water and sanitation can help developing countries grow their economies. Since industrial development and agricultural productions are dependent on the availability of adequate and quality water, nations lacking these will tend to produce few agricultural and manufactured goods. They may have to depend on importation at significantly higher prices (Hertel et al., 2013). Reliance on importation will lead to trade imbalance between nations, leading importing nations into debt and borrowing at lenders' conditions. Inadequate water for developmental activities will lead to loss of potential investors and lack of revenue and declining tax revenues, job loss, and shrinking economies. The United Nations has warned of the consequences of food production not meeting the demand of a nation. Such food scarcity may lead to civil war, political turmoil, terrorism and social unease. If food prices are too high due to insufficient production because of water scarcity, then civil unrest may increase at a global level (Guarino, 2017).

The polluted water sources have health implications with the economic attendant consequences. Dirty drinking water will cause an increase in the spread of infectious and incidences of non-communicable diseases. Diseases such as malaria, cholera, diarrhoea, typhoid and dysentery are endemic in areas with polluted water (Rodríguez-Tapia and Morales-Novelo, 2017). In developing countries, the lack of clean drinking water increases the spread of disease and death than in developed countries (Guarino, 2017). The problems of sanitation and waste disposal in developing countries affect water supplies. Consequently, the health of the labour force and a lot of lives and hours meant for productivity are lost to communicable diseases (Muta'a Hellandendu, 2012).

Tourism is a big business and a source of substantial revenue for nations that develop it. For many countries, oceans, lakes, seas, rivers and waterfalls are attraction centres for tourists, generating massive income for their governments. If these water bodies are polluted, they will no longer attract tourists.

1.8 The Need for Water Recycle

Water has become a scarce resource in many parts of the world. The water demands will be increasing while its availability is shrinking due to population pressure and pollution. The available surface water resources remain constant for a long time, but the quality may deteriorate, and the spatial and temporal distribution pattern change (Wada et al., 2016). An estimate puts global water demand as twice the global population growth rate (Chowdhury, 2008). It was projected that the world population would reach nine billion by the year 2070 (Young, 2001), with a fixed number of freshwater bodies, there will be the need for conservation of water. If that projection holds, it means that global water consumption must be well managed to satisfy the needs of that population. The United Nations observed that the agriculture sector uses a substantial portion (approximately 70%) of the global freshwater for growing and maintaining crops and fruit trees while other industries 22% and domestic activities about 8% (Boretti and Rosa, 2019). About 60% of the water used in the agriculture sector, is lost to the thirsty ground due to the irrigation systems, inefficient application methods, and crop cultivations (World Wildlife Fund, 2016). Both developed and developing nations are prone to the water crisis. They should be prepared to meet the water demand of megacities that will result from the increasing population. The water infrastructural systems are presently over-burdened in many nations (Guarino, 2017) with many countries already experiencing water scarcity conditions and many will experience reduced availability of surface water resources by 2050 (Veldkamp et al., 2017).

Water can be recycled and used for various purposes such as domestic, agriculture, maintaining landscapes, or green zones. The advantages of water recycling include relieving the stress of finding new sources of water, reduction in the amount of both treated and non-treated effluent into the environment, thereby preventing the degradation of existing water bodies (DeNicola, 2015). Reusing of the recycled water can help reduce the possibility of polluting rivers, streams, and lakes and improve the rest of the environment.

1.9 Justification for the Study

The human population is increasing, and so is the burden of pollutants in the aquatic environment. The pressure of the people on water and water resources makes it necessary to conserve and recycle water for sustainable use. South Africa is a water-scarce nation, and this makes water conservation necessary. It is essential to know the composition, concentration and the nature of pollutants in the rivers and wastewater to be able to conserve rivers and reuse wastewater. Improperly treated wastewater is a threat to the aquatic biota and human health. The proper management of water supplies requires in-depth analyses of constituents with modern analytical instruments. The continuous monitoring of water quality parameters will help water managers to assess the current state of water bodies to know where improvements are necessary. EDCs are not yet adequately studied in Eastern Cape rivers and wastewaters, and hence some of them were included in this study. The problems posed with the presence of heavy metals in environmental waters made them be among the parameters investigated in rivers and wastewaters in this study. The effectiveness of WWTPs at removing the pollutants from wastewater also requires monitoring to know where improvements are necessary. It is also essential to determine, from available records, whether the concentrations of these water quality attributes are increasing or decreasing in the environmental waters over the years. Where such a record does not exist, this study will serve as a baseline for future studies.

1.10 Aims of the study

This study aimed to investigate the quality of Bloukrans, Buffalo, Swartkops and Tyhume Rivers in the Eastern Cape Province of South Africa regarding the selected physicochemical parameters, heavy metals, and endocrine-disrupting compounds. These are the main rivers supplying domestic and agriculture water needs in their respective municipalities. The investigation also extends to the municipal wastewater released from WWTPs in the cities around these freshwater bodies. The following specific objectives were set to achieve these aims:

1. To determine the physicochemical parameters of the selected rivers, wastewater influents and effluents and compare the concentrations with the standards set by the regulating bodies.
2. To determine the chemical functional groups of organic compounds in the water samples.

3. To determine the concentrations of the endocrine-disrupting compounds in the water samples.
4. To determine the concentration of some selected heavy metals in the water samples and compare the results with the standards set by the regulating bodies.

CHAPTER 2
LITERATURE REVIEW

2.0 Introduction

This chapter reviews related literature on water quality, common physicochemical parameters, chemical pollution and emerging contaminants. The literature on the health and environmental implications of water pollutants were also examined in this chapter. Earlier works on the roles of chemical compounds and heavy metals in pollution were also reviewed.

2.1 Surface Water Quality

The surface water is currently in crisis globally, as the recipients of human-generated wastes (Cobbing and de Witt, 2018). When these wastes are released into the air or soil, they eventually found their ways into water bodies where they cause pollution, which has become a global problem (Inyinbor et al., 2018; Kjellstrom et al., 2006). Human activities and population increase have led to the generation of more wastes (World Bank, 2019). The United Nations Organization (UNO) SDGs article 6 is to "ensure availability and sustainable management of water and sanitation for all" (UNDP 2019). Water quality around the world has deteriorated due to the increasing population, which is threatening human health, food security and biodiversity (Rafi et al., 2019; World Bank, 2019). Nutrient enrichment due to runoff from agricultural inputs such as pesticides and other agrochemicals, mining wastes, untreated and poorly treated municipal wastewater are other primary sources of surface water pollution. The United Nations Environmental Programme (UNEP) estimated that 90% of the municipal wastewater released to water bodies around the world is untreated (UNEP, 2019). Corcoram et al. (2010) categorised wastewater as domestic (with excreta, urine and faecal sludge), institutional wastewater (from establishments, institutions and hospitals), industrial and urban runoff and agricultural wastewater. Poorly treated wastewaters alter the characteristics of the receiving water bodies thereby causing a shift in the natural balance onto which aquatic organisms already adjusted (Jim et al., 2017; Edokpayi et al., 2017).

2.2 The Oxygen Factor in Water Quality

Oxygen demand in water bodies is one of the most critical factors determining water quality. Chemical oxygen demand (COD) is the amount of oxygen available for consumption in oxidation

reaction in water bodies (Geerdink et al., 2017). Observing the COD dated back to about 150 years ago when colour changes on the addition of permanganate solution to water, was a method of monitoring water quality (Miller et al., 2001). COD is a necessary measure for wastewater effluents to ensure that they will not exert oxygen demand on the receiving water so as not to endanger the organisms in the receiving water body. In water research, COD is an indirect measure of the amount of organic compound or oxidisable pollutants in water (Dhanjai et al., 2019). In South Africa, the Department of Water Affairs set COD limit of 30 mg/L for wastewater effluents released to water bodies, while 75 mg/L is the limit for effluents discharged to other places, such as irrigation of farmlands (DWAF, Act No. 991 – 18 of May 1984). Dissolved oxygen (DO) is the amount of molecular oxygen available in the water for the respiration of aquatic organisms. Most aquatic organisms, except some mud-dwellers, require high oxygen concentration for survival (Pearce and Schumann, 2003). It is an index of the life-supporting ability of the water. The solubility of oxygen in water depends on temperature, depth, altitude and turbulence (Jantzen, 1978).

Oxygen may dissolve in water from the atmosphere. In water bodies, the solubility of oxygen falls in the night and continues to rise as the day breaks only to reduce with high temperature at mid-day. Undersaturation of oxygen in water is related to the decaying processes of plants, nitirifcation, and ecosystem metabolism, while supersaturation is controlled by photosynthetic activity (Prasad et al., 2014; Butterfield, 2018). Anoxic conditions will lead to the death of aerobic organisms that are necessary to break down organic matter (Sinkko et al., 2019). Bagherzadeh et al. (2013) observed a significant reduction in the growth rate and bodyweight of fishes under reduced oxygen concentration in the water. A decrease in the ambient oxygen in the water body may result in the modifications of phenotypic traits such as stunting, early maturation and high mortality of larger-sized individuals in the guppies (*Poecilia reticulata*) (Pauli et al., 2017).

The oxidation-reduction potentials (ORP) are the ability of a water body to purify itself or break down contaminants inside it (Al-Samawi and Al-Hussaini, 2016). The higher the ORP value, the healthier the water body because higher values encourage decomposing bacteria to thrive since oxygen will be made available to them (Horne and Goldman, 1994). Oxygen solubility in water is temperature-dependent, and solubility reduces with increasing temperature. Availability of DO improves the ORP value and hence easy for decomposers to function at optimum capacity. If the

ORP value is positive, the water has oxidation potentials, but negative value signifies a reducing ability. South African water quality index sets the daily minimum of DO as 3.3 mg/L and 4.96 mg/L for a seven-day minimum (DWAF, 1996).

2.3 Water temperature

Temperature is the primary determinant in water chemistry, biogeochemical processes, and in the physiology of the organisms inside the water (Dallas and Ross-Gillespie, 2015; Grab, 2014). Temperature also affects the solubility of oxygen in the water and determines the susceptibility of organisms to diseases and parasites (Bhateria and Jai, 2016). The temperature of the freshwater body depends on seasons, solar radiation, hyporheic exchange, turbidity, and the flow rate (Chikita, 2081; Leiss et al., 2015; Naresh and Rehana, 2017). The surface water temperature may also be influenced by the prevailing weather, industrial cooling water discharge, shade and stormwater (Bhateria and Jai, 2016; Spellman and Drinan, 2012). Naresh and Rehana (2017) attributed the rising water temperature in the rivers over time is due to climate change. Monitoring water temperature is critical to water management since organisms prefer optimum conditions for survival; extreme temperatures may lead to migration or death. A change in the optimum temperature will affect the enzyme systems of organisms in the aquatic ecosystem. Extreme temperatures may inactivate or denature the enzymes leading to a modification of behaviour, metabolisms and growth rates (Issak et al., 2015; Naresh and Rehana, 2017). Warmer temperatures will increase the nutrient loading from soil and sediment, thereby encouraging eutrophication in water (Xia et al., 2016). High water temperature will reduce the dissolved oxygen and lower the self-purification capacity and degradation coefficients of water. An increase in water temperature will promote stratification, and with the internal nutrient loading, a favourable environment may favour eutrophication (O'Neil et al., 2012). An increase in temperature of the water will accelerate microbial activity in the sediments of lakes and rivers, thereby releasing the internal phosphorus loading and increase the total nutrient load in the water (Valdemarsen et al., 2015). Warm temperature will lower the surface water viscosity and increase nutrient diffusion, creating a ground for competition for nutrients between species (Xia et al., 2016).

A change in the water temperature will affect sexual development, the time of maturation, and the reproductive performance of fishes (Pankhurst and Munday, 2011). It has been observed that fishes

that bred near the upper-temperature threshold had the number and quality of their offspring reduced (Donelson et al., 2011).

2.4 Electrical Conductivity and Dissolved Solids

The ability of water to conduct electricity is as a result of dissolved salts. The dissolved salts may originate from both natural and artificial processes. Salts are naturally present in rocks and soil through which rivers flow (Bhateria and Jai, 2016). When salts dissolve in water, they ionise to cations and anions, turning the water to a conductor of electrical current. The presence of strong ionising compounds such as NaCl (Na^+ and Cl^-) increases the electrical conductivity of water. In water quality assessment, EC is an indirect measurement of the dissolved salts. Freshwater has low EC, usually 0 - 1,500 µS/cm, but seas and oceans may reach up to 50,000 µS/cm. Conductivity is directly proportional to the TDS since it is a measure of the total inorganic salts (ions) in solution (Niekerk et al., 2014). Conductivity measures the activity of the ions with their electrical charge, but TDS is a measure of the amount of dissolved salts. EC must be low in rivers to support plant and animal lives. The relationship between TDS and EC is given as TDS (mg/L) = k_e x EC (µS/cm) where k_e is a constant of proportionality (Taylor et al., 2018). Increasing TDS in rivers will promote a decline in the community and species richness (Timpano et al., 2010). High salt content will render water useless for most organisms, including man. Salinity defines the saltiness of the water. Inland water may be salty if salts dissolved in it from weathered rocks, soils, and pollution from industries, acid rain, or high evaporation in arid and semi-arid regions (Mabidi et al., 2018). Salinity in freshwater is always near zero, while it may be as high as 35 mg/L in seas. Increase in the salinity of freshwater is an indication of pollution and will lead to the death of aquatic organisms because they will lose water to the environment by plasmolysis (Nel et al., 2015).

2.5 Hydrogen ion concentration (pH)

This parameter measures the acidity and alkalinity of the water. Several factors determine the pH of water. These include the bedrock, soil and topography through which the river flows (You et al., 2019; Zhang et al., 2019), types of wastes coming from adjacent communities and industrial inputs such as mining activities (Zouch et al., 2018). Rivers flowing through limestone rocks would likely be alkaline. Decomposition of organic materials and plants in the water will release carbon dioxide, which combines with water to form carbonic acid that lowers the water pH (Hasler et al.,

2018; Oram, 2019). Living things are sensitive to pH, and extremes of it can damage their internal and external structures. The pH of water determines the distribution of bacteria in wastewater (Tyson et al., 2004). The solubility and convertibility of some compounds in water are also pH-dependent. Metals are more soluble in water and mobilised from the sediments at lower or acidic pH (Xie et al., 2018; Potysz et al., 2017), while ammonium ion will be converted to ammonia (toxic) at pH above 8.5 (Leoni et al., 2018). It also determines the productivity of aquatic fauna (Wurts and Durborow, 1992). When metals are mobilised from sediments at low pH, they can accumulate in the gills of young fishes and cause deformities and death in young fishes (Oram, 2019).

2.6 Water Turbidity

Turbidity measures the ability of light to penetrate water. Water turbidity may be due to dissolved and suspended materials, microorganisms and organic materials in the water. Light penetration is vital in water bodies because it is the principal input in primary productivity (Hussain et al., 2016). The primary producers in the aquatic ecosystem require light penetration for their activities. However, light rays reaching the water may be reflected, scattered or absorbed by suspended and dissolved particles in the water. When light waves are intercepted and absorbed by suspended materials in the water, the absorbed light energy will raise the water temperature above normal, thereby making the environment unconducive for the survival of some organisms (Leiss et al., 2015). Turbidity also aids the proliferation of pathogens in freshwater (Hussain et al., 2015). Reducing the turbidity of wastewater before discharge is an essential aspect of water quality management.

2.7 Dissolved ions in Water

Dissolved ions in water are from point and non-point sources. The primary source of ions in the river is the bedrock, through the process of chemical weathering of the rocks. More ions enter and dissolve in the river as it flows through forest, farmland and settlements (Moore et al., 2017). Moore and his colleagues observed a 27-fold concentration of dissolved ions in a river as it passes through uncultivated forest to the urban environment. Shin et al. (2017) observed increasing levels of dissolved ions along the river reaches, with nitrate contributed mainly by farmlands, sulphate, and chloride ions contributed largely by sewage from treatment plants. Stets et al. (2020) observed

a decrease in the urban contribution to nutrients in a river over ten years, but such was not the case with agricultural sites over the same period. Observations pointed to the fact that the distribution of ions in the rivers is controlled in the upstream by soil weathering, midstream by farmlands and in the downstream by the sewage (Moore et al., 2017; Stets et al., 2020). The concentrations of ions in the surface waters are influenced by seasons of the year. At the same time, the summer is dominated by the soil weathering due to the effect of rain; the impacts of sewage is felt more in the winter (Shin et al., 2017).

Sulphate ions may enter freshwater naturally through the oxidation of sulphite ores, dissolution of gypsum (calcium sulphate) and anhydrite; shale rich in organic compounds, breakdown of plant parts, especially leaves that fall into the rivers and decaying algae (Bhateria et al., 2016; Wang et al., 2019). Other sources may be from acid rain as a result of atmospheric-borne sulphur dioxide and industrial wastes released to water bodies (Bhateria et al., 2016; Kumar and Kumar, 2018). Under anaerobic condition, especially in river sediments, bacteria may split sulphates to form hydrogen sulphides. Hydrogen sulphides are respiratory toxicants that create extreme environmental conditions and produce obnoxious odour in water bodies. Humans and fishes will avoid water with hydrogen sulphides (Tobler et al., 2018). A high concentration of sulphate in water may affect the taste and have laxative effects when combined with magnesium or sodium (Kumar and Kumar, 2018). World Health Organization (WHO) recommends that sulphate ions in water should not exceed 500 mg/L (Kumar and Kumar, 2018).

Chloride constitutes about 0.05% of the earth's crust; it is ubiquitous in nature and naturally universal in water sources (Hunt et al., 2012). It is present in rocks and mineral deposits, seawater, agricultural wastes, domestic and industrial wastewater and other sources. Its sources in freshwater bodies include runoff, wastewater from industries, agriculture and households (Brandt et al., 2016). Chloride concentration may reach up to 20 g/L in seas and oceans but less than 50 mg/L in freshwater, a range necessary for normal functioning of the ecosystem (Hunt et al., 2012). Increase chloride concentration in the freshwater may be as a result of runoff or pollution from wastewater. Elevated levels of chloride will result in various environmental problems such as acidification and mobilisation of toxic metals from the soils and sediments; reproduction impairment and mortality

of aquatic organisms; alteration of the steady-state of aquatic ecosystem; corrosion of pipes and taste problems (Muralikrishna and Manickam, 2017; Brandt et al., 2016; Hunt et al., 2012).

Phosphates, like most other ions, are present naturally in rocks and released to the environment through weathering. Phosphates may occur as organophosphates, metaphosphates (polyphosphates) or orthophosphates. All forms of phosphates occur naturally in living systems, dead organisms, and available in sediments and soils (Oram, 2014). Orthophosphates are present as components of living tissues and may be released to the environment after the death and decay of the organism (Griffin, 2017). Orthophosphates are available in low concentration in the environment through natural processes, especially in the soil, for plants uptake. Levels of orthophosphate in freshwater bodies may increase due to pollution from wastewater, stormwater, agricultural runoff, and direct waste dump (Griffin, 2017; Oram, 2014). Phosphates removal from wastewater has not been the target of wastewater treatment plants (WWTP) designers; this may account for its escape into the effluents (Dabrowski and de Klerk, 2013; Oberholster and Botha, 2013). Phosphates are necessary for plant growth, but its enrichment in the receiving rivers will cause eutrophication (Matthews, 2014).

Ammonium ions in freshwater bodies are from anthropogenic sources such as industrial emissions, fertilisers and wastewaters. The increasing levels of ammonium in freshwater bodies are becoming a global problem (Du et al., 2017; Wieben et al., 2013). Ammonium ions arise from an excess of nitrogen compounds released into freshwater from wastes, agriculture, and conversion of atmospheric nitrogen to ammonia by nitrogen-fixing bacteria, which dissolves in water to form ammonium ions (Gupta et al., 2015; Chang et al., 2018). The interest in ammonium is because it can exert oxygen demand on the aquatic ecosystem, transformed into nitrite ions, which in turn form ammonia (Du et al., 2017). Ammonia is toxic to fish and other aquatic animals, even at low concentrations (lethal dose 0.2 – 2.0 mg/L) (Gupta et al., 2015; Du et al., 2017). In the event of ammonia pollution in water, fishes will experience loss of equilibrium, high respiratory activities, increased oxygen intake, convulsion, coma and death (Oram, 2014).

Nitrates and nitrites are natural parts of the nitrogen cycle and enter the environment, mainly through industrial and agricultural wastewaters (Sahu and Patel, 2016; Nezhad et al., 2017). In all

living system, nitrogen is essential because it is a component of protein. It is the most abundant gas in the atmosphere. Bacteria can fix nitrogen to nitrates in the soil. Nitrates have no taste, colour or odour and may not be detectable without testing. The importance of preventing nitrates in water is its convertibility to nitrites in the gut (Oram, 2014). Nitrites can oxidise the haemoglobin iron, turning it into methaemoglobin, which cannot effectively transport oxygen (Tredoux and Talma, 2006).

2.8 Spectroscopy in Environmental Research

Infrared (IR) spectroscopy is an essential tool in environmental sample analysis of solid, liquid or gas (Thermo Fisher, 2018). Samples absorb IR radiation as a reflection of the functional groups of molecules present in them. These functional groups absorb IR radiation at different frequencies, hence useful for determination of the sample's chemical make-up (Simonescu, 2017). Various compounds show differential absorption of IR radiation, which can be used quantitatively and qualitatively to analyse environmental samples. Fourier-transformed infrared spectroscopy (FT-IR) can identify trace contaminants in high-purity gas samples and can determine up to 30 components in gases from combustion processes, such as diesel engine emissions or continuous emissions monitoring applications (Köhler et al., 2017). FT-IR spectroscopy can quantify gas samples over large concentration spans, from parts per billion up to 99.99% and monitor gas samples continuously, providing concentration versus time trend charts (Simonescu, 2017). Grube et al. (2006) observed compost processes, and Kowalski et al. (2018) monitored sewage sludge decomposition with FT-IR spectroscopy. Several documented reports for FT-IR analysis of the fluids include wine (Tagg et al., 2015), olive mill wastewater (El Hajjouji, 2008; Ladwani et al., 2016) and urban wastewater and treated effluents (Navalon et al., 2011). FT-IR has been used to monitor different stages of organic matter decomposition in abandoned landfills (Smidt and Schwanninger, 2005). Information obtained from FTIR spectroscopic analyses of freshwater is useful in monitoring water quality and track point and non-point source pollutants. FT-IR with multivariate analyses has been a valuable tool in spectra data analyses and interpretation (Singh et al., 2011). Rohman et al. (2015) combined FT-IR with multivariate analysis to determine the level of adulteration of avocado oil. Cell-wall composition of apple during development was monitored with FT-IR and multivariate analyses (Szymanska-Chargot et al., 2014). Various manufacturing industries use FT-IR in quality control of their products and effluents. Sujka et al. (2017) reported

the use of FT-IR in quality control of flour production. Amir et al. (2013) monitored different varieties of wheat with FT-IR spectroscopy.

The nuclear magnetic resonance (NMR) spectroscopy is a useful tool in molecular studies. Before the invention of NMR, structural elucidation of a molecule used to take days and months until the discovery of chemical shifts because of the variation in NMR frequencies (ACS, 2011). In the early days, NMR started with a continuous wave, a system whereby the oscillator frequency was constant while the magnetic field changes gradually and the signal amplitude measured as a function of frequency (Newton et al., 2017). Modern NMR consists of the cryo-magnet, user console and an electronic console (Marion, 2013). The magnet contains the vacuum chamber, probe, liquid nitrogen, helium vessels, probe, tuning and cryogenic shims covered with a radiation shield (Teng, 2012). The frequency of the proton (^{1}H-NMR) signals generated by the magnet determines its strength and always boldly written on the magnet (e.g. 400MHz). NMR makes it possible for the non-destructive analysis of samples during the determination of molecular structures and unveiling chemical identities (Rahman et al., 2016). It also makes analysis faster with rapid results and high accuracy (ACS, 2011). It can accurately measure the relative amount of different components in a mixture because the absorption coefficient of nuclei of the same species is the same, whether in a molecule or mixture of molecules. There is no need for calibrations as applicable to other optical spectroscopies where absorption coefficients need different calibrations for the components of a mixture (Anderson et al., 2002). The resolution of NMR is far better than other optical spectroscopies because there is usually little or no overlap (interference) of the observed peaks. The nucleus is effectively isolated from forces acting on the molecules, thereby reducing the linewidth compared to other spectroscopies.

NMR spectroscopy has become an evolving analytical tool in organic and inorganic chemistry and a versatile tool in the analysis and structural determination of bio-macromolecules (Pan et al., 2016). NMR spectroscopy is a useful tool in molecular biology, providing a reliable method for atomic resolution and structure determination of biological macromolecules in aqueous solutions similar to natural physiological environments that have posed a challenge to X-rays (Bieri et al., 2011). It has also proven to be the most powerful technique for quantifying the motional properties of bio-macromolecules, giving useful information in the rate of enzymatic conversion of substrates

to products (Lisi and Loria, 2016). NMR spectroscopy is a valuable tool in drug screening, identification, and determination of metabolites interactions with enzymes, receptors, and other proteins (Luzarowski and Skirycz, 2019). The high sensitivity of NMR to protein binding has made it possible for the screening of fragments, measurement and detection of the binding strength of weak affinity ligands (Dalvit and Vulpetti, 2018). NMR spectroscopy is a tool for structural identification and conformational analysis of chemicals, whether synthetic or natural (Harrell and Bergbreiter, 2017). Besides, NMR has been a useful tool in petroleum chemistry and industries to provide information about the chemical composition of crude petroleum and its products (Edwards, 2011). NMR Spectroscopy is a non-destructive technique with several advantages over other analytical methods, including fast speed of analysis, reproducibility of data, input recovery after analysis, little or no need for pre-treatment with several reliable results obtained in a single measurement (Mesquita et al., 2017; Filho et al., 2015). NMR spectroscopy has become a versatile tool in the study of chemical structures and interactions in the soil, water and air. Solid-state NMR is a useful tool in the analysis of soil, especially the chemical composition, moisture and organic matter contents (Hou et al., 2002); and in the determination of microbial constituent and their products in the soil (Simpson et al., 2007). NMR has been a tool in the study of soil humification processes, aggregate structure, stability, fertility and in the prediction of the response of soil carbon pool to land-use change, agriculture and climate change (Schmidt et al., 2011; Feng et al., 2010). NMR is a tool to monitor qualitative and quantitative changes of metabolites in the aquatic ecosystem and to examine the presence of external inputs such as contaminants or nutrient enrichment (Bundy et al., 2009; Sardans et al., 2011). It has been a tool in water quality assessment and monitoring of organisms' response to pollutants (Cappello et al., 2013). NMR is useful in monitoring ion exchange in water sediments and nutrient dynamics in the aquatic environment (Ahlgren et al., 2005). Navalon et al. (2011) analysed the chemical components of treated wastewater effluents with NMR, and Filho et al. (2015) used it to monitor the efficiency at which WWTPs remove chemical pollutants. Information obtained from NMR spectroscopy of wastewater analyses is useful in monitoring, processing and quality control, to ensure that the final effluent released after treatment is fit for public use and to optimise the performance of wastewater treatment plants (Wagner et al., 2019).

Mikhail S. Tswett, a botanist, pioneered the use of chromatography to separate plant pigments in the early 1900s and coined the name chromatography, from the Greek words *chroma* (colour) and *graph* (writing) to describe his 'colourful' experiment (Arsenault and McDonald, 2009; Ettre, 1990). The advances in chromatography in the 1940s led to the production of partition chromatography developed by Matin and Synge, which gave rise to improvements that led to a new form of technology used to analyse chemicals and separate mixtures (Ettre, 2001). That development used two liquid phases instead of one and helped researchers to separate liquids with different partition coefficients (Ettre, 2001). The development of pumps to push the liquid phase and the compounds through the stationary phase helped the compounds to pass through and separated more quickly (Karger, 1997). Pumping improved the performance of liquid chromatography (LC) considerably and hence called 'high-performance liquid chromatography' or HPLC. Coupling mass spectrometer (MS) to LC (LC-MS) made the system more efficient and sensitive compared to earlier detectors. In the MS, charging analytes ionise them, and the molecules are detected and analysed based on their mass to charge ratio (Pitt, 2009). While LC separates the ionised compounds, MS will detect the compositions both qualitatively and quantitatively (Pang et al., 2016). LC-MS can be used to analyse and identify different compounds in a mixture of many compounds at low concentrations.

2.9 Chemical Functional Group Analysis

Chemical functional groups are responsible for the reactivity, toxicity, persistence, polarity and other characteristics of a compound (Ertl, 2017; Ratnani et al., 2019). Organic compounds are composed of few elements. These elements are common to several organic compounds, which make elemental analysis not suitable in organic chemistry. There are many functional groups with each having unique characteristics such as alcohol, ketones, ethers, amides, esters, alkenes, alkanes and aldehydes (Malherbe and Meyer, 1999). An organic molecule may consist of one or more functional groups. The presence of a particular group may be diagnostic of the product expected and may be used to confirm the presence of that product in a sample. Understanding the chemical functional groups in a sample aids in identifying unknown compounds and can help prove the identity of suspected compounds. Knowledge of the chemical composition will help in monitoring water quality at any given point in the course of the rivers and provide a baseline for future quality assessment of water in the selected rivers and wastewater treatment plants. Chemical functional

groups in water samples give them uniqueness and can be useful to distinguish between samples from different sources and geographical locations.

2.10 Hormones and Physiological Regulations

The anterior lobe of the pituitary gland produces some hormones to carry out its regulatory functions. These hormones include the adrenocorticotropic hormone (ACTH) that regulates the release of the adrenal gland hormones. The follicular stimulating hormone (FSH) cooperates with luteinizing hormone (LH) to ensure the normal functioning of the gonads. The growth hormones (GH) regulate growth in young animals and maintain healthy bones and fat distribution in adults. Prolactin hormone stimulates milk production in mammary glands. Thyroid-stimulating hormone (TSH) regulates the secretion of the thyroid gland. The posterior lobe secretes the anti-diuretic (ADH) hormone that regulates the kidney's absorption of water. Oxytocin hormone is involved in stimulating uterus contraction and breast milk production during childbirth (Morley, 2019). Adrenocorticotropic hormone (ACTH) is a protein with 39 conserved amino acids sequence in mammals (Margioris and Tsatsanis, 2016). It serves as a mitogenic or differentiating factor in the development of the adrenal gland. It also controls steroidogenesis, aldosterone, glucocorticoid, and steroid secretions (Gallo-Payet, 2016). Follicular stimulating (FSH) and luteinizing (LH) hormones are glycoproteins that regulate follicle growth and ovulation and invariably, the ovarian cycle in women. These hormones bind to their specific receptors in the gonads (Carini et al., 2016). FSH stimulates the ovary to produce oestradiol (E2) and regulate key ovarian physiological processes during the early stage of development (Francois et al., 2017).

Growth hormones (GH) maintains the homogeneity of tissues and organs during development and have a high diversity of actions, which include activation of many proteins involved in cell signalling with diverse effects (Carter et al., 2016; Ban et al., 2012). This hormone is vital for female fertility because it stimulates progesterone and oestradiol production (Devesa and Devesa, 2016). Its primary function in reproduction is the regulation of the formation of the gonads. This hormone plays a vital role in hepatic triglyceride secretions in the liver. It is actively involved in neurogenesis in the juvenile brain, normal differentiation, and function of the blood cell (Devesa and Devesa, 2016). It also regulates skeletal development and mineral deposition on the bones

(Yakar et al., 2010). It is involved in the regulation of postnatal skeletal muscle expansion and hypertrophy (Weber, 2002).

The thyroid-stimulating hormone (TSH), produced by the thyrotrophs of the pituitary, stimulates the functions of the thyroid gland (Szkudlinski et al., 2002). TSH is a heterodimeric glycoprotein with α-subunit and a unique β-subunit (Brailsford et al., 2018). This hormone effects control by binding to a protein receptor on the cell membrane of the thyroid cells (Szkudlinski et al., 2002). The concentration of TSH in the blood serum correlates to the functions of the thyroid hormones in the body (De Groot et al., 2012). Evidence has linked TSH with ovulation (Du et al., 2019).

The posthypopysis of the pituitary gland produces the anti-diuretic hormone (ADH) or vasopressin as a result of stimulation by the hypothalamus gland. The hypothalamus, in turn, receives feedback from osmoreceptors. The secretion of ADH and its binding to its protein receptor on the renal cells make the cell membrane more permeable to water, and it can be absorbed according to sodium osmotic gradient (Nawal et al., 2019). Whenever the secretion of ADH is suppressed, the membrane becomes impermeable to water (Nawal et al., 2019). If the water and electrolytes in the body are depleted, the baroreceptors will transmit a message to the central nervous system (CNS), causing the hypothalamus to hinder further secretion of ADH by the pituitary gland (Morley, 2019).

The pineal gland secretes melatonin hormone that controls the circadian rhythms and the adaptation of the individual to its periodic environment (Tordjman et al., 2017). Melatonin is a neuroendocrine product. It has the functions of promoting DNA repair system, thermoregulation, and skin pigmentation in lower vertebrates. It has complex antioxidation, immunomodulation and antitumour properties (Slominski et al., 2018). With these functions, melatonin synchronises the central and peripheral oscillators such as the foetal adrenal gland, pancreas, liver, kidney, heart, lung, gut and fat among others (Tordjman et al., 2017).

The thyroid gland produces two main hormones: tetraiodothyronine (T4) and triiodothyronine (T3). Both hormones are necessary for the healthy development of the tissues and organs in foetuses and children (Hershman, 2019). The functions of these hormones are to regulate protein,

fat and carbohydrate metabolism (Hershman, 2019). The thyroid hormones are necessary for brain formation and function throughout life. Hypothyroidism will lead to anxiety, hyperreflexia, mood disorder, dementia and confusion (Bernal, 2015). Thyroid hormones (TH) trigger major transition and metamorphosis in vertebrates (Stepien and Huttner, 2019). Inadequate production, when needed during the developmental stage, may lead to brain damage and the severity determined by the stage of development of the organism (Bernal, 2015). Tyrosine amino acid bonded to iodine produces tyrosine hormones. Tetraiodothyronine (T4) is the more secreted of the two hormones, T3 is more active than T4, but T4 can be converted to T3 (Hershman, 2019). Figure 2.1 shows the structural difference between T3 and T4 molecules. Triiodothyronine (T3) can regulate cellular and tissue metabolism in the body by binding to thyroid hormone receptors in many organs, such as the liver (Chi et al., 2016). It can also induce autophagy to modulate lipid metabolism (Sinha et al., 2012).

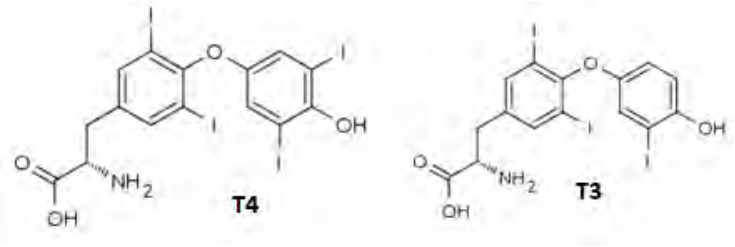


Figure 2.1: Structural differences between tyrosine T4 and T3 hormones

The parathyroid glands secrete parathormone (PTH), which stimulates the osteoblast to cause the breakdown of bone to release calcium when its level is low in the blood (Blaine et al., 2015; Civitelli and Ziambaras, 2011). PTH also activates vitamin D to aid the absorption of calcium in the intestine and its conservation by the kidneys (Hall and Guyton, 2005). PTH also promotes phosphate absorption through vitamin D in the intestine and inhibits its reabsorption on the proximal tubules of the kidneys when in short supply (Lanske and Razzaque, 2014; Hall and Guyton, 2005). Absence or too low PTH will cause hypercalcemia, and excess production will cause hypocalcaemia.

The thymus gland produces many hormones, of which four are essential. One of these hormones is thymulin, secreted by the thymic epithelial cells, and in the presence of Zn can couple with nonapeptide to induce T-cells and normalise the ratio of helper to suppressor T-cells (Haddad et al., 2005). Thymosin consists of TF5, thymosin-α and -β, which influence cyclic nucleotide level,

T-dependent antibody production, cell surface maturation and production of migratory inhibitory factor (Romani et al., 2012; Csaba, 2016). Epithelial cells of the thymus produce thymopentin hormone. It is essential in the immune regulation, production of lymphocyte precursors and maturation of these precursors to T lymphocytes (Zhu et al., 2015). Thymopentin influences neuromuscular transmission (Csaba, 2016; Zhu et al., 2015). Thymic humoral factor (THF) can increase the frequency of mitogen responsive T-cells and restore deficient T-cell functions, promotes T-helper cell functions and stimulate proliferation of lymphoid cells (Csaba, 2016).

The adrenal or suprarenal glands produce epinephrine, aldosterone (mineralocorticoid), and cortisol hormones (Santulli, 2015). Mineralocorticoid hormones regulate blood pressure and electrolyte balance in the body. The glucocorticoid hormones comprise of cortisol and cortisone. They control a wide range of metabolism and immune system suppression (Park et al., 2019). The adrenal glands produce androgens hormones, which converted to sex hormones in the gonads (Turcu et al., 2016) Androgen (testosterone) influences the development of primary male sex organs at an embryonic stage and secondary sex characteristics at puberty (Carlson, 2012). Androgen is present in the female at a lower level than males, and it serves as a precursor for the production of oestrogen (Carlson, 2012). The cortex of the adrenal glands produces corticoids hormones. The medulla produces catecholamine, adrenaline and noradrenaline, which help the body to act rapidly when necessary. The structures of some hormonal molecules produced by the adrenal glands are shown in Figure 2.2.

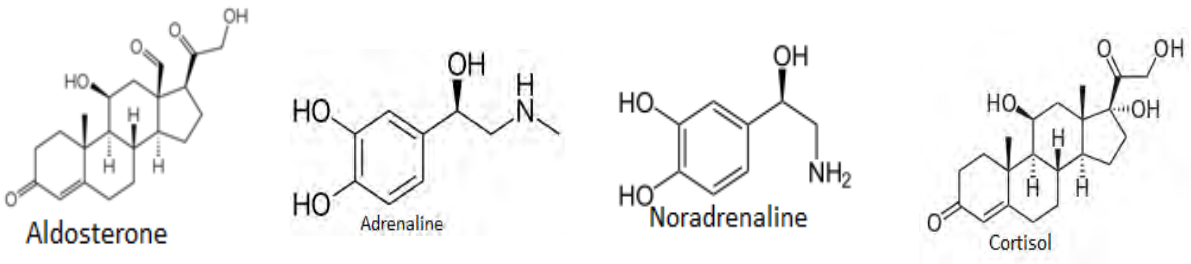


Figure 2.2: Structure of some hormones produced by the adrenal glands

The islets of Langerhans have five endocrine cells, alpha, beta, delta, epsilon, and upsilon, secreting five major hormones: glucagon, ghrelin, insulin, pancreatic polypeptides and somatostatin (El Sayed and Sandeep, 2019). Somatostatin may be produced from several glands and tissues, including the central nervous system (CNS), gastrointestinal tract and hypothalamus (O'Toole and Sharma, 2019). It has inhibitory effects on some other hormone secretions and has anti-proliferation effects on healthy and cancerous cells in the body (O'Toole and Sharma, 2019). The alpha cells of the islet of Langerhans secrete glucagon hormone in response to low levels of glucose and fatty acids in the blood, causing the liver to break down stored glycogen and oxidise stored fats, releasing them into the bloodstream, (Jones et al., 2012). The beta cells produce insulin hormone, which regulates the level of insulin in the body by promoting the conversion of glucose to glycogen for storage in the liver and muscles and triglycerides for storage in the adipose tissues (El Sayed and Sandeep, 2019).

The testes (male) and ovaries (female) produce sex steroids: androgens, oestrogens and progestogens. Oestrogen is the primary female sex hormone responsible for the development of the female reproductive system and secondary sex characters. It occurs as oestrone (E1), oestradiol (E2), oestriol (E3) and oestetrol (E4), but E3 is produced during pregnancy (Labhart, 2012). Figure 2.3 shows the structural differences in the oestrogen hormones. Oestrogens are present in both males and females but produced in higher concentrations in females than males. In the male, it regulates the maturation of sperm and maintains the libido (Hill et al., 2004).

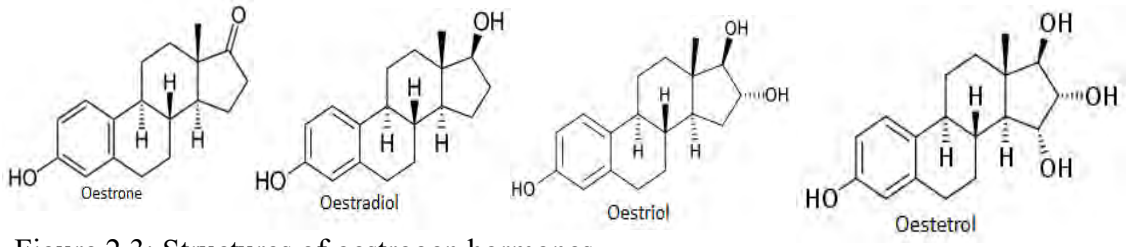


Figure 2.3: Structures of oestrogen hormones

In mammals, progesterone is produced from progestogens; it maintains the menstrual cycle, embryogenesis, and pregnancy (Jameson and De Groot, 2015). The progestogens are essential intermediates in the synthesis of other hormones and may act as neuroactive steroids (Baulieu and Schumacher, 2000). Progesterone and oestradiol regulate the immune response in women (Xiu et al., 2016). Since oestradiol can activate different but inter-related areas in the brain, it is involved

in mediating in sexual behaviours and display, promotion of nutrient absorption, social learning, and cognition (Sinchak and Wagner, 2012; Ervin et al., 2015; Luine, 2016). Oestrone and oestriol are weaker forms of oestrogen, with oestriol far weaker than oestrone (Labhart, 2012). Oestrone is convertible to oestradiol, serves as a precursor or pheromone of oestrone and might be a partial antagonist of it (Lundstrom et al., 2015). Figure 2.4 shows the chemical structure of the progesterone. The testes produce testosterone (Figure 2.4), the primary androgen hormone in men, which functions in the production of male organs, pubertal development, spermatogenesis and male secondary sexual characteristics. This hormone is also involved in the regulation of gene expression in muscles, bones and the immune system (Winters, 2016).

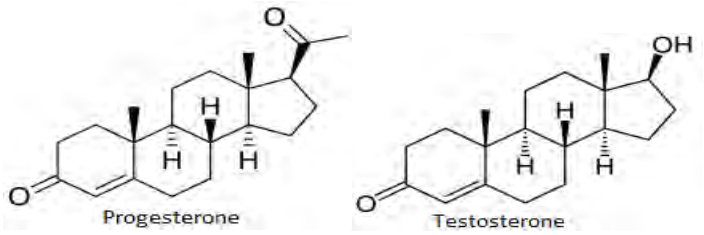


Figure 2.4: structures of progesterone and testosterone

Bisphenol A (BPA) and nonylphenol (NP) can compete with E2 in binding to oestrogen receptors with a similar preference and degree at nanogram concentration (Kuiper et al., 1998). BPA and NP can disrupt androgen hormonal functions and act as potent anti-androgen receptor (AR) antagonists. Both compounds can affect multiple steps in the activation and functions of androgen receptors, thereby inhibiting the binding of natural androgens to AR, hinder interaction with its co-regulator and its subsequent transactivation (Wang et al., 2017; Kuiper et al., 1998).

2.11 Mechanisms of Endocrine Disruption

An endocrine-disrupting compound (EDC) is any chemical that can mimic, obstruct the binding site of a hormone, or prevent the production and effects of such hormone. Such a compound can be of natural or artificial origin. They can cause temporary or permanent health problems in healthy organisms or their progenies (Stolz et al., 2018). There are about one thousand compounds with endocrine disruption abilities identified, and their numbers are growing (Gore et al., 2015). Since they are emerging contaminants, the activities of some potential endocrine disruptors are yet to be determined. Endocrine disruptors can mimic natural hormones by binding to hormonal receptors

and initiate normal responses at the wrong time. They can play agonistic roles by stimulating hormone production but not at the right time (Darbre, 2015). If the stimulation is at the right time, the feedback mechanism that controls the production of the hormone will be hindered, since EDCs are of exogenous sources, such stimulation will be excessive. In antagonistic action, EDCs may modify the binding site of a normal hormone by binding to hormonal receptors but not activate it. In this type, the natural hormone is prevented from binding to its site since EDC has occupied it (Darbre, 2019). EDCs may bind to hormone carrier or transport proteins in the blood, thereby reducing the amount of hormones in circulation. Another way by which EDCs affect the body is to interfere with the metabolic processes by changing the rate of synthesis or breakdown of natural hormones and disrupt the actions of enzymes involved in steroidogenesis (Yang et al., 2015).

2.12 Heavy Metal Pollution

Metals are part of the earth's crust and had been since the inception of the planet (Dyer, 2007). They are present in rocks and soils. The process of weathering and human activities such as mining, quarrying, among others release heavy metals to the soil from where they are washed to the rivers through runoffs. Those classified as heavy metals have densities that are greater than $5mg/cm^3$. Some heavy metals are environmental problems because they can persist for a long time in the environment. Some, like cadmium (Cd), lead (Pb), and arsenic (As), have half-life greater than ten years in the environment (Cooke, 2014). Humans are exposed to some of these heavy metals daily through various activities such as mining, smelting, refining, fossil fuel combustion, jewellery, children products, food and food packaging, surface water, and groundwater (Khan et al., 2016). Lead, especially, is common in children's products, and that makes the children more vulnerable to lead poison (Viet et al., 2013). Because of the chemistry and reactivity of this category of metals, they are highly mobile when entered into the ecosystem (Zhang et al., 2018).

Some of these heavy metals like copper (Cu), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) are necessary in trace amounts for normal physiology in living systems, especially as coenzymes and in redox reactions, but may cause damages when present in high concentrations (Tchounwou et al., 2012; Harmanescu et al., 2011). Non-essential heavy metals can be poisonous, even at very low levels (Orisakwe et al., 2012), causing damages as they accumulate in vital organs. Various pathological conditions are

traceable to heavy metal pollution. Some of these include renal dysfunction and lung diseases from cadmium poisoning (Satarug, 2015). Chronic exposure to manganese will cause dopamine depletion in the central nervous system (O'Neal and Zheng, 2015). Mercury damages the immune system, lung, kidney, digestive system, brain and the CNS (WHO, 2017) while exposure to arsenic will cause haemolysis, kidney and liver damages (Rajeswari and Namburu, 2014).

Recently the endocrine disruption abilities of some heavy metals are unfolding, especially on the gonads and their hormonal activities (De Toni et al., 2017). It has been observed that e-waste workers, exposed to various toxic metals in the electronic wastes, have significantly reduced levels of sex-related hormones. The affected hormones include luteinizing (LH), follicle-stimulating (FSH), testosterone, prolactin, progesterone and oestrogen (Igharo et al., 2018). Heavy metals disrupt the secretion, transportation and activities of steroid hormone receptors in rats and men. Zhang et al. (2018) demonstrated the inhibition effects of heavy metals on glucocorticoid and mineralocorticoid steroid receptors. They observed that Cd, Pb, Li, Mn, and Sn could antagonise aldosterone actions in the inhibition of hepatocellular carcinoma cells, thereby implicating them in cancer induction. Glucocorticoids form a group of a conserved family of steroid hormones, secreted in all vertebrates due to the activation of the hypothalamic-pituitary-adrenal (or inter-renal) axis; they serve numerous functions essential to survival (Pelt, 2011). Increased production of glucocorticoid hormones facilitates lung maturation during late gestation (Fowden et al., 2016).

The knowledge of the roles of lead (Pb) in reproduction disruption preceded modern science. It was alluded to cause problems and abnormalities in the development of the male reproductive system in ancient Greek and Roman empires (Haghighi et al., 2014). Experimental rats fed with water containing Pb experienced an alteration in the number and affinity of uterine oestrogen receptors and LH ovary receptors both in adult and pre-pubertal rats (Iavicoli et al., 2009). Exposure of the rats to doses of Pb during pregnancy shows an alteration in the binding between gonadotropins and their respective ovary receptors with a change in steroid production. Changes in hormone secretion and delayed puberty, reduction in plasma levels of insulin-like growth factor (IGF-1), LH, and oestradiol were observed in the offspring of rats treated with lead (Dearth et al., 2002). A recent study on some group of girls showed that prenatal and early-life exposure to lead is associated with delayed breast maturation, pubic hair growth and delayed first menstruation (Liu

et al., 2019). In male pups exposed to lead, there was a suppression of plasma levels of testosterone with plasma LH decreasing significantly, elevated pituitary LH content, and a decrease in plasma testosterone/LH ratios. In female pups, observation showed a suppression of the plasma concentrations of estradiol during puberty (Iavicoli et al., 2009).

Cadmium is a toxic heavy metal with disruptive effects on the reproductive system, growth retardation, sterility and defective embryo (Pant et al., 2014). Humans get exposed to cadmium through air pollution, green vegetables and grains, contaminated water and soil, industrial activities such as smelting and refining, mining, and manufacturing of batteries, paints and pigments (Donovan et al., 2016; Hayat et al., 2019). Tobacco can absorb and concentrates cadmium from the soil and when smoke, is passed through to the lungs of smokers, thereby raising blood cadmium levels three times higher than in non-smokers (Pant et al., 2014). Cadmium enters the body mostly through inhalation; only a small percentage is absorbed through food but can accumulate with age (Pant et al., 2014). Smoking has been linked to defective levels of male semen parameters and known to reduce the success of assisted reproduction techniques, such as in-vitro fertilisation (IVF) and intra-cytoplasmic sperm injection (ICSI) (Dai et al., 2015; Kovac et al., 2015; Akinloye et al., 2006). Occupational and long-term exposure to cadmium could modify the levels of LH, FSH, prolactin and adrenocorticotropic hormone (ACTH) in the body (Igharo et al., 2018). Cadmium can also reduce the levels of testosterone, sperm motility, and induce atrophy, necrosis, oedema, and haemorrhage (Allouch et al., 2009). Lafuente et al. (2003) showed that Cd differentially affected the secretory patterns of pituitary hormones. Cadmium decreased progesterone production in rats during a 48-hour exposure period, and the response was concentration-dependent (Iavicoli et al., 2009). Cadmium hinders the production of progesterone and testosterone. Thus, data suggested that Cd could interfere directly with hormone production in steroid-producing cells (Iavicoli et al., 2009).

Mercury is commonly found and used in manufacturing industries, smelting, mining and various other anthropogenic sources (Beckers and Rinklebe, 2017; Bourtsala and Themelis, 2019). It is present as a component of many electrical instruments, medical products and appliances such as the thermometer and dental amalgams, among others (Iavicoli et al., 2009). Mercury can spread through air pollution and runoff from mining industries. The general population is exposed to

mercury principally through the ingestion of contaminated foods. Fishes can accumulate mercury in the form of methylmercury and when consumed by man, becomes poison (Driscoll et al., 2013). Mercury can reduce hormone-receptor binding and can inhibit critical enzymes involved in hormone biosynthesis as in adrenal steroid biosynthesis and the inhibition of 21α-hydroxylase enzyme (Rice et al., 2014). Hormones that are the most affected by mercury are insulin, oestrogen, testosterone and adrenaline (Rice et al., 2014). Exposure of male and female fathead minnows (*Pimephaleas promelas*) to mercury lowered testosterone and 17β−oestradiol plasma levels (Iavicoli et al., 2009). Mercury also interferes with the process of spermatogenesis, in male rats, administration of $MeHgCl_2$ and $HgCl_2$ resulted in a reduction in sperm motility, sperm count and prolong ovulation in the female (Rhyaf et al., 2018; Jenardhanan et al., 2016; Iavicoli et al., 2009).

Arsenic is naturally present in the soil from where it gets to surface and ground waters. It is common in herbicides and fungicides, paint, foundry works and combustion of fossil fuels; with high exposure rate among workers in such industries (Singh et al., 2015; Iavicoli et al., 2009). Arsenic may be excreted through urine, sweat and milk, which makes milk and breastfeeding sources of contamination to young animal and man (Georgescu et al., 2011). It is the most common environmental poison contacted through food and water, most especially common in tap water in some countries (Hallauer et al., 2016). This metal is implicated in bladder, lung, skin, and other forms of cancers linked with degenerative changes in the kidney, liver, thoracic artery, and brain (Noman et al., 2015). Arsenic was implicated in diabetic conditions, neurological and reproductive defects (Georgescu et al., 2011). Arsenic lowers the production of LH and FSH (Igharo et al., 2018). It may impair foetal development by lowering birth weight, causing foetal malformations and death, blood vessel damage, lower IQ, reduced nerve function and may be responsible for an increase in mortality in young adults (Hallauer et al., 2016). Arsenic inhibits the nuclear transcription activity of the glucocorticoid receptor (GR). It may exert stimulatory effects on GR-mediated gene activation in rat hepatoma cells (Iavicoli et al., 2009). Arsenic inhibits the functions of the retinoic acid receptor (RAR) and thyroid hormone receptor (TR) necessary in vital metabolism in the body (Sun et al. 2016). Accumulation or chronic exposure to this metal causes male infertility and reduce erectile functions in older men (Mahajan et al. 2018; Shen et al., 2013; Alamdar et al., 2019). Arsenic in the gonad influences sex hormones, and the production of ovarian and testicular hormones that play various roles in gametogenesis (Sun et al., 2016).

Zinc is present naturally in the environment through weathering of rocks, runoff from soils and anthropogenic sources such as industrial processes, incineration of wastes and burning of fossil fuels (Nazir et al., 2015). Zinc in freshwater may range between 5 and 10 ppb. Algae may accumulate 20-700 ppm in their cells, lobster 7-50 ppm and oysters 100-900 ppm (Lenntech, 2019). A high concentration of zinc (2 mg/L or higher) will increase water turbidity and add unwanted flavour to water. Zinc is necessary for the normal functioning of over 300 enzymes in different categories of living things where it has structural, catalytic and coactive functions (Takeda, 2000). It is an essential trace element for normal reproductive functions in animals (Maipas and Stamati, 2015). Zinc influences cell division, protein synthesis, and DNA replication, thereby exerting a positive influence on spermatogenesis (Iavicoli 2009). It is present in low levels in oligospermic and azoospermic individuals. High concentrations of Zn are associated with oxidative stress in liver cells, abnormal sperm quality in mammals (Bian et al., 2019; Kasperczyk et al., 2015) and malformations in embryo and larvae of zebrafish (Maipas and Stamati, 2015).

Nickel is present in the soil, industrial wastewaters, and air pollution, from where it enters into rivers and sea sediments (Harasim and Filipek, 2015). It is one of the essential heavy metals required in plants and animals and very important in modern technology and infrastructure (Beshir et al., 2016; Harasim and Filipek, 2015; Mudd, 2010). Ni is a component of some enzymes, such as urease (Lenntech, 2019). It can be mobilised from the soil by acid rain (Georgescu et al., 2011), but inhalation is the most common route of entry to humans, while oral through food and water is secondary. Ni disrupts the process of steroidogenesis and spermatogenesis (Sun et al., 2011). It interferes with the production of testosterone, luteinizing and follicle-stimulating hormones (Apostoli and Catalani, 2010; Beshir et al., 2016). Reports suggested that Ni may cause a decrease in the testicular production of steroidogenic enzymes. It may reduce testicle, prostate epididymis, and seminal vesicle weights (Kong et al., 2014; Beshir et al., 2016). Danadevi et al. (2003) observed that elevated levels of Ni in the blood of welding workers correlated with their abnormal sperm production. Nickel can inhibit prolactin and growth hormone secretions, reduce iodine uptake by the thyroid gland and linked with reduced foetal development in rats and insulin resistance in dogs (Georgescu et al., 2011).

Chromium is the world's most strategic heavy metal, available in abundance in some countries like South Africa and useful for various industrial purposes like electroplating and chemical manufacturing (Jacob and Testa, 2016). It is also essential in textile, petroleum refinery, automobile manufacturing, wood preservatives, alloy preparations and tanning industries (Faisal and Hasnain, 2006). The various uses of chromium made it a pollutant of the air, soil and water bodies. It enters surface waters from mine wastes, industrial emissions, wastewaters and open dump. Chromium in blood levels correlated with the percentage of abnormal sperm count (Igharo et al., 2018). Chromium can accumulate in the mammalian placenta to cause abnormal foetal development (Li et al., 2018; Coetzee et al., 2018).

Heavy metals are part of cosmetic products. Fuad and Al-Momani (2018) analysed 112 cosmetics for heavy metals and observed that Ni, Cd, and Pb were present in the samples. Gao et al. (2018) found Cr, Mn, Co, Ni, Cu, Cd, Sb, and Pb in female lip cosmetics, thereby making the female more vulnerable to these metals through ingestion. Abrar et al. (2018) observed that Cr, Mg and Pb concentrations in lipstick and eye-shadows analysed exceeded the permissible limits set by the Food and Drug Administration, which could lead to serious health problems. Heavy metals such as Cd, Cr, Cu, Ni and Pb present in cosmetics may pose risks to users, as they are ingested daily through the mouth, and accumulate in the body with continuous usage (Massadeh et al., 2017).

In summary, poor water quality may promote non-communicable and affect the various developmental stages in animals. Therefore, proper water treatment may encompass reducing the microbial load, enhancement of physicochemical quality, and eliminating the emerging pollutants.

CHAPTER 3
MATERIALS AND METHODS

3.0 Introduction

This chapter presents the materials used in this research with their sources and purity (where applicable). This chapter also contains details about the sampling sites with their coordinates, supported with detailed site maps. Sampling procedure and general procedure for handling of samples were also highlighted. This chapter is presented for easy reproducibility of this research in the future.

3.1 Study Area

This study involved four major rivers in Eastern Cape Province of South Africa located in four municipalities with high population concentration and agricultural settlements. The Bloukrans River serves Makana municipality with a population of over a hundred thousand and the Grahamstown, which is the principal town had, as at midyear 2019, over 80,000 inhabitants (Department of Statistics 2019). The Tyhume River serves Nkonkobe municipality with over two hundred thousand inhabitants, with 127,000 people living in Alice, its main town (Department of Statistics 2019). Both Grahamstown and Alice have Universities locations. Buffalo River serves Buffalo City Municipality with a population of over eight hundred thousand and its main town; King Williams Town has over 227,000 inhabitants. Swartkops River serves a part of Nelson Mandela Bay Municipality with a population of about 1.4 million, Uitenhage had about 71,000 inhabitants with the moderate presence of industries (Department of Statistics 2019). The WWTPs in the listed towns were also sampled. Coordinates of the sample sites obtained with the aid of eTrex Vista HCx (Garmin Ltd, Kansas City, USA) are shown in Table 3.1. The atmospheric pressure recorded at the sites was converted to altitude with the online Pressure Altitude Calculator provided by the National Weather Service of National Oceanic and Atmospheric Administration available at https://www.weather.gov/epz/wxcalc_pressurealtitude. The site map was generated with ArcGIS 10.5 software (Redlands, California USA) and Google Earth (2018). The rivers and the WWTPs were sampled between autumn and spring in the year 2018 at different locations indicated in Figure 3.1.

Table 3.1: Coordinates and altitude of the sampling sites.

River		Coordinate/Altitude (metres above sea level)		
		Upstream	midstream	downstream
Bloukrans	Location	33º 19' 0.07" S; 26º 31' 20.9" E	33º 18'51.4"S, 26º 33'11.5" E	33º 19' 07.1" S, 26º 34' 05.7" E
	Altitude	480.7m	428.5m	421.4m
Buffalo	Location	32º 4 7' 23.74" S, 27º 22' 10.56" E	32º 53' 49.14" S, 27º 23' 34.08" E	32º 56' 3.6" S; 27º 26' 25.18" E
	Altitude	372.2m	272.6m	256.2m
Swartkops	Location	33º 42' 59.64" S 25º 17' 16.43" E	33º 47' 31.08" S, 25º 24' 26.96" E	33º 47' 31.92" S; 25º 29'26.26" E;
	Altitude	8.6m	5.9m	4.3m
Tyhume	Location	32º 36' 38.72" S; 26º 54' 34.15" E	32º 47' 42.95" S, 26º 50' 88" E	32º 50' 15" S, 26º 53' 31.27" E
	Altitude	782.5m	479.8m	452m

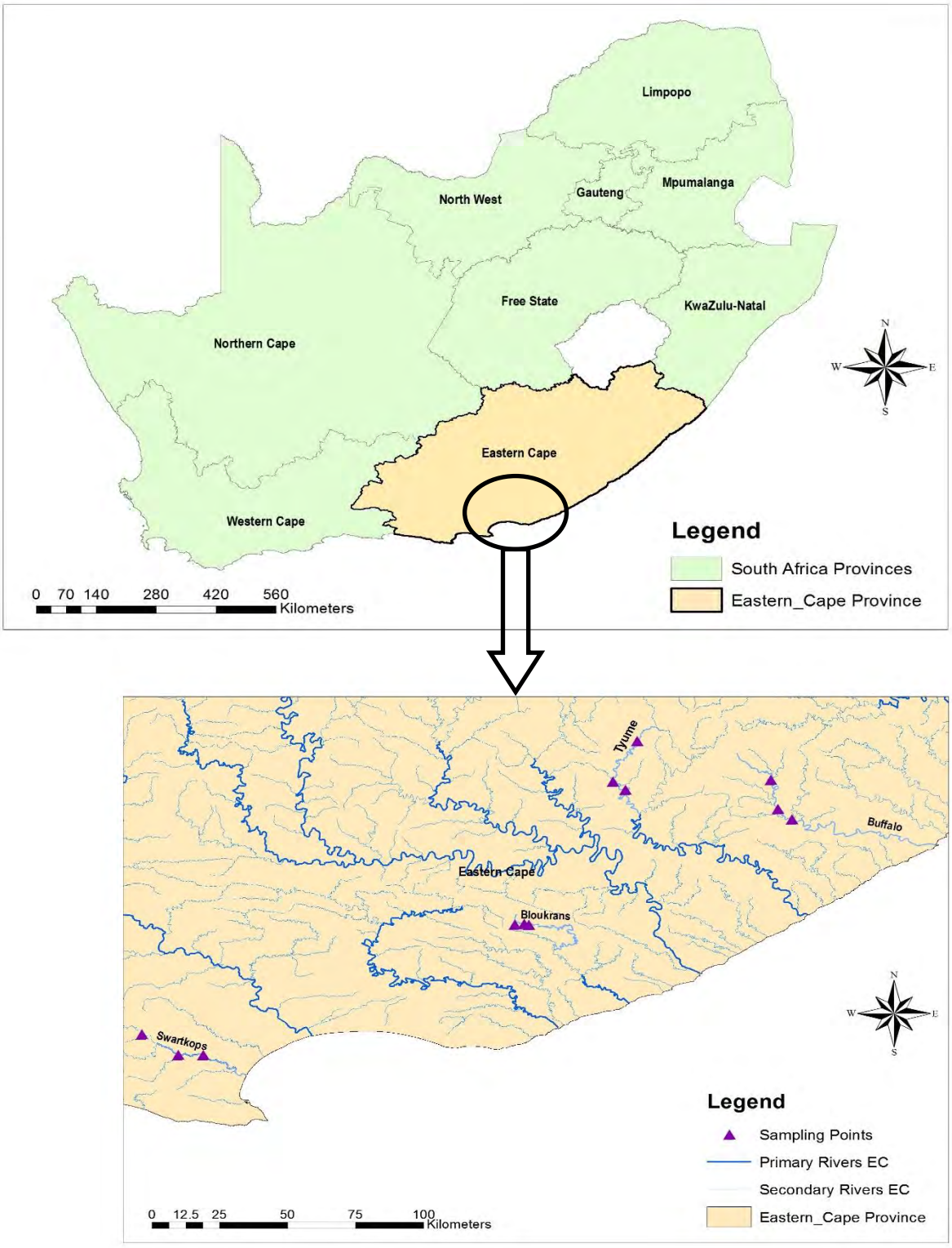


Figure 3.1A: Map of South Africa showing the sampled rivers (inset is the Eastern Cape Province).

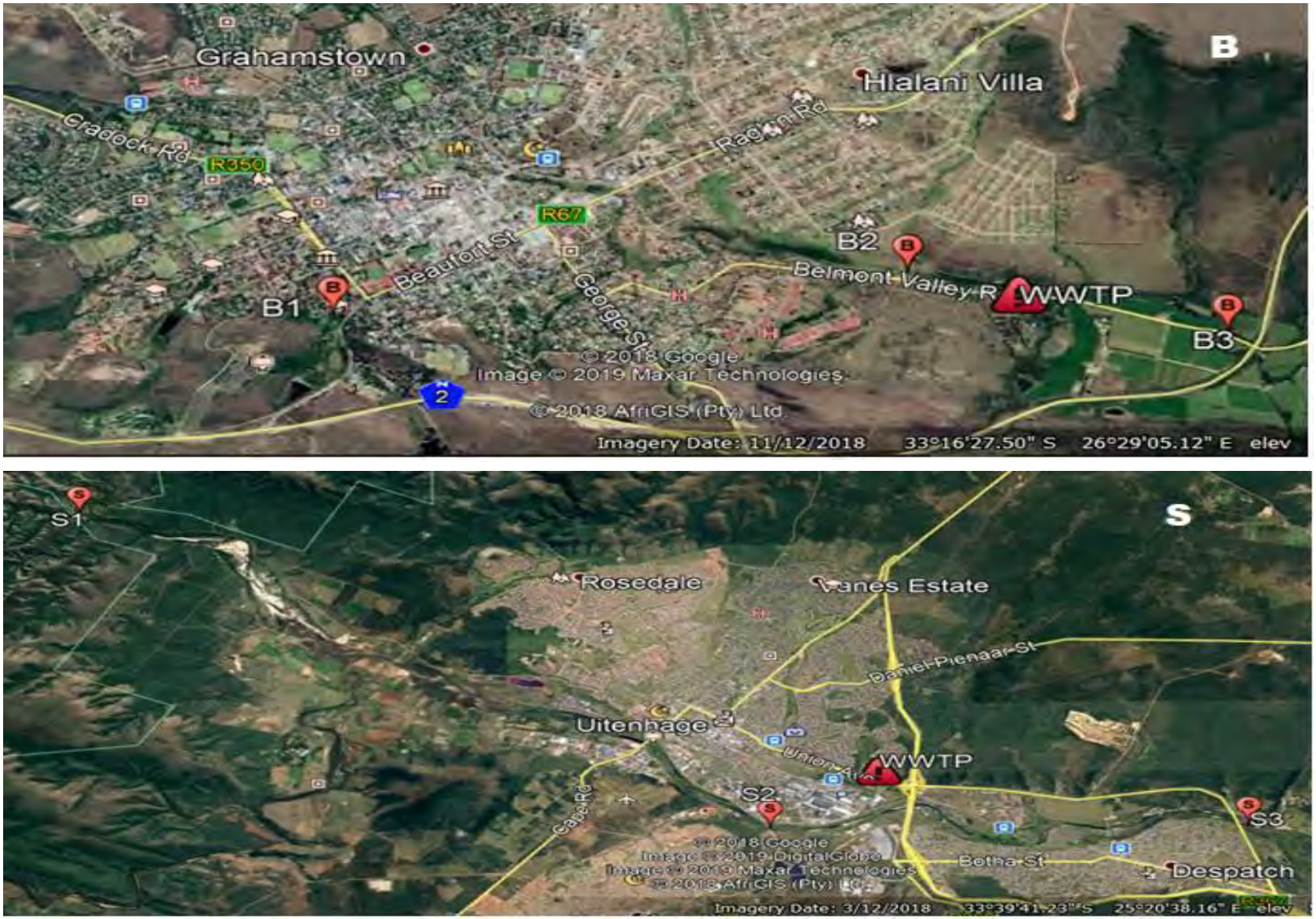


Figure 3.1B: Sampling sites along Bloukrans (B) and Swartkops (S) Rivers (1= upstream, 2= midstream and 3= downstream). WWTPs are shown in red triangles

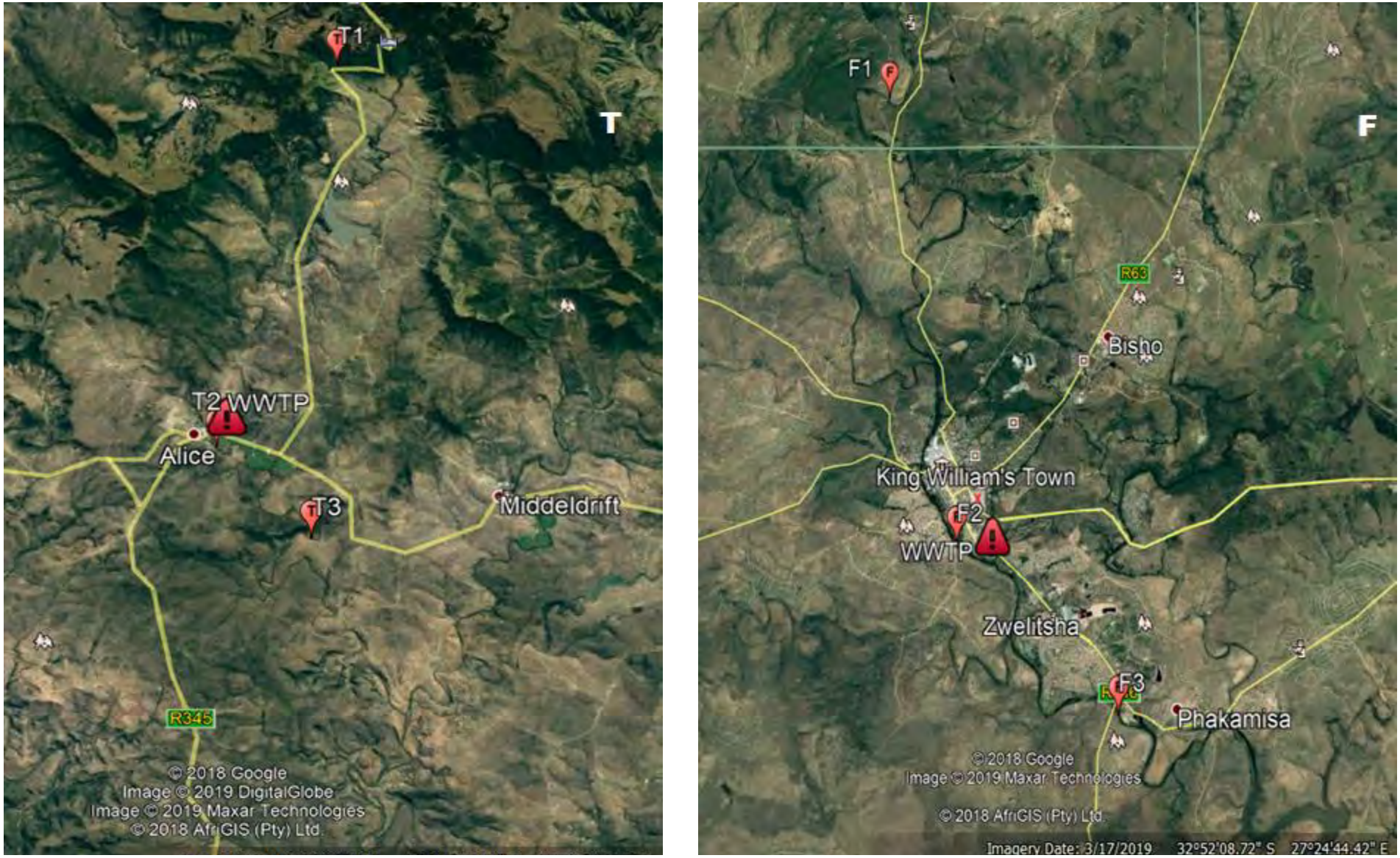


Figure 3.1C: Sampling sites along Tyhume (T) and Buffalo (F) Rivers (1= upstream, 2= midstream and 3= downstream). WWTPs are shown in red triangles.

3.2 Chemicals, Standards and Reagents

All solvents (hexane, methanol, acetone, chloroform, ethyl acetate, deuterated chloroform $CDCl_3$) used were of analytical standards and purchased from Sigma-Aldrich (Johannesburg, South Africa). Anhydrous sodium sulphate was purchased from Sigma-Aldrich. Chemicals used were of HPLC grade and purchased from Sigma-Aldrich (Johannesburg, South Africa). EDC standards, nonylphenol (NP) (Technical grade), dichlorophenol (DCP) (99%), oestrone (E1) (99%), 17β-oestradiol (E2) (98%), bisphenol A (BPA) (97%), octylphenol (OP) (99%), triclosan (TC) (99%), atrazine (AT) (99%), imidazole (IM) (99%) and 1,2,4-and triazole (TA) (98%) were also purchased from Sigma-Aldrich. Metal standards (copper, cadmium, chromium, lead, mercury, manganese, zinc, nickel and arsenic) were purchased from Perkin Elmer, Greenstone, South Africa. De-ionised water was produced with Millipore (Millipore SA, France).

Vir Tis BenchTop K freeze-drier, equipped with Elnor vacuum pump was purchased from SP Scientific, Pennsylvania USA. Solid-phase extraction (SPE) tubes (Supelclean LC-18) and vacuum manifold (Visiprep) was purchased from Sigma-Aldrich (Johannesburg, South Africa). Hanna HI9829 Multiparameter was purchased from Hanna Instruments, Woonsocket USA. FlowMate 2000 portable flow meter was from Marsh McBirney Inc, Maryland, USA. Spectroquant test kits were bought from Merck (Germany). Spectrophotometer UVmini-1240 was purchased from Shimadzu Corporation, Japan. Rotary evaporator Büchi Rotavapor R-210 with Büchi Heating Bath B-491 was bought from Büchi Labotechnik, Switzerland. NMR spectrometer Bruker AvanceTM III HD 400 MHz spectrometer, Topspin 3.5 pls and SampleXPress autosampler were from Bruker BioSpin, Rheinstetten, Germany. The Agilent 7900 ICP-MS was purchased from Agilent Technologies, Santa Clara, USA. Waters Acquity ultra-high-performance liquid chromatography (UPLC) coupled to Xevo tandem quadrupole spectrometer (TQ-S), forming UPLC-MS/MS for quantitative and qualitative analyses of samples was from Water Corporation, Wilmslow, United Kingdom.

3.3 Sampling Procedure

Each river was sampled at three different locations: upstream (closer to the river source), midstream (after passing through the listed major towns) and downstream (after receiving

municipal wastewater effluents). Samples of wastewaters (influents and effluents) were collected from the WWTPs of the listed cities.

Each water sample was collected into 1L Schott's bottle already prepared by washing with a phosphate-free detergent, dried and rinsed with acetone, rinsed again with deionised water and allowed to dry. The bottles were rinsed with sample water three times at the point of collection to condition the bottles (UNEP/WHO, 1996). The bottles were filled with the sample water, leaving no space between the sample and the cover to exclude air that may aid oxidation of materials of interest in the samples. The bottles were tightly covered and preserved in an icebox for further analyses in the laboratory. The icebox was to create a temperature that will discourage bacterial activities in the samples.

Water samples for heavy metal analysis were collected in polyethylene bottles (250 mL). The bottles, together with their lids, were prepared by washing with a phosphate-free detergent, rinsed with deionised water, soaked into 10% v/v concentrated HNO_3 for 24 hours, rinsed with deionised water again and later dried in a desiccator before sampling. Sample water was used to rinse the bottles three times at the point of collection. The bottles were filled with the sample water, acidified to 2% ultra-pure nitric acid (HNO_3) solution, labelled and preserved in an icebox and transported to the laboratory for elemental analysis. The samples were prepared by filtration through 0.45-μm filters.

Samples collected were labelled according to the sites and seasons as:
Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring).
Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring).
Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring).

Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring).
Grahamstown wastewater (influent) samples: G1A (autumn), G1B (winter) and G1C (spring).
Grahamstown treated wastewater (effluent) samples: G2A (autumn), G2B (winter) and G2C (spring).
King Williams Town influent samples: K1B (winter) and K1C (spring).
King Williams Town effluent samples: K2B (winter) and K2C (spring).
Alice influent samples: A1B (winter) and A1C (spring).
Alice effluent samples: A2B (winter) and A2C (spring).
Uitenhage effluent samples: U2B (winter) and U2C (spring)

3.4 Extraction of Organic Compounds

Samples for NMR and FT-IR analyses were extracted by liquid-liquid extraction (LLE) method (Adeniji et al., 2017; Brutti et al., 2016). There was a preliminary test to determine the best solvent for the extraction. Solvents of different polarity tested include hexane (non-polar), chloroform and ethyl acetate (medium polarity), ethanol and methanol (polar). Ethyl acetate and chloroform proved to be the best for the extraction. An exhaustive extraction was achieved by adding 300 mL of organic solvent to an equal volume of water sample (ratio 1:1), and the procedure was repeated three times, alternating chloroform with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4), filtered and the solvent removed *in vacuo* (at reduced pressure) on a rotary evaporator at 35 ^oC to obtain the extract. The extract was transferred to a vial and allowed to dry in an oven at 35 ^oC followed by spectral analysis on FT-IR and NMR spectrometers.

3.5 Statistical Analyses

Statistical and multivariate analyses were conducted with Statistica 13.4 (TIBCO Software Inc., Palo Alto, CA., USA) and MetaboloAnalyst 4.0 designed by McGill University, Parasitology Building, 21111 Lakeshore Road Ste. Anne de Bellevue, QC, Canada (http://www.metaboanalyst.ca). MestReNova 14 software (Mestrelab Research S.L., Santiago de Compostela, Spain) used for the analysis of NMR chemical shifts. Chemical structures and formulae were derived with ChemBio Draw 14 (Perkin Elmer Informatics). BioRad KnowItAll$^{(R)}$

Informatics 2018, Academic Edition (Bio Rad Laboratories Inc., Hercules, USA), was used for processing and analyses of FT-IR data obtained.

Descriptive statistics were used to generate the means and standard deviation for the data sets. Correlations of the sites were measured based on the parameters investigated with correlation statistics (r) significant at $p < 0.0500$ for physicochemical parameters and $p < 0.0100$ for EDCs. Association of the samples were tested with the principal component analysis (PCA). Hierarchical clustering analysis was achieved with the Ward algorithm and the distance expressed in Euclidean. The variable importance projection (VIP) scores for priority features in the samples were identified with partial least square-discriminant analysis (PLS-DA).

Data for Metaboanalyst were converted to ASCII files and imported to Microsoft Excel and saved as CSV comma-delimited (*.csv) files for upload to Metaboanalyst. MetaboAnalyst 4.0, an online, software (http://www.metaboanalyst.ca) was used for the visualisation, processing, analysis, and reporting of data. The spectral features were subjected to multivariate analyses (Ami et al., 2013; Szymanska-Chargot et al., 2015; Ildiz et al., 2016; Santos et al., 2017). The general procedures were carried out on the data include: checking for missing values, filtering using median intensity value, quartile normalisation with Log2 transformation, Pareto scaling, and cross-validation of the normalised dataset by permutation tests using leave-one-out cross-validation (LOOCV) method with the performance measure set at Q^2.

CHAPTER 4
INVESTIGATION OF THE PHYSICOCHEMICAL VARIABLES OF THE RIVERS AND WASTEWATERS

4.0 Introduction

Monitoring of changes occurring in water bodies is a tool to make a sound decision on water quality management. This chapter reports the result of the investigation of some common water quality parameters of the rivers and wastewaters selected for study in this work. The physicochemical parameters investigated include chemical oxygen demand (COD), hydrogen ion concentrations (pH), total dissolved solids (TDS), dissolved oxygen (DO), oxidation-reduction potentials (ORP), conductivity, turbidity, temperature and salinity. Others include nitrate, ammonium, chloride, sulphate and phosphate ion concentrations. This study is significant to assess the changes in some common water quality parameters at different reaches of the rivers and the influence of wastewater treatment plants on the quality of the receiving water.

4.1 Procedure

Pre-calibrated Hanna HI9829 Multiparameter instrument was used to measure pH, oxidation-reduction potentials, dissolved oxygen, conductivity, total dissolved solids, salinity and temperature directly at sampling sites. The river flow rate was measured with FlowMate 2000 portable flow meter. All readings were taken in triplicates at different points within each site. The physicochemical parameters measured in the laboratory include chemical oxygen demand (COD), phosphate, chloride, ammonium, sulphate, nitrate and nitrite ions. Standard solutions were prepared for the ions at different concentrations, analysed with Spectroquant (Merck, Germany) test kits and assayed on a spectrophotometer (UVmini-1240, Shimadzu Corporation, Japan). Light waves were filtered on spectrophotometer as recommended by the kit manufacturer; COD solutions were filtered at 610 nm wavelength, phosphate ion 660 nm, chloride ion 450 nm, ammonium ion 660 nm, and sulphate ion 420 nm. The optical densities (OD) of the solutions were recorded for the generation of the standard curves for different ions. The standard curves showed excellent linearity greater than 0.95 ($R^2 > 0.95$). The water samples were analysed for the presence of the ions on the spectrophotometer. The concentrations of nitrate and nitrite ions were measured

with Spectroquant (Merck, Germany) dipstick. All solutions were prepared in triplicates and analysed accordingly.

The results were pulled together according to the reaches (upstream, midstream and downstream) of the rivers. The wastewaters were analysed as influent (untreated) and effluent (treated) samples.

4.2 Results and Discussion

Tables 4.1 – 4.3 show the mean result for the physicochemical parameters recorded while Appendices 1A and B show the same for the individual river and wastewater sample. Most of the samples from the upstream (BU, FU, SU and TU) of the rivers have low COD values which were below the detection level (Table 4.1). Generally, the midstream samples of the rivers show higher COD values than other reaches. Among river samples, the midstream samples of Bloukrans River (BM) had the highest mean value of COD with 331.31 mg/L followed by midstream Buffalo River samples (FM) with 256.5 mg/L COD. The midstream samples were collected immediately after the rivers pass through their respective major towns but before receiving wastewater effluents. The high values of COD of the midstream samples may be due to the contributions of wastes from the towns, adjacent land and open dump of refuse into the rivers (as observed during sampling). The lower values of COD at the downstream of the rivers might be due to the natural cleansing capacity of the rivers. King Williams Town influents (KW) had the highest mean COD of 2337.53 mg/L, followed by Grahamstown influents (GW) with 299.66 mg/L. The high value of COD observed for KE might be related to population density compared to other towns in this study. King Williams Town has a population of 227,000 (Department of Statistics 2019), which is the highest among the towns. However, no COD was detected for King Williams Town effluents (KE), while Grahamstown treated effluents had 4.67 mg/L. Except for GE and KE; all other wastewater effluents had mean COD above the recommended value of 30 mg/L (DWA Act No 991- 18 May 1984). Apart from the upstream samples, all other freshwater samples have oxygen demand above the recommended value for a healthy aquatic ecosystem. Agoro et al. (2018) observed COD range of 17-394 mg/L in their study of three WWTPs in Eastern Cape Province of South Africa, with the least values observed in the effluent samples. In another study, COD values between 14 and 20 mg/L were observed for effluents in the study of three WWTPs in the Eastern Cape while the recipient rivers had values between 7 and 15 mg/L (Osode, 2007). Edokpayi et al. (2015), in a study of peri-urban WWTPs, recorded COD of effluents range from 50 – 105 mg/L. Igbinosa and

Okoh (2009) recorded COD range between 36 and 238 mg/L for effluents in their studies of a WWTP in the Eastern Cape Province. The observations in this study favourably compare with the cited works.

Table 4.1: Concentrations of oxygen-related parameters and temperature of the samples (values are means ± standard deviation; ND = not detected)

Sample	COD (mg/L)	DO (mg/L)	ORP (mV)	Temp (^{O}C)
BU	6.30 ± 8.40	4.36 ± 1.66	88.41 ± 46.88	14.56 ± 2.68
BM	331.31 ± 16.34	3.97 ± 1.02	70.20 ± 16.45	15.26 ± 2.69
BD	141.81 ± 47.60	3.93 ± 1.30	66.13 ± 25.58	14.71 ± 3.28
FU	ND	2.63 ± 0.72	69.77 ± 7.31	14.08 ± 2.93
FM	256.5 ± 28.8	3.89 ± 0.93	77.32 ± 8.75	14.84 ± 3.92
FD	96.98 ± 24.66	3.92 ± 0.75	68.70 ± 11.93	14.90 ± 2.51
SU	17.12 ± 11.42	3.23 ± 2.01	123.65 ± 34.58	16.11 ± 2.58
SM	169.86 ± 37.86	3.59 ± 0.51	99.20 ± 14.73	17.59 ± 1.81
SD	94.17 ± 32.78	3.81 ± 1.00	85.10 ± 11.69	19.95 ± 2.26
TU	19.90 ± 26.53	3.87 ± 2.48	92.00 ± 21.62	9.56 ± 2.18
TM	122.36 ± 41.87	5.48 ± 1.11	80.84 ± 17.07	12.04 ± 0.05
TD	47.48 ± 19.84	6.73 ± 2.21	87.26 ± 5.70	14.80 ± 1.46
GW	299.66 ± 90.17	2.76 ± 1.60	-85.77 ± 11.09	19.31 ± 2.21
GE	3.11 ± 2.07	6.35 ± 1.18	87.14 ± 29.04	17.45 ± 2.9
KW	2337.53 ± 570.81	2.28 ± 0.09	-33.69 ± 23.52	17.08 ± 0.31
KE	ND	3.55 ± 0.47	121.00 ± 50.47	16.1 ± 1.31
UE	70.31 ± 10.31	4.04 ± 0.40	105.19 ± 16.22	18.86 ± 0.63
AW	286.84 ± 40.74	3.84 ± 0.52	-75.72 ± 15.89	17.0 ± 0.28
AE	125.99 ± 21.99	5.48 ± 0.52	129.88 ± 41.15	14.25 ± 0.60

Bloukrans River: BU (upstream), BM (midstream), BD (downstream). Buffalo River: FU (upstream), FM (midstream), FD (downstream). Swartkops River: SU (upstream), SM (midstream), SD (downstream). Tyhume River: TU (upstream), TM (midstream), TD (downstream). Grahamstown wastewater: GW (influent), GE (effluents). King Williams Town wastewater: KW (influents), KE (effluents). Alice wastewater: AW (influents), AE (effluents) Uitenhage wastewater UE (wastewater effluents).

All the river samples show positive values for oxidation-reduction potentials (ORP) (Table 4.1) with the highest values observed in the upstream reaches. Positive values indicate the oxidation potentials or the ability of the water to cleanse itself (Al-Samawi and Al-Hussaini, 2016). All the

wastewater influents had negative ORP values, showing reduction ability or high levels of oxygen-demanding compounds. The effluents had positive ORP values, indicating the removal or reduction of oxygen-demanding compounds from the wastewater during treatment.

The mean range of dissolved oxygen (DO) observed for the rivers in this study was 2.63 mg/L to 6.73 mg/L. (Table 4.1). The midstream and some downstream samples have higher values of dissolved oxygen than upstream samples. Oxygen reduces with altitude (Huang et al., 2017), and this might be the reason the upstream reaches did not have the highest concentrations of dissolved oxygen. Table 3.1 shows the altitudes of the sites. In the wastewater category, effluents show higher DO values than influents, an indication of a positive impact of aeration during treatment. Low levels of DO in rivers will cause severe stress in the aquatic ecosystem with reduced physiological activities of fishes and increased mortality when the DO fell below 9.5 mg/L (Sharma and Gupta, 2016; Huang et al., 2017). Igbinosa and Okoh (2009) observed DO values ranging from 4.15-11.22 mg/L in a study of Eastern Cape Rivers, Omole et al. (2016) recorded DO range from 5.42-6.98 mg/L, Fatoki et al. (2003) reported 2.70 – 3.60 mg/L and Mathebula (2015) observed a range of 7.26-12.21 mg/L. The results of DO obtained for freshwater in this study compare favourably with those reported by these workers.

The temperature observed for the rivers in this study ranges from 7.11 to 19.99 ^{o}C. The upstream reach of Tyhume River, with the least mean temperature, had plant canopy and located in high altitude (Table 3.1) of Hogsback Mountain, hence the low mean temperature of 9.56 ^{o}C observed. Temperature determines the water chemistry, and the physiology of the living things inside the water (Dallas and Ross-Gillespie, 2015; Grab, 2014), it also affects the solubility of oxygen and some compounds in water and determines the susceptibility of organisms to diseases and parasites (Bhateria and Jai, 2016). The extreme temperature may lead to migration or death of organisms since they prefer optimum conditions for survival. The temperature of the water may be influenced by the prevailing weather and the time of sampling. The water temperature will be higher in the afternoon than morning and winter than other seasons. DWAF (1996) recommends a maximum temperature of 25 ^{o}C for effluent discharge to the environment. The temperatures recorded in this research are within the permissible limit for effluent discharge into water bodies.

Tables 4.2 shows more results of the physicochemical measurement recorded in this work. Hydrogen ion concentration (pH) observed among the river samples ranged from 7.39 – 9.65 with midstream Swartkops River (SM) having the highest value. Appendix I shows the contribution of each water sample to the pH values. Swartkops River, at all reaches, shows higher pH values than other rivers. Uitenhage wastewater effluents (UE) had a high mean value of pH (9.25), which might have contributed to the elevated level of pH in downstream Swartkops River (SD). The observed pH values of the rivers did not show any variation with seasons. Appendix 1A shows upstream samples S1C, T1A, S1B and F1C with high pH values. These high values may be due to weathering rather than wastes dump. Some of the pH values were above the recommended values between 6.5 and 8.5 for aquatic life by DWAF (1996).

The salinity observed for the rivers, in this study was near zero as characteristic of freshwater. The midstream and downstream reaches of the Swartkops River had values above 1 mg/L. Salinity values above 0.5 mg/L in freshwater is an indication of pollution and salinization of the river (Lee et al., 2013). Grahamstown wastewater influents and Uitenhage wastewater effluents had salinity above 0.5 mg/L. Salinization of rivers has been attracting attention as a global problem stemming from irrigation, mining activities, de-icing salts and so on (Cañedo-Argüelles et al., 2013). Salinisation will lead to loss of species diversity and compromise the ecosystem.

Table 4.2: Concentrations of some physicochemical parameters in the samples (values are means ± standard deviation)

Sample	pH	Salinity (PSU)	Conductivity (µS/cm)	Turbidity (NTU)
BU	7.39 ± 0.42	0.15 ± 0.03	305.22 ± 62.96	2.25 ± 3.00
BM	8.06 ± 0.27	0.79 ± 0.06	1566.44 ± 109.92	214.24 ± 125.40
BD	7.61 ± 0.54	0.70 ± 0.07	1386.56 ± 127.26	40.18 ± 9.44
FU	7.91 ± 0.75	0.26 ± 0.10	540.89 ± 193.85	2.84 ± 3.79
FM	9.03 ± 0.79	0.245 ± 0.025	503.17 ± 48.5	22.49 ± 17.22
FD	7.67 ± 0.60	0.31 ± 0.03	757.11 ± 116.37	289.55 ± 340.30
SU	8.73 ± 1.75	0.09 ± 0.00	198.67 ± 3.56	0.00
SM	9.65 ± 0.97	1.50 ± 0.46	2857.50 ± 830.83	11.34 ± 11.34
SD	8.92 ± 1.20	1.46 ± 0.12	2806.56 ± 212.59	23.98 ± 0.32
TU	8.70 ± 1.33	0.03 ± 0.00	63.33 ± 3.11	22.59 ± 15.06
TM	9.35 ± 1.03	0.17 ± 0.02	350.67 ± 31.34	222.84 ± 222.84
TD	8.13 ± 0.37	0.18 ± 0.07	375.44 ± 133.92	125.00 ± 133.78
GW	7.63 ± 0.47	19.31 ± 2.21	1203.56 ± 155.63	539.45 ± 165.85
GE	7.65 ± 0.22	17.45 ± 2.90	661.33 ± 436.89	31.16 ± 4.75
KW	8.49 ± 0.57	17.08 ± 0.31	820.67 ± 53.67	642.34 ± 206.67
KE	8.16 ± 0.82	16.10 ± 1.35	483.50 ± 5.50	10.79 ± 10.79
UE	9.25 ± 0.99	18.86 ± 0.63	1093.11 ± 176.57	28.00 ± 4.24
AW	8.26 ± 0.87	17.00 ± 0.28	678.67 ± 18.34	492.17 ± 110.84
AE	8.05 ± 0.22	14.25 ± 0.60	474.67 ± 3.34	13.79 ± 13.79

Bloukrans River: BU (upstream), BM (midstream), BD (downstream). Buffalo River: FU (upstream), FM (midstream), FD (downstream). Swartkops River: SU (upstream), SM (midstream), SD (downstream). Tyhume River: TU (upstream), TM (midstream), TD (downstream). Grahamstown wastewater: GW (influent), GE (effluents). King Williams Town wastewater: KW (influents), KE (effluents). Alice wastewater: AW (influents), AE (effluents) Uitenhage wastewater UE (wastewater effluents).

Conductivity is a function of the dissolved salts; hence both parameters are related. Electrical conductivity was generally low in Tyhume River compared to others. Swartkops River had the highest EC, followed by Bloukrans. Upstream reaches of the rivers showed lower values of conductivity compared to other reaches. Wastewater influents had higher conductivity values than

their corresponding effluents. Grahamstown wastewater influents (GW) had a mean conductivity value of 1203 µS/cm while Uitenhage effluents had 1093.11 µS/cm. Matshakeni (2016) cited conductivity ranging from 0-4000 µS/cm in a review of some water quality parameters in South African rivers. Igbinosa and Okoh (2009) recorded EC range of 225.53 – 490.80 µS/cm for freshwater and 268.33 – 298.50 µS/cm for treated effluents. Edokpayi et al. (2015) recorded EC range of 320.30 – 1360.80 µS/cm for wastewater influents and 340 – 1250.3 µS/cm for effluents. DWAF recommended a maximum of 250 µS/cm EC for treated effluents before discharge to water bodies. The result obtained in this study shows higher values than the recommended level.

Turbidity values observed for upstream reaches of the rivers were closer to zero except for the upstream samples of Tyhume River (TU) with a mean of 22.59 NTU (Table 4.2). The downstream Buffalo River samples (FD) had the highest mean turbidity of 289.55 NTU, followed by midstream samples of Tyhume River (TM) with 222.84 NTU. The turbidity values of midstream and downstream reaches of the rivers were higher than 50 NTU recommended for agricultural and recreational purposes (DWAF, 1996). The turbidity values of the wastewater influents were expectedly high but mostly removed during treatment as reflected in the effluent values. The turbidity observed by Fatoki et al. (2003) range from 14.9 – 90 NTU and Edokpayi et al. (2015) observed 52.9 - 180.9 NTU for wastewater influents and 4.3 - 14.6 NTU for effluents. Dissolved and suspended materials, microorganisms, and organic materials in the water contribute to the turbidity of water and may intercept or absorb light waves. Turbidity determines light penetration into the water. Light penetration is vital in water bodies because it is the principal input in primary productivity. Photosynthesis of aquatic plants depends on sunlight penetration. Absorbed light energy will raise the water temperature above normal. Reducing the turbidity of wastewater before discharge is an essential aspect of water quality management.

Table 4.3 shows the concentrations of some chemical parameters of water quality. The downstream samples of the Swartkops River (SD) had the highest mean concentration of phosphate ions, with 19.27 mg/L. Phosphates were generally low in the river samples and below the limit of detection in upstream samples. In the wastewater category, Grahamstown influents (GW) had the highest mean concentration of phosphates (28.08 mg/L), followed by King Williams Town influents with 26.70 mg/L. The recommended level of phosphates in effluents is 5.0 mg/L (DWAF, 1996). The

mean concentrations observed for wastewater effluents in this research were higher than the recommended value. Matshakeni (2016) reported phosphate range of 0.0 – 200 mg/L from eight years study of Eerste River. Osode (2007) in a study of rivers in Buffalo city and Nkonkobe municipalities of Eastern Cape, reported phosphate concentrations ranging from 3.70 to 11.58 mg/L, and 0.07 to 4.81 mg/L was reported by Igbinosa and Okoh (2009). Edokpayi et al. (2015) reported a range of 0.552 – 4.646 mg/L for wastewater influents and 1.572 – 32.554 mg/L phosphate concentration for the effluents. The levels of phosphate in the midstream samples suggested other environmental sources. The WWTPs did not remove all the phosphates in the wastewater influents before releasing the effluents to the receiving rivers. An elevated phosphate level in the rivers will promote eutrophication (Matthews, 2014).

Table 4.3: Concentrations of some chemical parameters in the water samples (values are means ± standard deviation; ND = not detected)

Sample	PO_4^- (mg/L)	Cl^- (mg /L)	NH_4^+ (mg /L)	SO_4^- (mg /L)	NO_3^- (mg /L)	TDS (mg /L)
BU	ND	27.13 ± 6.31	20.38 ± 1.43	ND	ND	152.56 ± 31.41
BM	13.39 ± 7.47	215.49 ± 248.87	95.02 ± 23.23	174.71 ± 77.91	4.44 ± 1.85	782.89 ± 55.19
BD	9.33 ± 2.93	196.68 ± 227.12	88.17 ± 42.07	171.02 ± 134.74	5.0 ± 1.11	693.22 ± 63.70
FU	ND	15.02 ± 20.03	18.07 ± 2.45	ND	1.67 ± 2.22	275.89 ± 93.41
FM	ND	351.36± 10.04	18.69 ± 2.77	ND	10.83 ± 4.17	251.32 ± 24.34
FD	7.75 ± 2.24	380.85 ± 55.43	17.07 ± 1.86	ND	2.78 ± 2.59	319.56 ± 33.63
SU	0.08 ± 0.06	40.00 ± 53.34	28.39 ± 5.19	ND	1.11 ± 1.48	99.67 ± 1.78
SM	5.28 ± 2.88	274.23 ± 210.10	48.71 ± 15.58	36.45 ± 36.45	4.17 ± 2.5	1428.67 ± 415.34
SD	19.27 ± 5.72	179.03 ± 204.66	38.05 ± 3.82	ND	5.56 ± 1.85	1402.55 ± 106.15
TU	ND	39.07 ± 44.69	22.87 ± 3.02	ND	ND	32.00 ± 1.33
TM	1.90 ± 1.00	137.57 ± 84.51	32.71 ± 11.37	3.56 ± 3.56	3.33 ± 1.67	175.50 ± 15.50
TD	0.86 ± 0.57	146.81 ± 80.98	49.66 ± 29.96	57.88 ± 77.18	5.56 ± 1.85	188.11 ± 67.26
GW	28.08 ± 6.62	215.13 ± 253.21	63.21 ± 38.96	4.31 ± 1.91	3.89 ± 1.48	603.22 ± 76.74
GE	20.53 ± 3.29	201.19 ± 255.32	67.06 ± 15.06	48.69 ± 47.02	8.33 ± 4.44	330.56 ± 218.37
KW	26.70 ± 5.04	443.72 ± 10.42	114.33 ± 40.33	17.60 ± 15.46	3.33 ± 1.67	410.50 ± 26.50
KE	16.18 ± 0.43	354.21 ± 28.44	21.69 ± 7.07	2.17 ± 0.69	4.17 ± 4.17	241.84 ± 2.83
UE	25.83 ± 1.19	254.79 ± 224.78	94.72 ± 50.82	9.65 ± 7.06	5.83 ± 0.83	956.50 ± 128.17
AW	23.01 ± 0.26	155.33 ± 148.84	137.15 ± 11.98	5.04 ± 2.95	31.67 ± 11.67	339.34 ± 9.34
AE	12.43 ± 2.43	148.22 ± 100.15	51.85 ± 25.34	6.82 ± 6.57	30.83 ± 9.17	234.17 ± 4.83

Bloukrans River: BU (upstream), BM (midstream), BD (downstream). Buffalo River: FU (upstream), FM (midstream), FD (downstream). Swartkops River: SU (upstream), SM (midstream), SD (downstream). Tyhume River: TU (upstream), TM (midstream), TD (downstream). Grahamstown wastewater: GW (influent), GE (effluents). King Williams Town wastewater: KW (influents), KE (effluents). Alice wastewater: AW (influents), AE (effluents) Uitenhage wastewater UE (wastewater effluents).

Chloride ions concentrations in the river samples were highest in downstream Buffalo River (FD) with a mean of 380.85 mg/L, followed by the midstream samples (FM) with 351.36 mg/L. Chloride concentrations were low in the upstream samples (Table 4.3). Appendix IA shows the chloride concentrations in the individual sample. The level of chloride in the midstream samples was an indicator that non-point sources contributed to its presence in the rivers. King Williams Town influents (KW) had a mean concentration of 443.72 mg/L chloride and the effluents 354.21 mg/L. Grahamstown wastewater influents (GW) had a mean concentration of 322.70 mg/L chloride, and the effluents 301.79 mg/L. The results show little impact of the WWTPs at removing chloride ions from the wastewater, thereby contributing to their concentrations in the downstream of the receiving rivers. Fatoki et al. (2003) reported a maximum of 25.7 mg/L chloride concentration in the Keiskamma River. Edokpayi et al. (2015) reported a range of 18.933 – 51.972 mg/L chloride concentration in wastewater influents and 15.293 – 56.524 mg/L in the effluents. The result shows that chloride concentrations recorded in this study were higher than those reported above workers. The limit set by DWAF (1996) for chloride in wastewater effluents is 100 mg/L, but the observation from this study shows that the limit was exceeded in most samples. Huizenga (2011) observed that chloride and sulphate contamination are the main factors that characterise South African surface water chemistry. Uncontrolled levels of chloride in freshwater will promote mobilization of toxic metals from the soils and sediments. A high concentration of chloride causes the formation of soluble complex chlorides of heavy metals that it is often used to extract heavy metals from residues, known as brine leaching (Stec et al., 2020). Chloride ions will also cause reproduction impairment and mortality of aquatic organisms, alteration of the steady-state of the aquatic ecosystem, corrosion of pipes and taste problems (Muralikrishna and Manickam, 2017; Brandt et al., 2016; Hunt et al., 2012).

Ammonium ions were present in all the water samples, but the concentrations in the upstream samples of the rivers were lesser (Table 4.3). The highest mean concentration of ammonium was recorded in the midstream samples of Bloukrans River (BM) with 95.02 mg/L, followed by the downstream sample (BD) with 88.17 mg/L. In the wastewater category, Alice influents (AW) had the highest mean concentrations of ammonium, with 137.15 mg/L, followed by King Williams Town influents (KW) with 114.33 mg/L. Appendices 1A and 1B show the contributions of each sample to the concentrations in different reaches of the rivers and wastewaters respectively.

Mathebula (2015), in a 12-year study, observed an increasing trend of ammonium in rivers from 2 mg/ L to 10 mg/L over the years. Matshakeni (2016) observed ammonium concentration in the rivers in the range of 0-55 mg/L. The observed levels of ammonium in this study show higher values than those recorded by earlier workers. Ammonium is not toxic at pH near neutral but may convert to poisonous ammonia at pH above 11 (DWAF, 1996). Ammonium can exert oxygen demand on the aquatic ecosystem and transformed into nitrile ions, which in turn form ammonia (Du et al., 2017). Ammonia is toxic to fish and other aquatic animals, even at very low concentrations of 0.2 – 2.0 mg/L (Gupta et al., 2015; Du et al., 2017). In the event of ammonia pollution, fishes will lose equilibrium, increase in respiratory activities and oxygen intake, convulsion, coma and death (Oram, 2014).

Sulphate ions were below the detection limit in the upstream samples of the rivers (Table 4.3). The Bloukrans River had a high concentration of sulphate with a mean value of 174.71 mg/L in its midstream samples (BM) and 171.02 mg/L in the downstream (BD). The Bloukrans River passes through the centre of Grahamstown and Belmont valley where there are many farm settlements. Wastes were indiscriminately dumped into the Bloukrans River that made the water stink. Sulphate ions were below the detection limit in the Buffalo River. The safe limit of sulphate recommended for a healthy aquatic ecosystem is 100 mg/L (DWAF, 1996). Sulphates, in river sediments, may split under anaerobic conditions to form hydrogen sulphides, which produce obnoxious odour, thereby, render the water useless to man and fish (Kumar and Kumar, 2018).

Alice wastewater influents (AW) had the highest mean concentration of nitrates, with 31.67 mg/L (Table 4.3). Among the rivers, midstream Buffalo River sample (FM) had the highest mean nitrate concentration of 10.83 mg/L. The mean nitrate concentrations in Grahamstown (GE) and King Williams Town (KE) effluents were higher than the influents values. The observed higher values were contributed by the autumn and spring samples in both cases (Appendix I-B). The higher amounts of nitrates in the effluents might have resulted from contamination of the waste stabilisation ponds. Animal wastes are rich in nitrates and when allowed to graze around the waste stabilisation pond, may contaminate the effluents. Nitrate ions were not detected in the upstream samples. Matshakeni (2016) reported a range of 0-9 mg/L of nitrate ions in a project; Igbinosa and Okoh (2009) reported a range of 1.82 – 13.24 mg/L while Edokpayi et al. (2015) reported 0.499 –

2.31 mg/L for wastewater and 7.454 -19.413 mg/L for treated effluents. DWA (1996) recommends 15.0 mg/L of nitrates in effluents before releasing to the environment. No nitrite ion detected in the samples.

The midstream samples of Swartkops River (SM) had the highest mean concentration of dissolved solids (TDS) with 1428.67 mg/L, followed by its downstream samples (SD) with 1402.55 mg/L. The upstream samples of Tyhume River (TU) had the least amount of dissolved solids, with a mean of 32.0 mg /L. The wastewater effluents from Uitenhage were high in TDS with a mean value of 956.50 mg/L. High values of the physicochemical parameters in Uitenhage wastewater influents may be related to the industrial activities in the town. Although the researcher was not permitted to take the inflow samples, analyses of the outflow shows that the WWTP was unable to cope the volume and content of the wastewater. The high values of TDS in Swartkops River samples correlate with other parameters measured in this study. Dissolved solids are naturally present in environmental waters and may comprise organic materials, minerals from rocks and mining activities, and toxic metals (Weber-Scannell, 2007). Water bodies must dissolved solids moderately because a very low TDS may discourage some aquatic macrofauna (Olson and Hawkins, 2017) and, when high, poses a threat to aquatic ecosystem health (Cañedo-Argüelles et al., 2016). The DWAF recommended a maximum of 450 mg/L TDS for surface waters.

Table 4.4 shows the efficiency of WWTPs at removing some pollutants identified in this study. The efficiency was determined by the ratio of the concentration of each parameter in the effluent to to that of influent multiplied by hundred. The attribute best removed from wastewater was the COD but poorly removed in Alice WWTPs. Generally, chloride and nitrate ions were poorly removed in all the WWTPs. Sulphate ions were poorly removed at Grahamstown WWTP. The overall rating of the WWTPs showed that King Williams Town WWTP performed best among others in the removal of physicochemical attributes of wastewater while Grahamstown was least. The performance of the WWTPs may not be related to population or volume of the wastewater.

Table 4.4: Efficiency of WWTPs at removing some pollutants from the wastewaters

	Removal Efficiency (%)		
Parameter	Grahamstown WWTP	King Williams Town WWTP	Alice WWTP
COD	98.96	100	56.08
Phosphate	26.88	39.40	45.97
Chloride	6.48	20.17	4.58
Sulphate	6.10	81.03	62.19
Ammonium	NA	87.70	NA
Nitrate	0	0	2.65
TDS	45.20	41.09	30.99
Average performance	30.60	52.77	33.74

Table 4.5 shows the correlation statistics (r) for the samples as a measure of site correlation, indicating the similarity of the measured features. All the samples show positive correlation values with each other but at different degrees. Correlation values above 0.5 were strong, while below that is weak. Samples BM and BD had a correlation value above 0.9, while upstream samples BU and SU above 0.8. BU and TU were both upstream samples of Bloukrans and Tyhume Rivers, respectively, but with correlation just above 0.5. Tyhume midstream samples (TM) and Alice wastewater influents (AW) had a correlation value above 0.9, indicating a strong similarity of features. King Williams Town effluents (KE), and the downstream Buffalo River sample (FD) had a correlation value above 0.9. Alice effluents (AE), and Swartkops River upstream sample (SU) had a correlation value greater than 0.8. The correlation value between midstream Bloukrans River (BM) and King Williams Town wastewater (KW) was near zero, same with samples KW and BU. High correlation coefficients show similarities in the concentrations of the attributes under study. When a freshwater sample strongly correlated with wastewater or other samples, it means that their qualities were similar.

Table 4.5: Correlations coefficient of the sites (Marked correlations are significant at p < .05000)

Variable	BU	BM	BD	FU	FM	FD	SU	SM	SD	TU	TM	TD	GW	GE	KW	KE	AW	AE	UE
BU	1.000000																		
BM	0.933093	1.000000																	
BD	0.943619	0.986189	1.000000																
FU	0.987971	0.954842	0.963834	1.000000															
FM	0.773092	0.872636	0.843210	0.760567	1.000000														
FD	0.832057	0.925093	0.886568	0.836713	0.902518	1.000000													
SU	0.876693	0.798351	0.811017	0.809857	0.856664	0.819478	1.000000												
SM	0.968852	0.980288	0.984859	0.991157	0.813931	0.872280	0.799812	1.000000											
SD	0.968993	0.978134	0.981048	0.991548	0.812794	0.874843	0.799704	0.999647	1.000000										
TU	0.540118	0.475530	0.428606	0.426249	0.680998	0.563491	0.798326	0.411128	0.408996	1.000000									
TM	0.786254	0.879927	0.809659	0.784083	0.818791	0.956719	0.744680	0.806234	0.808585	0.613386	1.000000								
TD	0.841700	0.921282	0.926469	0.842268	0.799617	0.891524	0.785537	0.867294	0.859051	0.516027	0.863077	1.000000							
GW	0.819872	0.944144	0.890634	0.856764	0.825243	0.958317	0.682919	0.893356	0.895857	0.414830	0.954458	0.867249	1.000000						
GE	0.924251	0.941465	0.959160	0.922364	0.885805	0.925343	0.900790	0.941924	0.941938	0.515481	0.816732	0.895936	0.864286	1.000000					
KW	0.174419	0.400074	0.278047	0.186999	0.563606	0.410754	0.192385	0.259643	0.255346	0.468457	0.510553	0.309749	0.473340	0.191285	1.000000				
KE	0.842789	0.847369	0.865388	0.816968	0.903572	0.901857	0.937374	0.839798	0.841379	0.606950	0.772084	0.825241	0.777780	0.966995	0.169068	1.000000			
AW	0.676591	0.841733	0.756879	0.719620	0.714715	0.880222	0.525329	0.759713	0.762532	0.372274	0.938854	0.778504	0.960922	0.711579	0.574998	0.610255	1.000000		
AE	0.876590	0.921500	0.886822	0.862228	0.935856	0.854657	0.850385	0.886969	0.884568	0.700142	0.826306	0.809278	0.834776	0.860909	0.587798	0.815652	0.739131	1.000000	
UE	0.928870	0.942173	0.943154	0.945884	0.823080	0.841985	0.805407	0.956058	0.956385	0.446326	0.788079	0.828449	0.861978	0.922903	0.273764	0.843676	0.731366	0.870983	1.000000

Bloukrans River: BU (upstream), BM (midstream), BD (downstream). Buffalo River: FU (upstream), FM (midstream), FD (downstream). Swartkops River: SU (upstream), SM (midstream), SD (downstream). Tyhume River: TU (upstream), TM (midstream), TD (downstream). Grahamstown wastewater: GW (influent), GE (effluents). King Williams Town wastewater: KW (influents), KE (effluents). Alice wastewater: AW (influents), AE (effluents) Uitenhage wastewater UE (wastewater effluents).

Figure 4.1 shows the result of the hierarchical cluster analysis as a dendrogram. This analysis describes the closeness of the samples based on the parameters measured. Closely related samples occur on the same cluster. Ward algorithm was used in the clustering analysis with the distance expressed in Euclidean. There are four main clusters in the dendrogram. Samples SM and SD shared the same cluster. Wastewater samples clustered together with UE, BD and FD, but KW was alone. Other samples clustered together but upstream samples TU, BU and SU occupied a sub-cluster of the group. The clustering pattern reiterated the observation that some rivers samples were not of better quality than wastewaters.

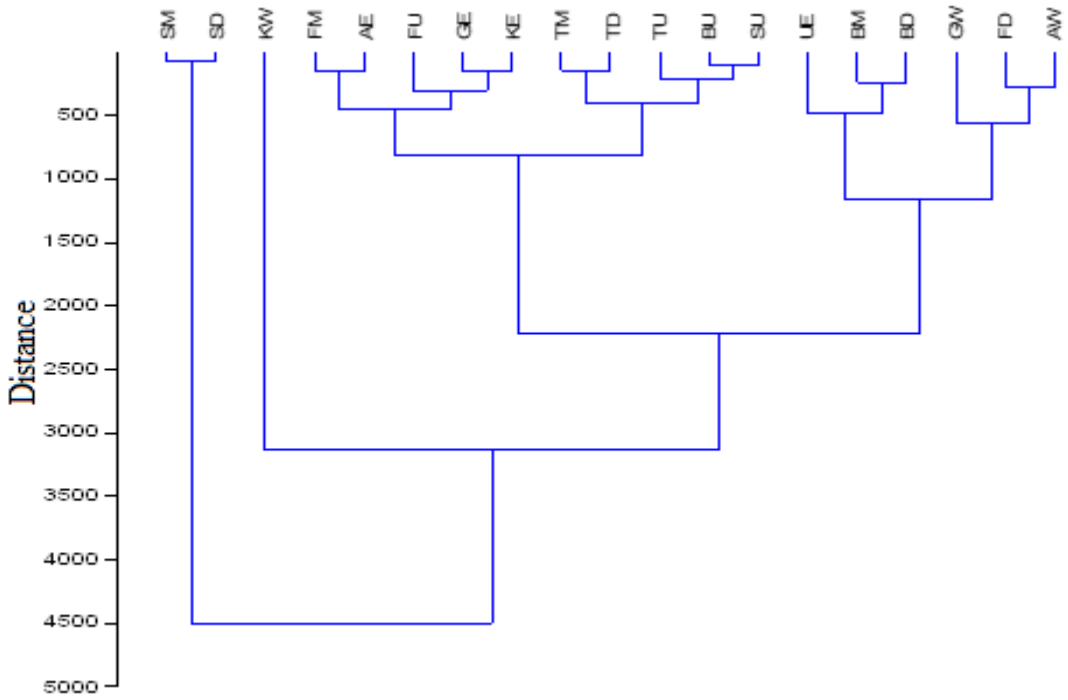


Figure 4.1: Hierarchical clustering of the samples shown as a dendrogram.

4.3 Conclusion

Based on the result of the physicochemical parameters studied in this work, the quality of the four rivers was generally poor, and the concentrations of the attributes were above the recommended values for agriculture and aquatic ecosystems. This study shows that some of the parameters investigated in the effluents have concentrations above the recommended levels, thereby influencing the physicochemical qualities downstream. Among the 33 freshwater samples, only

four had pH near neutral; the rest are at alkaline range. Most of the rivers, at lower reaches, show characteristics similar to the municipal wastewater near them. The WWTPs were unable to remove chloride and nitrate ions from the wastewater. On the average, King Williams Town WWTP performed better than the others in removal of physicochemical attributes of the wastewaters.

CHAPTER 5

INVESTIGATIONS OF THE CHEMICAL FUNCTIONAL GROUPS IN THE RIVERS AND WASTEWATERS

5.0 Introduction

This chapter focuses on the examination of chemical functional groups in the freshwater and wastewater samples. Thus, the section reports the analyses of water samples with data obtained from proton and carbon chemical shifts of ^{1}H-NMR and ^{13}C-NMR using nuclear magnetic resonance (NMR) and Fourier-transformed infrared (FT-IR) spectroscopies. The procedure for processing NMR chemical shifts and FT-IR absorption peaks were stated, and the spectra obtained analysed with the aid software and functional group tables. The chemical shifts of both ^{1}H-NMR and ^{13}C-NMR of the metabolites in the samples were compared with the standard chemical shift Table to determine the chemical composition of the samples. The same way FT-IR absorption peaks were analysed with appropriate software and compare with established functional groups absorption peaks Table. Conclusions were drawn based on the observations from NMR and FT-IR analyses.

5.1 Procedure

5.1.1 FT-IR analysis

Extracted samples were dried and analysed on Perkin Elmer 400 FT-IR spectrometer operated with Spectrum Quant (version 10.5.4) software mounted on Window XP. FT-IR absorption peaks of the samples were recorded at mid-IR radiation, between 4000 and 650 cm^{-1}. The IR spectra obtained were smoothened (smooth factor 2.00), subjected to baseline corrections and the peaks automatically labelled. The spectral were analysed with Knowitall software. The absorbance (A) was measured as the logarithm (base 10) of the reciprocal of the transmittance (T):

$A = \mathrm{Log}_{10}\left(\frac{1}{T}\right) = \mathrm{Log}_{10}\left(\frac{I0}{I}\right)$. Transmittance is the ratio between the intensities of the transmitted (I) and incident (I_0) beams (Moraes et al., 2008). Infrared Spectrum Tables obtained from Sigma-Aldrich (2018), BioRad software with Sadler's note (Bartels, 1978), Talari et al. (2017), Coates (2016), Socrates (2004) and Larkin (2011) were used to determine the functional

group absorption peaks. Data collected on spectrometer were saved as ASCII files for statistical analysis. Saved spectra were imported into Metaboanalyst 4.0 for multivariate analyses.

5.1.2 NMR analysis

^{1}H NMR and ^{13}C NMR chemical shifts of the water extract in $CDCl_3$ were acquired at 300 K on NMR spectrometer using a PULprog Zg30. For the ^{1}H-NMR, the spectra were obtained at 400.13 Hz by taking 16 scans without prior dummy scans, spectra width of 20.0254 ppm, receiver gain of 32 with time and frequency domain of 32767 and 262144 points, respectively, and acquisition time of 4.096 s. While the ^{13}C-NMR spectra were acquired at 100 MHz, taking 2068 scans without prior dummy scans, spectra width of 20.0254 ppm, receiver gain of 32 with time and frequency domain of 32767 and 262144 points, respectively, and acquisition time of 4.096 s.

The ^{1}H-NMR and ^{13}C-NMR spectra were processed and analysed using MestReNova 14. The NMR signals were calibrated with the chemical shift of the residual $CDCl_3$ signal at δ values of 7.26 ppm and 77.16 ppm for ^{1}H-NMR and ^{13}C-NMR respectively, relative to zero value of tetramethylsilane (TMS). The processing of the ^{1}H- and ^{13}C- NMR spectra involved phase correction by global algorithms, full automated baseline correction with Bernstein polynomials at degree 5, smoothing using Whittaker Smoother method at a normal mode, zero filling along t1 from 32768 (32k) to 65536 (64k) and normalized by the highest peak set at a value of 100. After that, the analysis of the spectra was carried out, including positive peak picking with a noise factor of 50 using an interactive default option and parabolic interpolation with a maximum number of peaks of 10000. Each of the ^{1}H- and ^{13}C-NMR spectra were stacked and aligned (to compensate for the intrinsic acidity of the samples).

5.2 Results and Discussion

5.2.1 Carbon (^{13}C) chemical shifts

Standard NMR chemical shifts Tables were consulted before arriving at point-by-point analysis shown in this result (Reich, 2019; Kennpohl et al., 2016; Silverstein et al., 1991). Table 5.1 shows the chemical shifts (δ) recorded for carbon 13 (^{13}C) in the samples, for freshwater samples and Table 5.2 for wastewater influents and effluents samples. The reference point (0 ppm) is the chemical shift of carbon in tetramethylsilane, [TMS or $(CH_3)_4Si$]. Common to all the samples are

^{13}C chemical shifts in primary alkyl (methyl group), secondary alkyl (methylene) and tertiary alkyl. Carbon chemical shifts as obtainable in alkenyl and aromatic rings were frequent on the spectra. Aromatic and alkene regions overlap to a significant extent. Signals from quaternary carbons (having no hydrogen) are usually quite weak hence may reduce the number of the visible carbon peaks, and the proton decoupling process gives rise to an enhancement that quaternary carbons do not experience (Gable, 2014).

Table 5.1: River samples with NMR chemical shifts of ^{13}C.

chemical shift (ppm)	**Type of Carbon**	**Sample**
2.0		B1B, T1A, T2B
10-30	R-CH3 Primary Alkyl (methyl)	B1A, B1B, B1C, B2B, B3A, B3B, B3C, F1A, S1A, S1B, S1C, S2A, S2B, S3C, T1A, T1B, T1C, T2A, T2B, T3A, T3C.
15-55	R-CH2- R Secondary alkyl (methylene)	B1B, B1C, B2A, B2B, B2C, B3B, B3C,F1A, F2B, S1A, S1B, S1C, S2A, S2B, S3C, T1B, T1C, T2A, T2B, T3C
20-60	R3C-H; CR_4 Tertiary or quaternary alkyl	B1B, B1C, B2A, B2C, F2B, S1A, S1B, S1C, S2A, S2B, T1B, T1C, T2A.
40-80	C- O Attached to oxygen	F2A
65-90	RC≡CR Alkynyl	F2A, S1A.
100-150	R2C=CR_2 Alkenyl	B1B, B1C, B2A, B2B, B2C, B3A, B3B, B3C, F1A, F2A, F2B, S1A, S1B, S1C, S2A, S2B, S3C, T1B, T1C, T2A, T2B,T3C.
110-170	 Aromatic (phenyl ring C)	B2A, B2B, B2C, B3A, B3B, B3C, F1A, F2A, F2B, S1A, S1B, S1C, S2A, S2B, S2C, S3C, T1B, T1C, T2A, T2B
165-185	RCOOH; RCOOR; $RCONH_2$ C=O, carboxylic acid, ester, amide	B2C, B3A, F2A.
185-220	ROR RCOH C=O, ketone or aldehyde	F2A

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring).

Chemical shifts of carbon bonded to oxygen (C-O) as obtainable in C-OH and C-OR were present in the midstream samples of Buffalo River, Grahamstown and King Williams Town wastewater influents and effluents. The characteristic chemical shifts of ^{13}C attached to oxygen (C=O) in carbonyl group (carboxylic acids, amides, esters) were observed in the midstream samples of Bloukrans and Buffalo Rivers, and also in Grahamstown, King Williams Town and Alice wastewater influents. Samples from midstream Buffalo River showed the shifts of ^{13}C-NMR in ketones and aldehydes.

Table 5.2 shows the wastewater samples with ^{13}C-NMR. The chemical shift around 2 ppm alludes to C-I because iodine tends to shift the carbon resonance closer to TMS. Sample A2B (Alice wastewater effluents) showed the ^{13}C-NMR shift of C-N bond. All the wastewater influent samples showed the chemical shifts of ^{13}C-NMR in C-Cl. Both chemical shifts (C-N and C-Cl) were not present in river samples.

Table 5.2: Wastewater samples with NMR chemical shifts of ^{13}C

chemical shift (ppm)	**Type of Carbon**	**Sample**
0.5-1	C-I	G2A, U1C.
10-30	R-CH3 Primary Alkyl (methyl)	A1B, A2B, A2C, G1A, G1B, G1C, G2B, K1B, K1C, K2B, U1C, U2C.
15-55	R-CH_2- R Secondary alkyl (methylene)	A1B, A1C, A2B, A2C, G1A, G1B, G1C, G2A, G2B, K1B, K1C, K2B, K2C, U1C, U2B.
20-60	R_3C-H; CR_4 Tertiary or quaternary alkyl	A1B, A1C, A2B, G1A, G1B, G1C, G2B, K1B, K1C, K2B, U1C, U2B, U2C.
40-60	C- N attached to nitrogen	A2B.
35-80	C- Cl attached to chlorine	A1C, G1A, K1B, K1C, K2B, U1C,
40-80	C- O attached to oxygen	A2B, G1A, G1B, G2B, K1B, K2B,
65-90	RC≡CR Alkynyl	K1B,
100-150	R_2C=CR_2 Alkenyl	A1B, A1C, A2B, A2C, G1B, G1C, G2A, K1B, K1C, K2B, U1C, U2B, U2C.
110-170	 Aromatic (phenyl ring C)	A1B, A1C, A2B, A2C, G1A, G1B, G1C, G2A, K1B, K1C, K2C, U1C, U2B, U2C.
165-185	RCOOH; RCOOR; $RCONH_2$ C=O, carboxylic acid, ester, amide	A1B, G1A, G1B, G1C, K1B, K1C.

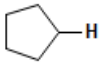
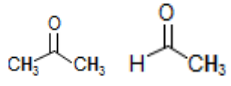
Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

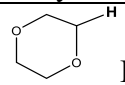
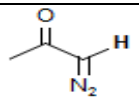
There are usually overlap in carbon chemical shifts, which make it not suitable for functional group analysis. Some other functional groups not listed might be present in the samples but hidden due to overlapping. Appendix III shows the spectra obtained from MestReNova analysis of the ^{13}C-NMR for the samples.

5.2.2 Proton (^{1}H-NMR) chemical shifts

Table 5.3 shows the ^{1}H-NMR chemical shifts of the functional groups in the river samples and Table 5.4 for wastewater samples. The reference point (0 ppm) is the chemical shifts for protons in tetramethylsilane (TMS). Proton chemical shifts below zero show absorption of higher energy than the TMS value. These shifts are present in a sample of midstream Buffalo River (F2A). Dimethylzinc [$(CH_3)_2Zn$)] is a synthetic compound from industrial processes and its presence in the water samples is an indication of industrial pollution (Stuhl et al., 2018).

Table 5.3: ^{1}H NMR chemical shifts (relative to TMS at δ=0) of freshwater samples. The shifting protons are bold.

δ (ppm)	Compound	Sample
-0.4	$(C\mathbf{H}_3)_2Zn$	F2A
0.1	Cyclopropane	B2A, B3A, F1A, F2A, S1A, S3B, T1A, T1B, T2B, T3A, T3B.
0.8-0.9	$(C\mathbf{H}_3)_4C$ $(C\mathbf{H}_3)_3CH$	B1A, B1B, B1C, B2A, B2B, B2C, B3A, B3C, F1A, F1C, F2A, F2B, F2C, F3A, F3C, S1A, S1B, S1C, S2A, S2B, S2C, S3B, S3C, T1A, T1B, T1C, T2B, T2C, T3A, T3B, T3C,
1.0-1.2	$C\mathbf{H}_3CH_2OH$ $(C\mathbf{H}_3CH_2)_2CO$ $(C\mathbf{H}_3)_2COH$	B1A, B2C, F1A, F1C, F2C, F3C, S2A, T1C, T2B, T3A, T3C,
1.2-1.3	$CH_3C\mathbf{H}_2CH_3$	B1A, B1B, B1C, B2A, B2B, B2C, B3A, B3C, F1C, F2B, F2C, F3A, F3C, S1A, S1B, S1C, S2A, S2B, S2C, S3B, S2C, T1A, T1B, T1C, T2B, T2C, T3A, T3B, T3C,
1.3-1.4	 $CH_2P(C\mathbf{H}_3)_3$	B1B, B1C, B2A, B2B, B2C, B3A, F2B, F2C, F3A, S1B, S1C S2A, S2B, S2C, S3B, T1A, T1B, T2B, T3A, T3B, T3C.
1.4-1.5		B2B, B2C, B3A, B3C, F2B, F3A, S2A, S2B, S2C, S3B, S3C, T2B, T2C, T3B,
1.5-1.69	Chlorinated alkane $(C\mathbf{H}_3)_3C-$ Cl	B2A, B2B, B2C, B3A, B3C, F1A, F2A, F2B, S2C, S3B, T1B, T2B, T3B, T3C
1.7-1.8	Brominated alkane $BrC(C\mathbf{H}_3)_3$; $BrCH_2C\mathbf{H}_3$,	B1A, B2A, F1A, F1C, S2C, S3C,
1.8-1.9	$C\mathbf{H}_3CH_2I$	B1A, B1B, B1C, B2C, B3A, B3C, F2C, F3C, S1C, T3A, T3C,
1.9-2	Propyne $\mathbf{H}C\equiv C$-Me $(\mathbf{H}C\equiv C)_2CH$	B1C, B2B, B2C, F1A, F2B, F3A, S1B, S1C, S2A, S2B, S2C, S3B, S3C, T1B, T1C, T2B, T2C, T3B,
1-4	RNH_2 amino	B2A, B2C, F2B, S2B,
2-2.05	Acetonitrile, methacrylonitrile $C\mathbf{H}_3$-C≡N	B1A, B1C, B1B, B2B, B2C, B3C, F1A, F1C, F2A, F2B, F3A, F3C, S1A, S2A, S2B, S2C, S3B, S3C, T1A, T1B, T1C, T2B, T2C, T3A, T3B, T3C,
2-2.2	 carbonyl compounds	B1A, B1C, B2A, B2C, B3A, B3C, F1A, F1C, F2B, F2C, F3A, S1B, S2B, S1C, S2C, S3B, S3C, T1B, T3B, T3C.
2.3-2.4	$\mathbf{H}C\equiv C\mathbf{H}$ acetylenic	B1C, B2B, B2C, F1A, F2B, F2C, F3C, F2C, F3C, S2C, T1A, T1C, T2C, T3A,

2.4-2.5	$(CH_3C\mathbf{H}_2)_2CO$ $(CH_3C\mathbf{H}_2)_3N$	B1C, B3A, F1A, F3C, S1A,
2.2-3	Ar–C–**H** benzylic	F1C, F2B, S1A, S1C, S2B, S2C, S3C, T1C, T3A,
2.7-2.8	$C\mathbf{H}_3Br$ bromides	B2C, B3A, F1C, S3C,
2.8-2.9	$(C\mathbf{H}_3)_2SO_2$	S3C,
3-3.1	$(C\mathbf{H}_3)_2CHCl$ chlorides	B1A, B2A,F1A, F1C,
3.3-4	**H**C–OH alcohols **H**C–OR ethers Alkyl halides	B1A, B1B, B1C, B2A, B2C, B3A, B3C, F1A, F2A, F2C, F3A, , S1A, S2A, S2B, S2C, T1A, T1C, T2B, T3A, T3B, T3C.
3.5	 Dioxane	B1C, F1A,
3.6-3.7	$BrC\mathbf{H}_2C\mathbf{H}_2Br$	B1B, B2C, F1A, F2C, F3C, S1A, S2B, S3B, S3C, T1A, T1B, T3A,
3.7-4.1	RCOO–CH esters	B1C, B2C, B3A, F1A, F3C, S1A, S1B, S1C, T1A, T2C, T3A,
4-4.5	**H**C–F fluorides	B2A, B2B, B2C, B3A, F1C, F2B, F2C, S1A, S1C, S2C, S3C, T3A, T3C,
4.9-5	$C\mathbf{H}_2Br_2$	F3A, S3C,
4.5-5.2	ArO**H** phenolic	B1B, B1C, B2A, B2B, B3C, F2A, F2B, F3A, S1B, S2A, S2B, S2C, S3B, S3C, T1A, T1B, T1C, T2B, T2C, T3B, T3C,
4.6-5.5	 vinylic	B1B, B1C , B2A, B2B, B2C, B3A, B3C, F1A, F2B, F3A, S1A, S1B, S1C, S2A, S2B, S2C, S3B, S3C, T1A, T1B, T1C, T2B, T2C, T3A, T3B, T3C,
5.0-5.1	$PhC\mathbf{H}_2Cl$ chlorides	F3A, S2C, B1B, B1C, B2B, B2C, B3C, F1A, F2B, S1B, S1C, S2A, S2B, S3B, T2B, T3B, T3C,
6.9-8.5	C=C**H** shift in heterocyclic compounds	B1B, B1C, B2A, B2C, B3A, B3C, F1A, F1A, F1C, F2A, F2B, F3A, S1B, S1C, S2B, S3B, S3C, T1B, T1C, T2B, T2C, T3A, T3B,

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring).

The chemical shift of hydrogen in cyclopropane was observed mainly in midstream and downstream samples of Tyhume River. Cyclopropane is used in the manufacturing of pyrethrins, quinolone antibiotics (e.g., ciproflaxin, sparfloxacin), and some other biomolecules (Heeb et al., 2011). Pyrethrin is widely used as insecticides and produced naturally by some plants (*Chrysanthemum cinerariaefolium and C. coccineum*), to protect against insects (Soderlund et al., 2002; Todd et al., 2003). Its sources in the samples may be from runoffs and waste-dump. Treated effluents might have contributed to its presence in the downstream samples because it was present in the corresponding wastewater influents and effluents (Table 5.4).

Between 0.8 ppm and 0.9 ppm, is the region of chemical shifts of protons in primary aliphatic alkanes. These shifts were common to all the samples. ^{1}H-NMR chemical shifts in carbonyl compounds between 1.0 ppm and 1.2 ppm were frequent in the midstream, downstream and few upstream samples. Some authors reported that carbonyl compounds enter the environment through various sources, including bacteria activities in the sediments, and that these compounds are widely used in industries as raw materials (Reuss et al., 2005; Kohlpaintner et al., 2008).

^{1}H-NMR chemical shifts in cyclic pentane and phosphine occurred between 1.3 ppm and 1.4 ppm. These compounds are members of organophosphates primarily used in pest control. Cyclic pentanes are raw materials in the manufacturing of synthetic resins, rubber adhesives and also as blowing agents in the production of polyurethane insulating foam for the lining of refrigerators and freezers because it is environment friendly than previously used compounds such as chlorofluorocarbon (CFC-11) (UNEP, 1994). The proton shifts in cyclopentane and cyclohexane were observed in most of the freshwater and wastewater samples.

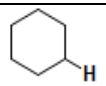
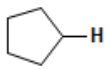
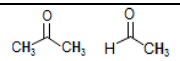
Proton chemical shifts of brominated primary aliphatic alkanes (1.7-1.8 ppm), appeared restricted to Bloukrans, Buffalo and Swartkops Rivers. Proton chemical shifts in secondary CH_2Br were present in the downstream samples of Buffalo (F3A) and Swartkops (S3C) Rivers. ^{1}H-NMR shifts in chlorinated primary aliphatic alkanes were present in Bloukrans and Buffalo upstream samples. Proton shifts in phenolic chlorides ($PhCH_2Cl$) were observed in all the river samples except Tyhume upstream samples. Proton shifts in CH_3CH_2I appeared restricted to the lower reaches of the rivers. Generally, halogenated primary alkanes have proton shifts between 1.5 ppm and 1.9

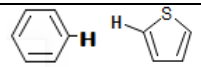
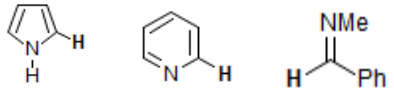
ppm. Pesticides are the primary source of halogenated compounds in the environment (Jeschke, 2017). Some biomolecules, for example, organochlorides, are natural sources of halogenated compounds (Raven, 2016).

Midstream samples of Bloukrans, Buffalo and Swartkops showed ^{1}H-NMR chemical shifts characteristic of amino-bonded alkanes (R-NH_2). Proton shifts in alkynes (1.9 - 2.4 ppm) were present in almost all the samples. Proton shifts in triethylamine (2.5 ppm) occurred in Bloukrans (B1C, B3A), Buffalo (F1A, F3C) and Swartkops (S1A) River samples. Proton shifts in Dioxane, an ether, occurred in Bloukrans (B1C) and Buffalo (F1A) upstream samples. Dioxane is used as a solvent for many applications and as a stabiliser for chlorinated hydrocarbons (Wisconsin Department of Health, 2013). Proton shifts in HC-OH were frequent among midstream, and downstream samples, as observed with FT-IR analysis. ^{1}H-NMR chemical shifts of vinylic and heteroaromatic compounds were common to the river samples. Heteroaromatic compounds are present naturally in organic molecules such as DNA, drugs and cellulose (Kowalski et al., 2018) from where they get into the water (see FT-IR absorption peaks).

Table 5.4 shows the ^{1}H-NMR chemical shifts of compounds in wastewater samples. Sources of some of the compounds were as discussed under the freshwater analysis above. ^{1}H-NMR chemical shifts below 1 ppm for cyclopropane and CH_3 (alkyl compounds) were present in most of the samples. Proton shifts in cyclopropane compounds occurred more in wastewater influent than effluent samples.

Table 5.4: ^{1}H NMR chemical shifts (relative to TMS at δ=0) of wastewater samples. The shifting proton is bold.

δ, ppm	Description	sample
0.1	Cyclopropane	A1C, A2C, G1A, G1B, G2A, K1B, K1C, K2B, K2C.
0.8-0.9	$(C\mathbf{H}_3)_4C$ $(C\mathbf{H}_3)_3C$	A1B, A1C, A2B, A2C, G1A, G1B, G1C, G2A, G2B, G2C, K1B, K1C, K2B, K2C, U1C, U2B, U2C.
1.0-1.2	$C\mathbf{H}_3CH_2OH$; $(C\mathbf{H}_3)_2COH$ $(C\mathbf{H}_3CH_2)_2CO$	A1C, G1A, G1C, G2A, G2B, G2C, K1B, K1C, K2C, U1C,
1.2-1.3	$CH_3C\mathbf{H}_2CH_3$	A1B, A1C, A2B, A2C, G1A, G1B, G1C, G2A, G2B, G2C, K1B, K1C, K2B, K2C, U1C, U2B, U2C.
1.3-1.4	 $CH_2P(C\mathbf{H}_3)_3$	A1B, A1C, A2B, A2C, G1A, G1B, G1C, G2A, G2B, G2C, K1B, K1C, K2B, U1C, U2B, U2C.
1.4-1.5		A1B, A1C, A2C, G2B, G2C, K1B, K1C, K2B, U1C, U2B, U2C.
1.5-1.69	Chlorinated alkane $(C\mathbf{H}_3)_3C$- Cl	A1B, A2B, A2C, G1A, G1B, G1C, G2A, G2B, G2C, K1B, K1C, K2B, U1C, U2B, U2C.
1.7-1.8	Brominated alkane $BrC(C\mathbf{H}_3)_3$; $BrCH_2C\mathbf{H}_3$,	G2B, G2C,
1.8-1.9	$C\mathbf{H}_3CH_2I$	G1A, G1C, G2A, K1C, K2C,
1.9-2	Propyne $\mathbf{H}C{\equiv}C$-Me; $(\mathbf{H}C{\equiv}C)_2CH$	A2B, A2C, G1A, G1B, G1C, G2A, G2B, G2C, K1B, K1C, K2B, U1C, U2C.
1-4	RNH_2 Amine	A1B, A1C, G1A, G1B, G1C, G2B, G2C, U1C.
2-2.05	Acetonitrile, methacrylonitrile $C\mathbf{H}_3$-C≡N	A1B, A1C, A2B, A2C, G1A, G1B, G1C, G2A, G2C, K1C, K2B, U1C, U2B, U2C.
2-2.2	 carbonyl compounds	AIB, A2B, A2C, G1A, G1B, G1C, K1B, K1C, K2B, K2C, U1C, U2B,
2.3-2.4	$\mathbf{H}C{\equiv}C\mathbf{H}$ acetylenic	A1C, G1C, G2A, K2C,
2.4-2.5	$(CH_3C\mathbf{H}_2)_2CO$ $(CH_3C\mathbf{H}_2)_3N$	A1B, A1C, A2C, G1B, G2A, G2B, K1B, K1C, K2B, K2C, U1C, U2C.
2.2-3	Ar–C–**H** benzylic	A1C, A2B, G1B, G2A, G2B, G2C,
2.7-2.8	$C\mathbf{H}_3Br$	G1A, G1C, K1B, K1C.

2.9-3	**H**C≡C-Ph	K2C,
3.3-3.5	PhCOC≡C**H**	U2B,
3.6-3.7	Br$C\mathbf{H}_2C\mathbf{H}_2$Br	G1A, G1C, G2A, G2C, K1C, K2B, K2C.
3.4-4	$C\mathbf{H}_2$: Alkyl halides, Alcohols, Ethers	GS, KS, A1B, A1C, A2B, A2C, G1A, G1B, G2A, G2B, K1C, K2B, K2C, U1C, U2B, U2C.
4-4.1	$MeCOOC\mathbf{H}_2CH_3$ $(CH_3)_2C\mathbf{H}Cl$	GS, KS, A2C, G1A, G1B, G2A, G2B, K2C, U1C,
4-4.5	$RC\mathbf{H}_2OH$	GS, KS, AS, A2C, G1B, G2A, G2B, G2C, K1B, U1C, U2C.
4.5-5	$PhC\mathbf{H}_2Cl$; $C\mathbf{H}_2Br$ $PhCH=C\mathbf{H}_2$	A1B, A2B, A2C, G1B, G2C, K1B, K2B, U2B, U2C.
5.0-5.1	$PhC\mathbf{H}_2Cl$	A1B, A2B, A2C, G1B,
5.3-5.5	$RCH=CH_2$ $(CH_3O)_2C\mathbf{H}_2$	A1B, A1C, A2B, A2C, G1A, G1B, G1C, G2A, G2B, G2C, K1B, K1C, K2B, U1C, U2B, U2C.
5.32	$C\mathbf{H}_2Cl_2$	G1A, G1B.
6.2-6.7	$RC\mathbf{H}=CH_2$	A1B, A2B, G1A, G1B, G2A, K1B, K2B, K2C, U1C, U2B, U2C.
5.5-7.5	Phenolic compounds	KSB, A1B, A2B, A2C, G1B, G2A, G2B, G2C, K2B, U1C.
7-7.2		A1B, A2B, G1A, G1B, G2B, G2C, KS, K1B, K1C, K2B, K2C, U1C, U2B, U2C.
7.4-7.9	Furan Naphthalene Methenamine Imidazole	A1B, A2B, G1A, G1B, G1C, G2B, K1B, K2B. U2C.
8.1-8.7		G2A

Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring), King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring), Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring), Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring)

The wastewaters and their treated effluents show proton shifts in cyclic pentane and phosphine (1.3-1.4 ppm). Proton shifts in brominated alkanes were only present in Grahamstown wastewater effluent samples (G2B and G2C) while alkyl iodide was present in Grahamstown (G1A, G1C, and G2A) and King Williams Town (K1C and K2C) samples. Brominated alkanes are common disinfectants in water treatments (Nalepa and Shelton, 2003). Reports show that some bromides (e.g. brominated trihalomethane) are environmental carcinogens (WHO, 2018). Alkyl halides are widely used in industries for the production of refrigerants, propellants, fire retardants and drugs (Gal et al., 2016) from where they enter into the environment. Proton shifts of amino bonded alkanes were present in wastewater influents and effluents samples. Also, ^{1}H-NMR chemical shifts in alkynes (1.9 - 2.4 ppm) were present in almost all the samples. Proton shifts in triethylamine (2.5 ppm) occurred in nearly all wastewater influent samples.

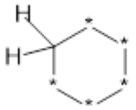
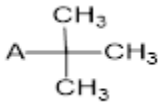
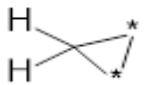
^{1}H-NMR chemical shifts of acetonitrile and methacrylonitrile (2 - 2.05 ppm) were common to all the samples. Acetonitrile is a by-product of methacrylonitrile with various uses as analytical materials in laboratories (e.g. LC-MS), battery production, as solvents in pharmaceuticals and photographic films. Methacrylonitrile is essential in the preparation of amides, amines and plastics among other uses. Proton shifts similar to **H**C=C**H** in furan, imidazole and methenamine were observed in some wastewater influent and effluent samples. These compounds are components of various drugs, which constitute a group of emerging contaminants in the surface waters.

5.2.3 FT-IR spectroscopy

Appendices III A - Q are the spectral peaks for all the samples. The functional group region (4000-1500 cm^{-1}) of the spectra was similar except the differences in the intensity between 1800 and 1500 cm^{-1}. Characteristically all the spectra show uniqueness at the fingerprint region (1500-650 cm^{-1}). Table 5.5 shows the samples that exhibited absorption peaks similar to alkane group bonds. Alkane bonds are prevalent among organic compounds and therefore are not very useful in determining structures in IR spectroscopy (Bartels, 1978). The spectra of cyclic alkanes of five or more ring carbons show ring CH_2 stretching frequencies, which overlap those of CH_3 and CH_2 groups of their alkyl substituents (Bartels, 1978). These frequencies also overlap those of the CH_3 and CH_2 stretching of acyclic alkanes. Numerous authors noted that the spectral region of 2800-2600 cm^{-1} confirms the presence of saturated simple ring structures (Talari et al., 2017; Coates,

2016; Larkin, 2011). Absorption at this region consists of a weak band or bands whose pattern and locations help confirm or indicate the presence of these rings. Although such absorption features have a limited diagnostic value, it is most reliable when the absorption occurs in the spectra of simple saturated aliphatic hydrocarbons. Alkane peaks around 1380 - 1375 cm^{-1} were limited to Grahamstown and Bloukrans samples. C-H vibrations in cyclohexyl, 920 - 880 cm^{-1} were present in all the spectra samples, suggesting that they might have been broken down due to microbial activities. Alkanes are common in organic compounds. Their presence in the water samples is due to organic sources such as microbial products, decayed organic matter, aquatic plant products, industrial and household wastes, oils, fossil fuels, and natural gas, among others (Guo and Fang, 2012). Natural waters may not be free of alkanes due to their various sources.

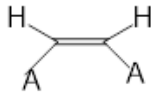
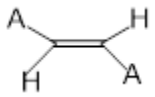
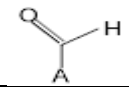
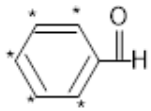
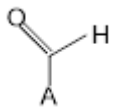
Table 5.5: Samples with absorption peaks similar to the alkane group

Peaks(cm^{-1})	Intensity	Functional group and bond	Sample
3000-2900	strong	 C-H vibrations in cyclohexyl *any attachment	All samples
2936-2916	strong	C-H vibrations in normal alkanes ($A\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}C^*$) A=any element except for H; *any attachment	All samples
2863-2843	strong		
1490-1430	variable	C-H vibrations in $R\text{-}CH_3$ (R= any element except H)	All samples
1485-1445	medium	As in 2936- 2916 cm^{-1} above	All samples
1380-1375	medium-weak	C-H vibrations in R-CH	Bloukrans and Grahamstown samples
1350-1320	weak	Stretching deformation of C-H in alkanes $(R)_3CH$ R= any attachment except H	G1B, A1B, K1C.
1258-1200	medium	Skeletal vibrations of C-C in branched alkanes. $C\text{-}(CH_3)_3$  A= any element except H	B2B, B3B, B2C, F3B, T1A, T1B, A1B, G1C, G1B, K1B, K1C, K2B, K2C.
1048-1000	medium	 Ring deformation vibration in cyclopropyl alkanes *any attachment	B1A, B2A, B3A, G2B, K2C.
920-880	Medium-strong	C-H vibrations in cyclohexyl	All samples except.
750-720	medium	C-C vibrations in normal alkanes	B1A

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

Table 5.6 shows the samples with absorption peaks related to alkenes, alkynes and aldehyde bonds. The C=C stretching vibration of alkene molecules absorbs very weakly, if at all, in the infrared region and, always, difficult to detect especially in the trans isomers and the tetrasubstituted C=C linkages (Bartels, 1978). When two or more olefinic groups occur in the hydrocarbon molecule, the infrared absorption spectrum shows the additive and combined absorption of the unsaturated groups (Bartels, 1978). If the unsaturated groups are subject to conjugation, the C=C stretching frequency, usually, is lowered and splitting of the C=C stretching frequency band occurs. Conjugation also intensifies the C=C stretching frequency of trans unsaturated groups. Alkene group absorption peaks in the samples were shown in Table 5.6. These peaks were limited to wastewater influents and effluents, with rare presence in midstream, and downstream samples of the rivers. Peaks 1662-1631 cm^{-1} (vibrations of C=C bond in vinylidene) occur mainly in wastewater effluents and rarely in influents (only in Alice sample A1B). Alkenes in water originated from hydrocarbon pollutants, mostly from plastic products and plastic industries. Some algae, especially members of chrysophytes and diatoms, can produce certain alkenes in the aquatic environment by enzymatic breakdown of polyunsaturated fatty acids (Satchwill et al., 2007). Alkenes are toxic and carcinogenic pollutants, which may enter into the food through packaging materials. Acute or chronic exposure to some alkenes can cause significant damages to organs and systems (Zhang et al., 2016). The vinylidene peaks in the samples might be due to pollutants, biotic, and abiotic degradation of polyvinylidene products in the industrial and municipal wastewaters (Benson, 2003). Their presence in treated effluents shows that they were not effectively removed from wastewater during treatment; this will harm the organisms in the receiving water bodies.

Table 5.6: Samples with absorption peaks similar to alkenes, alkynes and aldehydes groups.

Peaks (cm^{-1})	Intensity	Functional group and bond	Sample
Alkenes Peaks			
1662-1631	weak	 C=C bond stretching vibrations in asymmetric substituted alkenes (vinylidene) A=any attachment but not H	B2A, K2B, K2C, A1B, A2B, A2C, U2B.
1310-1295	Variable-weak	 C-H in-plane deformation in disubstituted alkenes RCH=CHR A=any attachment but not H	B2C, G1C, G1B, K1B, K1C, A1B.
980-960	weak		F3C
Alkyne Peaks			
2260-2190	weak	C≡C stretching vibrations in disubstituted alkynes (RC≡CR') and C≡N in nitriles.	B2C, B3C, F2C, F3C, T2C, T3C, U2C.
2165-2110	weak	C≡C stretching vibration in monosubstituted Alkynes (RC≡CH)	G1C, G1B, GS, G2A, G2B, K2C.
Aldehydes Peaks			
1740-1720	Weak-medium	 C=O stretching vibration in saturated aliphatic aldehydes. A=any element except H	B2A, A2B, K1B, K2B.
1715-1685	strong	 C=O stretching vibrations in Aryl aldehydes (Ph-CHO) *any attachment	B1A, B1B, A1B, A1C, AS, A2C, G1B, G1C, G1A, G2A, G2B, K1B, K1C, KS, K2B, K2C, U1D, U2B.
1210-1150	medium	Stretching vibration of C-C bond in aromatic aldehydes (as above).	B1A, B2A, B2B, B3B, B1C, B2C, B3C, A1B.
980-780	Weak-medium	 C-H deformation vibration in aliphatic aldehydes R-CHO A=any element except H	B2C, B3C, G1A, G1C, GS, F2C, F3C, S1C, S2C, S3C, T2C, T3C.

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

Alkyne group peaks observed on the spectra were limited to downstream rivers samples, wastewater influents and effluents (Table 3.4). Few numbers of alkyne peaks found in this study may be due to symmetrical substitution of the alkyne, and if the internal alkyne is symmetrical, the peaks may be absent (Bryan, 1999). Monosubstituted alkynes were present mainly in Grahamstown samples. Their absence in the upstream reach of the river might be an indication that they are associated with pollution. Alkynes are present in some drugs such as Efavirenz (an antiretroviral), calicheamicin (antitumour), antifungal terbinafine, and they are also an essential component of both the natural and synthetic hormone oestradiol (Walker et al., 1992; Stevenson et al., 2019). Most oral contraceptives contain synthetic oestradiol, which may be passed to household wastewaters through urines. The presence of this functional group in the treated effluent samples might be an indication of inadequate treatment. Since this functional group is associated with emerging contaminants in water, they will affect the physiological processes of organisms that depend on these rivers, especially at midstream and downstream.

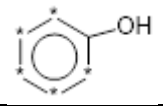
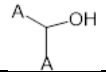
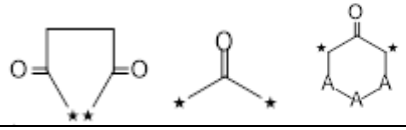
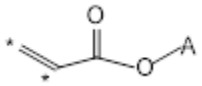
Aldehydes, ketone, ester, carboxylic acids and amides are characterised by C=O bond. Carbonyl stretching has a strong absorption on IR and very useful in structure determination (Bartels, 1978). Table 5.2 shows the peaks identified with aldehydes in the samples. The commonest of the aldehydes identified is aryl. Aryl compounds are present in some products such as dyes in textile industries, cosmetics and various drugs such as antibacterial, antiviral and antifungal pharmaceuticals (Ali et al., 2018). Their sources in water are from various wastes. Saturated aliphatic aldehydes are present in wastewater and treated effluents. The aliphatic aldehydes with C8 – C10 are common in natural products such as fruits, honey of different floral origin, oils, mushrooms, coffee and other products (Cullere et al., 2011; Lopez-Galilea et al., 2006). They are common in wines (Cullere et al., 2011). Their sources in the water samples are from industrial, household and agricultural wastes. Aryl aldehydes peaks were present in wastewater effluents, an indication of partial or non- removal. Aliphatic aldehydes were present mainly in the midstream and downstream reaches of the rivers but not in treated effluents, indicating sources other than wastewater effluents.

Table 5.7 shows the samples with alcohol, ketone and ester absorption peaks. Most of the peaks characterising alcohol vibrations were not common in spectra of upstream river samples except C-O vibrations observed in some upper samples. They were present mainly in the spectra of wastewater influents and effluents samples. Peaks 1390-1330 cm^{-1} (OH deformation) appeared limited to samples from the midstream reach of the rivers. Peaks 1260-1180 cm^{-1} occurred only in river samples. Alcohol groups are present in wastewater, which may be due to the fermentation of organic substrates by microorganisms (Silva-Bedoya et al., 2016). Their presence in the treated effluent samples is an indication of poor treatment of the wastewater.

Ketone peaks are typical of carbonyl groups especially ketones and aldehydes, but in IR absorption of a single compound, aldehyde has a broad impurity absorption between 2700 cm^{-1} and 2800 cm^{-1} but not ketone (Smith, 2017). Alcohols have broad absorption between 4000 cm^{-1} and 3000 cm^{-1} but do not have carbonyl double bond peaks. Since the samples in this work are mixtures, they may contain ketones. The peaks from 1725-1705 cm^{-1} (saturated aliphatic open-chain ketones) are present in the spectra of freshwater samples only but not in wastewater or treated effluents. Ketones are common as sugars (e.g. fructose), generated in the body and sometimes pass out with urine (Grabacka et al., 2016; Ho et al., 2019). They have many applications as solvents in chemical industries, laboratories, rubber, paint and perfumes manufacturing, printing and pesticides from where they find their ways to the environment (William, 2019).

Aliphatic and olefinic esters show strong stretching vibrations of the C-O bond between 1300 cm^{-1} and 1160 cm^{-1} and usually with one or more weaker peaks in the region from 1300 cm^{-1} to 1000 cm^{-1}. Formates have peaks near 1185 cm^{-1}, acetates near 1256 cm^{-1}, propionates near 1194 cm^{-1} (Bartels, 1978). Table 5.7 shows the samples with ester group peaks. The strong peak around 1705 cm^{-1} is alluded to C=O bond stretching in various compounds and not only esters. It may also apply to ketones and others with such bond. It is common to all the samples in different intensities.

Table 5.7: Samples showing absorption peaks similar to alcohols, ketones and esters

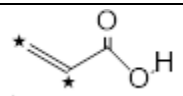
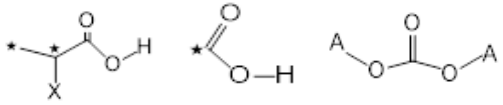
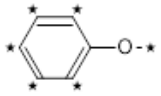
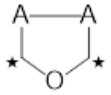
Peaks (cm^{-1})	Intensity	Functional group and bond	Sample
Alcohol Peaks			
3400-3200	variable	-OH vibrations in alcohols R-CH_2-OH	A2C, A2B, G2B, B2A, B3A, K2C, S1A, S3A, S1B, U1D, U2B, U2C, U1C.
1480-1410	medium	As above	T2B, T3B, T1C, T3C, U1D, U2B, U2C, A1C
1390-1330	medium	 OH deformation in alcohols (phenols). Mostly double peaks. *any attachment	B1A, K2B, A2B, A2C, U2B.
1260-1180	strong	C-O bond vibrations in alcohols (phenols) (structure as above)	B1C, FIA, F1B, F3A, F3B, S1A, S3A, S1B, S2B, S3B, S3C, T1A T1B, T2B, T3B, T1C.
1125-1090	medium	 C-O bond vibrations in alcohols $(R)_2CH$-OH A=any attachment except H	B1A, B2A, U1D.
1075-1000	strong	C-O bond vibrations in RCH_2OH	F2C, B3A, T1A, T2B.
Ketone Peaks			
1725-1705	Variable-strong	C=O stretching vibrations in saturated aliphatic open-chain ketones (C-(C=O)-CH; ketones C-(C=O)-C and 6-7C ring ketones.  *any attachment A=any element except H	B3B, B3A, B2A, B1A, B1C, B2C, B3C, F1A, F3A, F3B, F3C, S1A, S3A, S2B, S3B, S2C, S3C, T1A, T2B, T1C, T3C.
Ester Peaks			
1740-1715	strong	 Stretching vibrations of C=O in aliphatic and olefinic esters; peak near 1720 is for α and β unsaturated acids. A=any element except H; *any attachment	B3B, B3A, B2A, B1A, B1C, B2C, B3C, F1A, F3A, F3B, F3C, S1A, S3A, S2B, S3B, S2C, S3C, T1A, T2B, T1C, T3C.
1300-1160	strong	C-O-C stretching vibration in aliphatic and olefinic esters.	B2A

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

Table 5.8 shows the samples with absorption peaks related to carboxylic acids and ethers. Carboxylic acids are characterised with O-H stretch, a weak and broad-spectrum between 3200 cm^{-1} and 2500 cm^{-1}. Since this is the same region of the strong C-H stretching vibrations of alkyl and aromatic compounds, they always obscure O-H stretch (Bartels, 1978). O-H peaks were not reported in this region in this work, but other complementary peaks of carbo-acids were listed. C-O stretching and OH in-plane deformation, coupled, near 1430 cm^{-1} and around 1300 cm^{-1} (two bands). Carbo-acid absorption peaks observed in this study were present in Grahamstown and Swartkops River samples. Carboxylic acids enter the environment from various sources. They are essential constituents of foods such as fruits and vegetables and used as food preservatives, flavours and antioxidants (Badea and Radu, 2018). They are present in various metabolic pathways in animals, various pharmaceuticals and personal care products (Kalgutkar and Daniels, 2010; Lukic et al., 2016). They are constituents of atmospheric aerosol, which is one of their sources in the surface waters (Mkoma et al., 2014). They are the dominant organic acids in the ambient air of many cities and contribute to the acidity of rainwater (Guo et al., 2015). Other sources include the burning of fossil fuels, photochemical oxidation of organic compounds, industrial and household wastes (Guo et al., 2015). Their presence in the upstream samples, in this work, might be due to plant products in the rivers, the upstream reach of Swartkops River, for example, had a lot of algae growth and the upstream of Bloukrans River had diverse aquatic macrophytes.

The diagnostic peaks for ethers are C-O vibrations. Ethers have double C-O bonds in their structures. Heterocyclic ethers appeared to be associated with river samples at all courses (Table 5.8). Ethers are ingredients in flame-retardants and present in many consumer products like furniture upholstery, car, television sets and other household products. Ethers had been isolated in foods, household dust, human serum and milk (Czerska et al., 2012). Ethers are raw materials in the production of insecticides, fumigants and medicine as anaesthetics and pain relievers (Wade, 2019). Their applications in many products have made them available in the environment, especially in air and water as pollutants (Pan et al., 2018).

Table 5.8: Samples with absorption peaks related to carbo-acids and ethers.

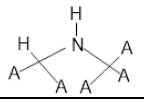
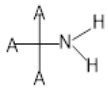
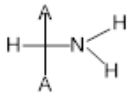
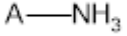
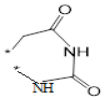
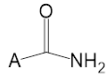
Peaks (cm^{-1})	Intensity	Functional group and bond	Sample
Carbo-acids Peaks			
1725-1700	strong	 C=O stretching vibration in saturated aliphatic open chain carboxylic acids, C=C-COOH *any attachment	B1A, B1C, G2A, G2B, S1A, S3A, S3B.
1440-1395	weak	Coupled C-O stretching vibration and O-H deformation vibrations: C-O stretching and OH in-plane deformation, coupled, around 1430 cm^{-1} and 1300 cm^{-1} (two bands)	B1A, B1C, G2A, G2B, S1A, S3A, S3B
1320-1211	medium	C-O stretching vibration of various carboxyl groups: C-CX-COOH; COOH;  R-OCOOR X=halogen *any attachment A=any element except H	B1A, B1C, G2A, G2B, S1A, S3A, S3B
960-875	weak	O-H out-f-plane deformation, H-bonded in dimerised acids.	B1A, B1C, G2A, G2B, S1A, S3A, S3B
Ethers Peaks			
1310-1210	Variable-strong	 =C-O-C stretching vibrations in aromatic ethers, usually from O-atom attached to C- atom of the aromatic ring. *any attachment	B2A
1050-1010	strong	Symmetric stretching of C-O-C in the five-membered ring of aromatic ethers, usually near 1050 cm^{-1}. See structure below.	B1A, S1A, AS, A1C, A2C.
940-860	medium	 C-O-C symmetric stretching vibrations in heterocyclic ethers (5-membered rings). A=any element except H; *any attachment	B1C, B2C, B3B, B3C, F1A, F1B, F3A, F3B, S1B, S3A, S2B, S3B, T1B, T1C, T2B, T3B, T3C.
850-810	Medium-strong	 C-O-C symmetric stretching vibrations in aromatic ethers (3-membered rings). A=any element except H	B1A, B2A, S1A.

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

Table 5.9 shows the samples with absorption peaks for amines and amides. Primary amines usually have two strong peaks at 3400 and 3300 cm^{-1} while secondary has only ones, and tertiary none (Bartels, 1978). The samples had no amine peaks above 3000cm^{-1}. N-H bond absorption peaks appear around 1600-1500 cm^{-1} for primary and secondary amines, and around 1400 cm^{-1} and 1300 cm^{-1} for all amines (Coates, 2016). Most of the amine peaks were present in wastewater influents, midstream and downstream rivers samples. Aliphatic secondary amine peaks were present in the Bloukrans River samples for winter. Amine salt peaks appeared restricted to Grahamstown and King Williams Town wastewater influent samples. Aromatic amines are raw materials in the manufacturing of chemicals such as pesticides, dyes such as aniline, pharmaceuticals, cosmetics, rubber and textiles (Ferraz et al., 2012). Bacterial decarboxylation of amino acids in proteins, decaying plants, and animals will produce biogenic amines in contaminated water (Poste et al., 2014). Amines are natural components of many organic molecules such as histamine, dopamine, and some hormones such as epinephrine and norepinephrine; they may be available in urines (Poste et al., 2014). Amines enter the environment through natural processes, wastewater, and industrial sources. Tertiary amines are resistant to degradation and hence persist in the environment (Eide-Haugmo et al., 2009). Some amines such as aniline, aminophenols, naphthylamines and chloroanilines are carcinogenic pollutants (Ferraz et al., 2012). The distribution of the absorption peaks suggests that amines in the water samples are pollution indicators.

Amide group combines the features of amines and ketones because of the presence of both C=O and N-H bond. They show strong to medium peaks around 3200 cm^{-1} for N-H and another peak around 1710 cm^{-1} for C=O bonds (Bryan, 1999). Table 5.9 shows the absorption peaks of amides on the spectra. Hydantoin amide peaks are not present in the upstream river samples. Their presence in wastewaters influents, midstream and downstream river samples is an indication that they are associated with pollution. Their presence in wastewater effluents suggests the inability of WWTPs to remove them effectively from the influents. Vibrations of C-N bond in primary amides were limited to effluents. Amides are present as structural components in materials such as nylons; they serve as heat-resistant and fire retardants in synthetic fibres.

Table 5.9: Samples with absorption peaks related to amines and amides.

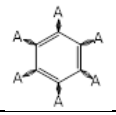
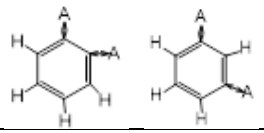
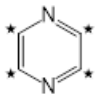
Peaks(cm^{-1})	Intensity	Functional group and bond	Sample
Amines			
1625-1585	Variable-weak	 In-plane N-H deformation vibration in aliphatic secondary amines. A=any element except H	B1A, B2A
1240-1170; 1038-1022	medium-weak	 C-N stretching vibrations in aliphatic primary amines. A=any element except H	G1A, G1C, G1B, G2A, G2B, K1B, K2B, K1C, K2C, A1C, U2B.
1191-1171	weak	C-N stretching vibrations in aliphatic secondary amines. (structure as in peak 1625-1585)	B2B
1140-1080	medium-weak	 C-N stretching vibrations in aliphatic primary amines. $(R)_2CH-NH_2$ A=any element except H	G1C, G1B, G2A, G2B, G2C and K1B.
1043-1037	weak		B2A, B2B, B3C, GIB, G2A, A1C, A2C, T2B, T3C, K2B, K2C, F3C, S3A, S3B
850-750	weak	 NH_3 rocking vibrations in primary amines salts. A=any element except H	G1A, G1C, G2A, G2B, K1B, K1C.
1305-1200	Medium-weak	NH bending vibrations in amine salts	B2B
Amides Peaks			
3300-3100	medium	 NH bond stretching vibrations in hydantoin amides. *any attachment	B2C, B3C, G2C, F2C, F3C, S2C, S3C, T2C, T3C, A1C, A2C, K1C, K2C, U2C.
1725-1705	Variable-strong	C=O stretching vibrations in saturated aliphatic open-chain ketones (C-(C=O)-CH; ketones C-(C=O)-C and 6-7C ring ketones.	B3B, B3A, B2A, B1A, B1C, B2C, B3C, F1A, F3A, F3B, F3C, S1A, S3A, S2B, S3B, S2C, S3C, T1A, T2B, T1C, T3C.
1680-1630	strong	C=O stretching vibrations in amides; R-CO-NH-C (See 1570-1515)	S1B
1420-1400	medium	 C-N stretching vibrations in primary amides R-CO-NH_2 A=any element except H	A2C, K2B, K2C.

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

Amides are present in many drugs, such as paracetamol, penicillin, and lysergic acid diethylamide (LSD), among others (Zumstein and Helbling, 2019; Scott and Njardarson, 2019). Amides are also present as organic molecules in living things. The presence of hydantoin amide peaks in wastewater and treated effluents samples is an indication of pollution with biocidal agents such as pesticides (Rai and Jayakrishnan, 2018). The presence of these peaks in treated effluent samples A2C, K2C and U2C is an indication that the amides were not effectively removed from the influents. Vibrations of C-N in primary amides were limited to wastewater effluents. Amides and amines are micropollutants that enter into surface and wastewater from various sources (Gulde et al., 2016; Zumstein and Helbling, 2019).

Table 5.10 shows the samples with absorption peaks related to various substituted aromatic compounds. P-disubstituted, 1,3,5- trisubstituted and 1,2,4-trisubstituted benzene appeared to be associated with wastewaters. Peaks at 1290-1250 cm^{-1} (ortho- and meta- disubstituted benzene) and 1105-1065 cm^{-1} were present river samples. Sources of these compounds in wastewater include, but not limited to, dyes (natural and synthetic), organic molecules such as DNA, drugs and cellulose (Talari et al., 2017; Kowalski et al., 2018). Their presence in the treated effluents samples is an indication of incomplete removal during treatment.

Table 5.10: Samples with absorption peaks related to substituted aromatic compounds

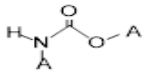
Peaks (cm^{-1})	Intensity	Functional group and bond	Sample
1525-1470	variable	 C-C stretching vibration in pentasubstituted benzene. A= any element except H	B1A, B2A, K2B, K2C, U1D, U2B, U2C
1290-1250 1105-1065	weak	 In-plane CH- bending vibrations in o- and m-disubstituted benzene. A= any element except H	B1B, B1C, B2B, B3B, B2C, B3C, F1A, F1B, S2B, S3B, T1B, T1C, T2B, T3B
1270-1250 1275-1255	weak	In-plane bending vibrations of C-H bond in p-disubstituted and 1,3,5-trisubstituted benzene.	G1B, G2A, GS, K1B, K1C, K2B, A1B, A2C, U2B.
1220-1200	weak	1,2,4- trisubstituted benzene	G1B.
1150-1110	weak	H in-plane bending vibration in disubstituted benzene	B3B
1085-1065 1030-1010	weak	In-plane bending vibrations of H in 1,2,3 trisubstituted benzene	B2A, G1A, G1C, K1C, U2B.
1050-1020	medium	In-plane bending vibrations of H in o- disubstituted benzene	B2A
1040-995	weak	In-plane bending vibration of H in 1,2,5-trisubstituted benzene.	A2B, B2B, B2A, B3A, B1C, T1A, T2B.
1010-990	weak	In-plane bending vibration of H in m-substituted benzene.	G1A, K2B, U2B, U2C
995-985	weak	H- in-plane bending vibrations in disubstituted benzene.	G1B, B1B, B3B, B1C, B3C F1A, F1B, F3B, F3A, F3C, S3A, S2B, S3B, S1C, S3C, T1B, T2B, T3B, T3C
950-925	weak	In-plane bending vibration of H in 1,2,4-trisubstituted benzene.	B2B, G2A, K1C, F3C.
720-690	medium	Out-of-plane deformation of C-H bond in 1,2,4-trisubstituted benzene	A1B, A1C, A2C.
710-680	weak	Ring deformation vibration: ring deformation, pentasub710-695 cm^{-1}.	B2A, K2B
1500-1400	strong	 C=C and C=N stretching vibration in heteroaromatics *any attachment	U2C

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

Table 5.11 shows the absorption peaks of urethanes and halogens. Urethanes are products of isocyanates, non-isocyanates and polyether, polyester or caprolactone glycol (Kim et al., 2016). They serve as monomers in the production of polyurethanes used in the manufacturing of various products, including foam. In addition to the peak at 1225 cm^{-1}, they are characterised with C=O stretching vibrations at 1744-1739 cm^{-1} (strong intensity), vinyl and phenyl esters near 1770 cm^{-1}, esters of α and β unsaturated acids near 1720 cm^{-1} (Bartels, 1978). Table 2.13 shows the peaks identified with urethanes in the water samples. The peaks were present in the midstream and downstream samples of Bloukrans River (B2B, B3B) but not in the upstream or the corresponding Grahamstown wastewater influents and effluents. Urethanes might have entered into the river through non-point sources. It was present in King Williams Town wastewater influents and effluents (K1C, K2C) and Buffalo River downstream samples.

Halogen peaks were present mainly among the wastewater effluents, few influents and downstream rivers samples (Table 5.11). It was present in Alice wastewater influents but not in the effluents probably due to the effect of waste stabilisation pond, where further degradation of compounds takes place before releasing the effluents into the environment. Most treated effluents contain halogen peaks probably because of chlorination of wastewater during treatment. They were likely to have entered the watercourse through effluent discharge. They can serve as a pollution index in streams since chlorine is toxic to aquatic organisms (da Costa et al., 2014; Mattingley, 2017). Absorption peaks related to C-F bond (organoflourides) were present in Grahamstown wastewater influents (G1A), Swartkops (S3A, S3B, and S3C) and Bloukrans (B3A) Rivers downstream samples. Organoflourides have many uses such as herbicides, pesticides, foams, refrigerants and propellants from where they found their ways to water bodies and serve as pollutants with health implications (Khanna and Nag, 2019). The halogen peaks reported here may not be exhaustive of the actual peaks that should be available because of the problems explained by Bartels (1978). The infrared absorption spectra of the carbon-halogen bond (C-X) are not always distinct. The difficulty of locating and recognising absorption bands that arise from the C-X bonds may be due to the following factors:

Table 5.11: Samples with Absorption peaks related to urethanes and halogen.

Peaks (cm^{-1})	Intensity	Functional group and bond	Sample
Urethane Peaks			
1265-1200	medium	N-C-O stretching vibrations in urethanes (carbamate esters) R-NHCOOR.  A= any element except H	B2B, B3B, S3A, S1B, K1C, K2C.
1740-1680	strong	C=O stretching in many compounds with C=O bond.	B2B, B3B, S3A, S1B, K1C, K2C
1540-1530	Medium-strong	Deformation of CHN in R-NHCOOR.	S1B
Halogen Peaks			
1300-900	Variable-strong	C-F stretching vibration in fluorinated aliphatic hydrocarbons	G1A, B3A,
1280-1120	Variable-strong	Stretching vibration of CF_2 in halogen compounds	U2B, U2C, S3A, S3B, S3C, K2B, K2C
830-600	strong	C-Cl stretching vibrations in chlorinated aliphatic hydrocarbons	G2C

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

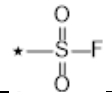
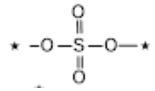
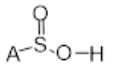
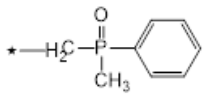
1. A variety of C-X bonds exist,
2. The C-X bonds are subject to considerable alteration in vibrational frequency through interaction with neighbouring groups,
3. Different conformational isomers have different stretching frequencies,
4. Equatorial and axial C-X bonds of ring structures have different stretching frequencies,
5. Multiple absorption bands related to the C-X bonds usually occur in the infrared spectrum.

Because of these factors, the C-X bonds do not always possess a constant vibrational frequency, nor do they always have unique absorption band features (Bartels, 1978). Elemental analysis is the most reliable proof of halogen in a compound (Bartels, 1978).

Table 5.12 shows the samples with sulphur and phosphorus peaks present in the sample spectra. Sulphur peaks observed were associated mainly with treated effluents and few wastewater samples. The peaks were present in the spectra of midstream and downstream river samples. Sulphur compounds might have entered into the river bodies through run-offs and wastewater effluents. Sulphur is present in amino acids, some pharmaceuticals and personal care products and agrochemicals from where they find their ways to water bodies through wastewater and runoffs (Faleye et al., 2017; Kebede et al., 2019) They are indicators of pollution in water bodies.

The spectra of phosphorus-containing functional groups vibrate at the same wavenumbers for different compounds. For example, peaks 910-900 cm^{-1} are present in ethers P-O-C, (O=P)-O-C in esters, P-OH in alcohols, (O=P)-OH in carbo-acids and P=S in Sulphur Compounds. Table 5.12 shows the observed phosphate group peaks among the samples. While P-CH_3 bond peaks appeared in all the river samples, P=S appeared limited to wastewater influent and effluent samples. Compounds with P=S bonds are components of insecticides such as acephate, malathion, dementon-S (Lim and Bolstad, 2019; Ali et al., 2012; Lai et al., 1995). Such compounds are additives in the production of plastics and as plasticisers (Li et al., 2019). They enter the environment, especially water, during production and usage (Khan et al., 2016; Lee et al., 2016). Other organophosphates in water may come from different sources such as microbial activities during decomposition of organic matter (Richardson and Simpson, 2011).

Table 5.12: Samples with absorption peaks related to Sulphur and Phosphorus.

Peaks(cm^{-1})	Intensity	Functional group and bond	Sample
Sulphur Peaks			
1415-1390	strong	 SO_2 asymmetric stretching in sulphonyl halides. *any attachment	T3C, G2A and A2C
1200-1187	strong	 S=O asymmetric stretching in sulphur compounds CO-SO_2-OC *any attachment	B2A, B2B, B2C, T3C, GIC, K1C, A2C, U2C
1090-990	strong	 S=O - stretching vibrations in sulphinic acids (-SO-OH) A= any attachment except H	B2B, B3A, G2B.
870-810	Strong-medium	S-O stretching vibrations in sulphonic acids. –SO-OH	B2A, B3A
700-600	Variable-weak	S-C stretching vibrations in sulphates	A1C
Phosphorus Peaks			
910-900	medium-weak	 P-CH_3 rocking vibrations in phosphorus compounds. *any attachment	B3B, B1C, B2C, B3C, F1A, F1B, F3A, F3B, S3A, S1B, S2B, S3B, T1B, T2B, T3B, T1C, T3C.
840-810	medium	 PH_2 wagging vibration in phosphorus compounds, R-PH. A= any element except H	B1A
800-580	variable	P=S stretching vibrations in phosphorus compounds	G1A, G1C, G1B, G2A, G2B, G2C, K1B, K1C, K2B, K2C, A2B, U2C.

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

Multivariate analyses are tools to properly analyse, classify and authenticate each water sample based on the composition of organic (metabolites profile) or chemical fingerprints. Such tools can help to trace samples from different sources (Messai et al., 2016). Binned FT-IR spectra data were arranged in rows and the features in columns before uploading to *MetaboAnalyst* 4.0. The uploaded file contains 51 samples with 3351 (spectra bins) data matrix. All the peak intensities loaded have positive values. The samples were not filtered, and there were no missing values in the loaded data.

Sample normalisation was according to the method of Dieterle et al. (2006). The procedure for normalisation was row-wise, by sample median. Generalised log transformation (glog 2) was adopted for a better comparison of the features. Pareto (mean-centred and divided by the square root of the standard deviation of each variable) was used for data scaling (Dieterle et al., 2006). Figure 5.1 shows the result of data normalisation with the boxplots showing 50 features due to space limitations. The density plots were based on all the samples. The chart on the left shows the features before normalisation and that on the right after normalisation.

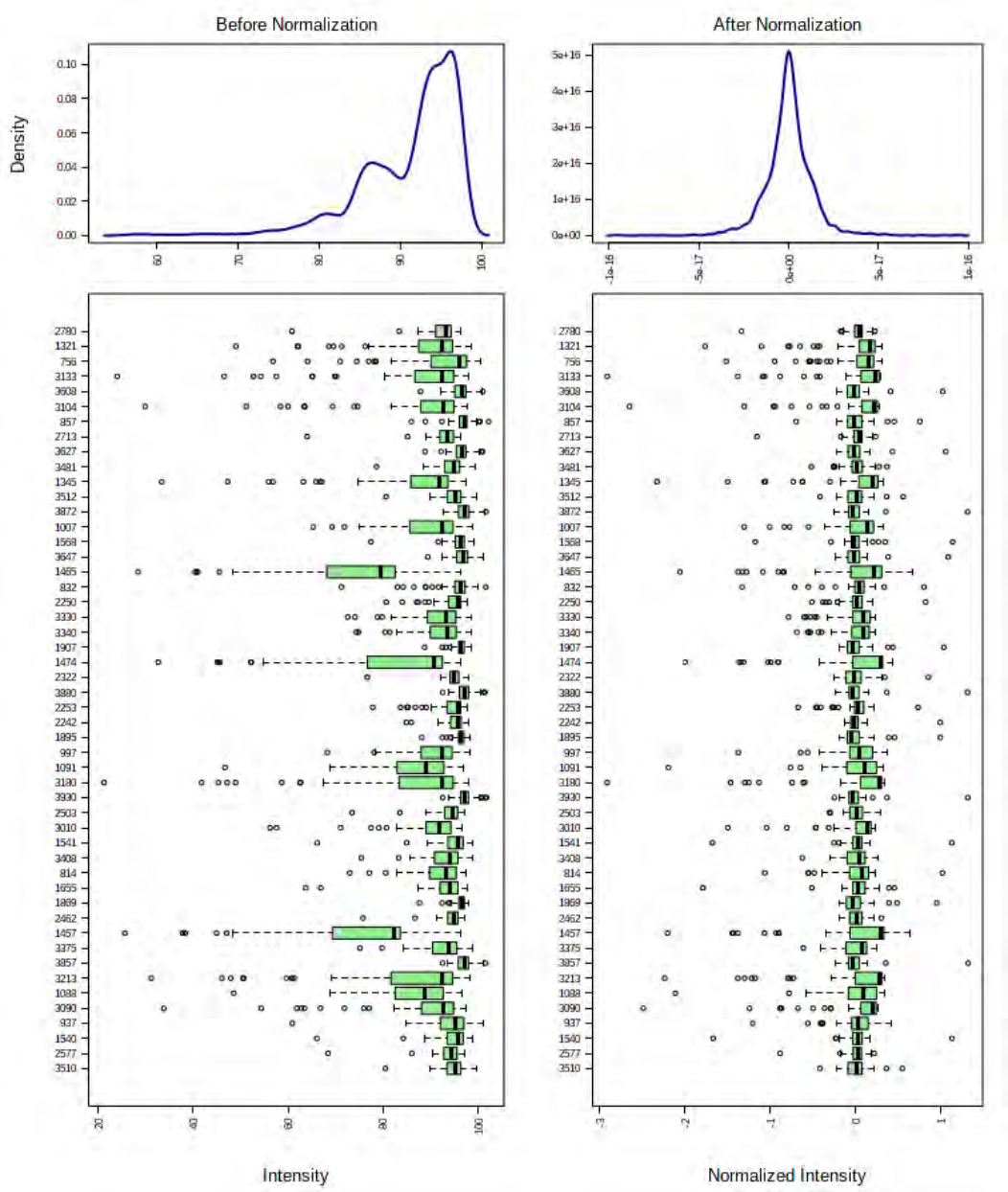


Figure 5.1: Box plots and kernel density plots before and after normalisation.

Figure 5.2 shows the result of correlation analysis, as a heatmap for the samples, samples with positively correlated features shown in brown, negative correlation in blue and white coloured samples were not correlated.

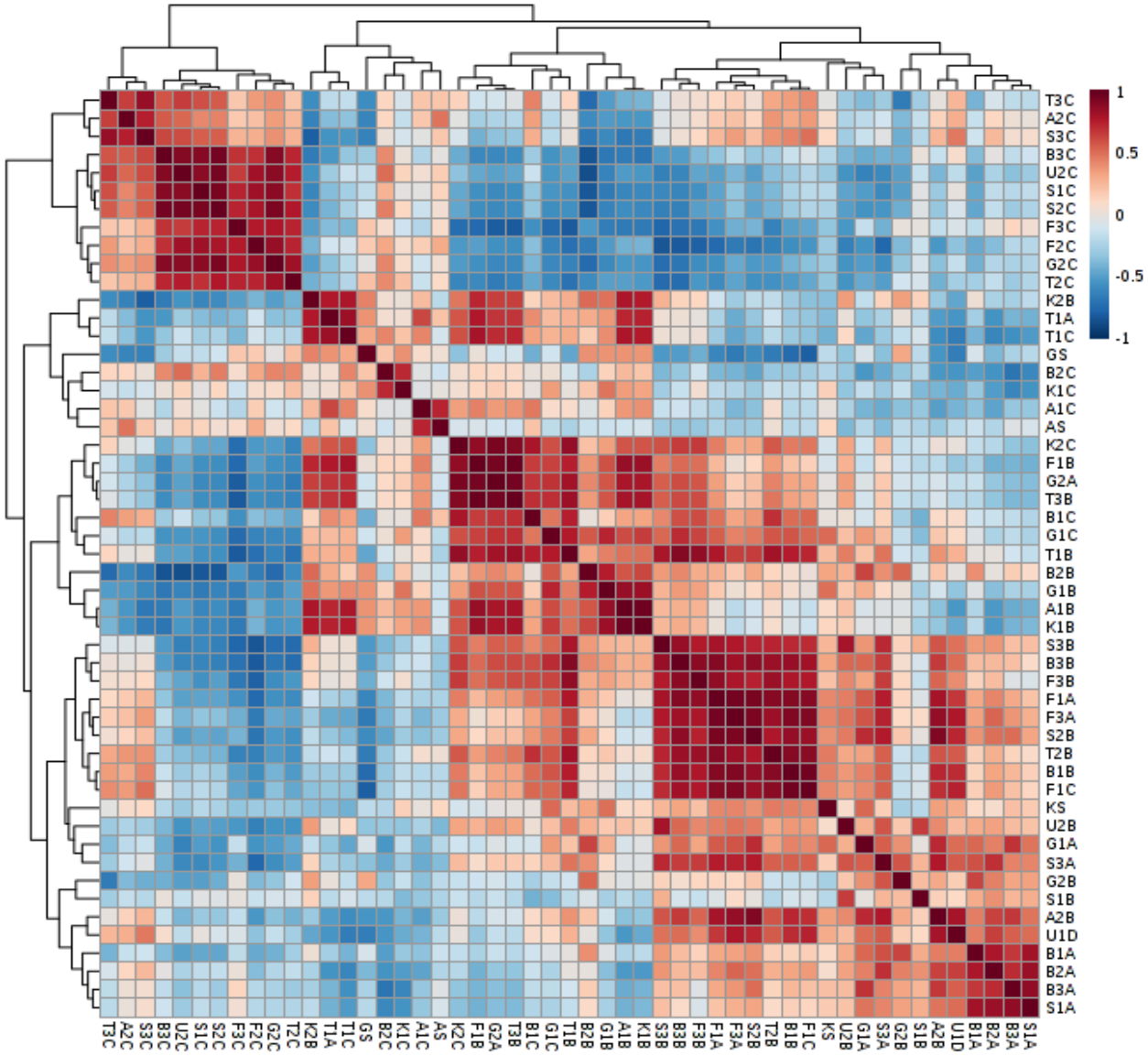


Figure 5.2: Correlation Heatmaps of the samples. Samples in brown colour are positively correlated.

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

The results of the ordination of the spectral features with the principal component analysis (PCA) were shown in Figures 5.3 and 5.4. PCA summarises the data in fewer variables called scores, which are weighted average (loadings) of the original variables (Chong and Xia, 2018). It is a multivariate tool to observe trends graphically, guided by correlation of components (Messai et al., 2016). The uniqueness of each water sample is due to the organic compound constituents, which vary in ranges and trends according to geographical origin. Ordination methods, including PCA, can show these differences. Figure 5.3 shows the scree plot for the principal components with their eigenvalue. The line at the top (green) shows the cumulative variance while the line passing through the bottom (blue) shows the variance explained by individual PC. The scree plot shows that 44.7% of the components fall within the first index and 79.6% within the first three. At a cumulative index of 79.6%, the first three factors (PC index 1- 3) were able to explain the variability of the spectra, meaning that normalisation has not removed the essential features of the spectral.

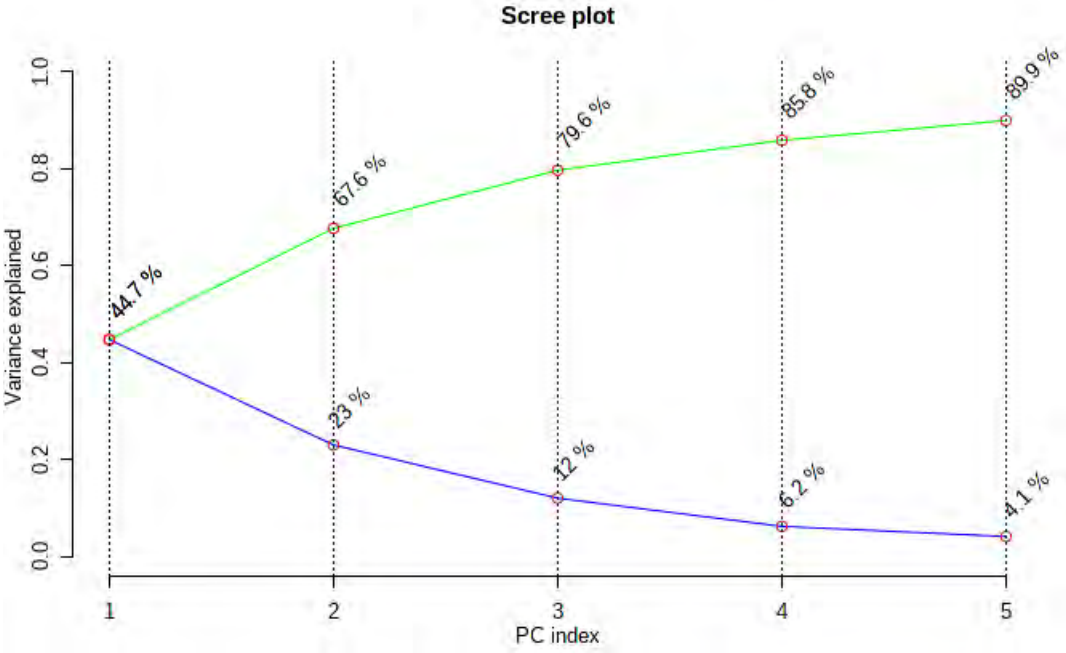


Figure 5.3: Scree plot shows the variance explained by PCs. The green line on top shows the accumulated variance explained; the blue line underneath indicates the variance explained by individual PC.

Figure 5.4 shows the 2-D scores plot between selected PCs. The same way midstream Buffalo River sample (F2C) at stood alone at PC2. The closer together the samples, the more related their peak features. Samples that cluster together showed similarities of features. Samples K2B and T1C

clustered, same were G1A and S1A, B3C, U2C, S2C and S1C. Their closeness on the PCA plot shows the similarity of some features. Most other samples form a cluster.

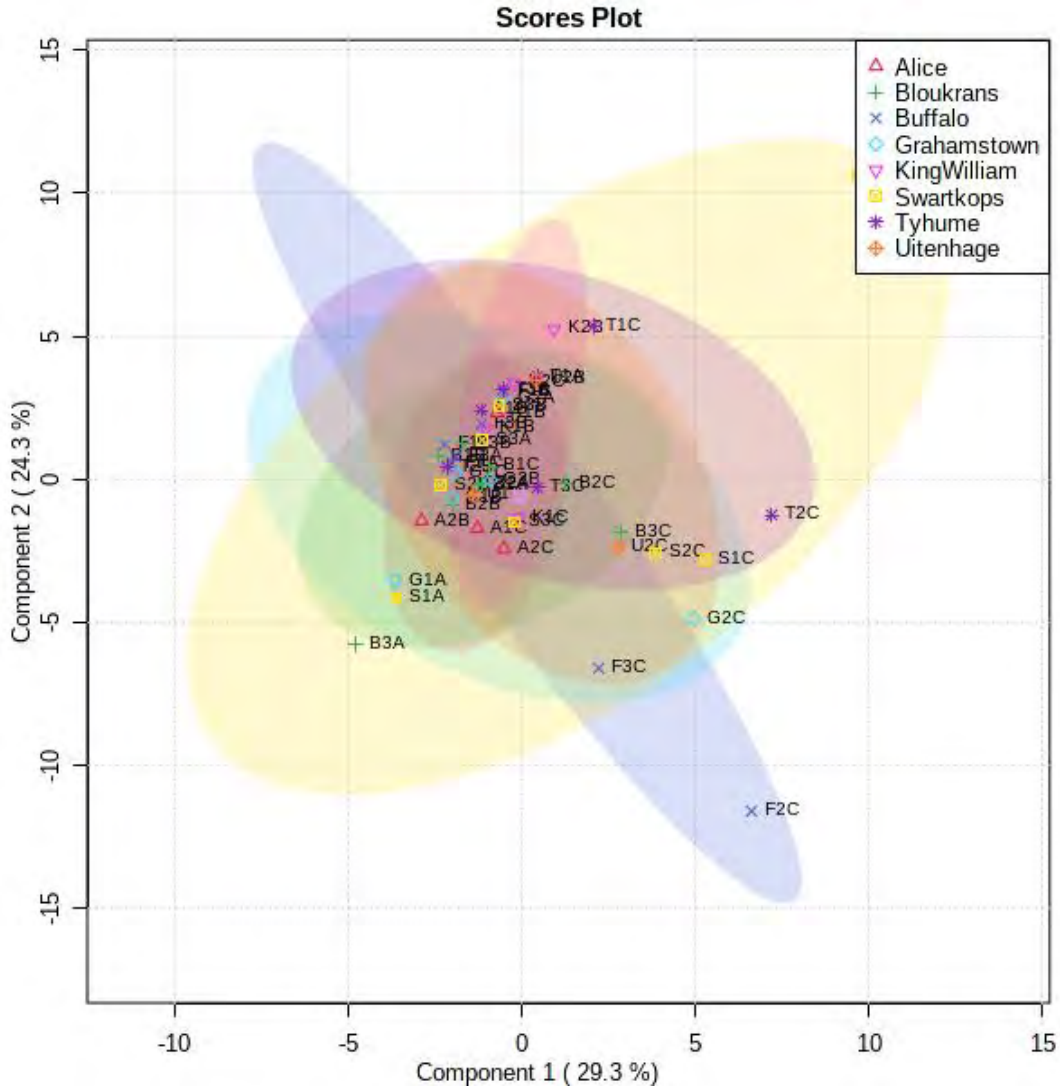


Figure 5.4: Scores plot between the selected PCs. The explained variances are in brackets.

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

Figure 5.5 shows the VIP scores for fifty priority features identified by PLS-DA. PLS-DA is a supervised method, guided by preliminary information, which serve as a reference for the target result (Messai et al., 2016). Variable Importance in Projection (VIP) is a weighted sum of squares of the PLS loadings taking into account the amount of explained Y-variation in each dimension (Bijlsma et al., 2006). King Williams Town samples had the highest concentration of these fifty features (1010-1036; 1358-1360). These features correspond to the spectra of various substituted aromatic compounds reported in Table 5.10 and phenols in Table 5.7. Grahamstown samples had features 794-804, which are peaks of aliphatic aldehyde (Table 5.6) and chlorinated aliphatic hydrocarbon compounds (Table 5.11). Features 1007 to 1009, associated with substituted aromatic compounds (Table 5.10), were more abundant in Tyhume River samples. The VIP features may serve to distinguish these samples if taken further through elemental analysis.

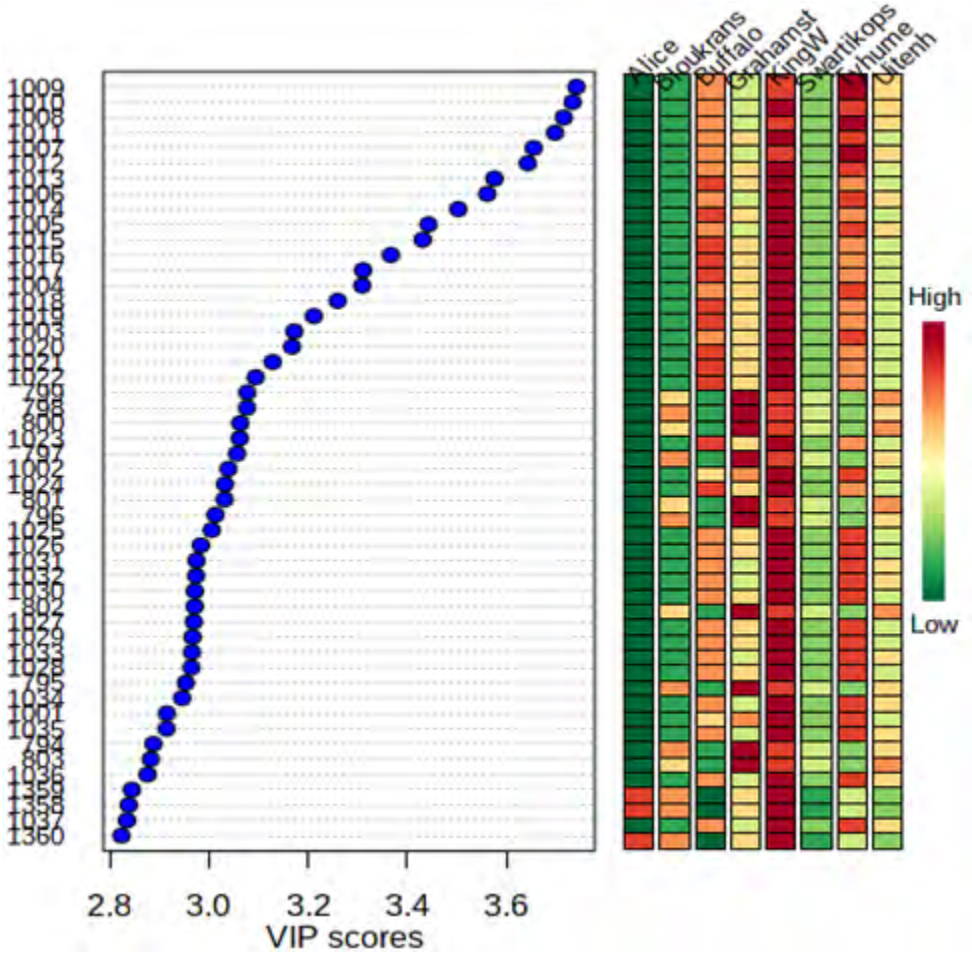


Figure 5.5: Important features identified by PLS-DA. The coloured box on the right indicates the relative concentrations of the corresponding metabolite in each group under study.

Figure 5.6 shows the result of Hierarchical clustering analysis of the samples as a dendrogram. Closely related samples (in term of chemical composition) clustered together. The similarity is measured with Euclidean distance and clustering algorithm with Ward's linkage (clustering to minimise the sum of squares of any two clusters) (Murtagh and Legendre, 2014). At a distance of 80, there are two clusters, but at 20, there are 7 clusters.

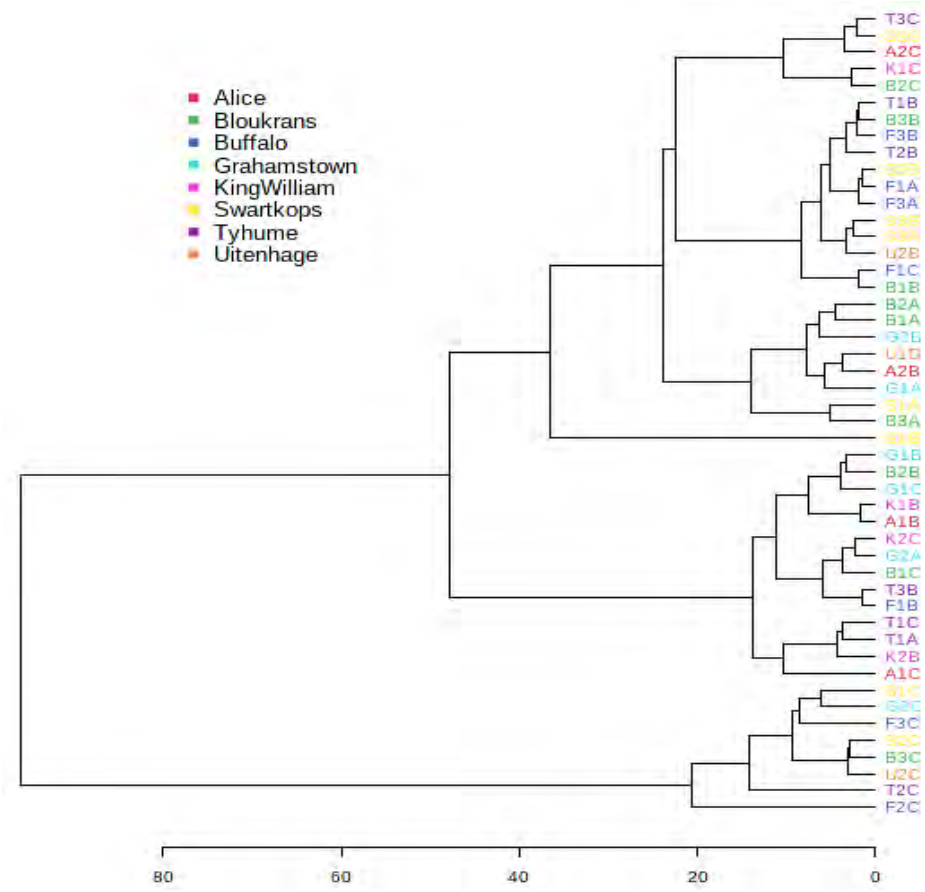


Figure 5.6: Clustering result is shown as a dendrogram (distance measure using Euclidean and clustering algorithm using ward.D).

Bloukrans River samples: B1A (upstream, autumn); B2A (midstream, autumn); B3A (downstream, autumn); B1B (upstream, winter); B2B (midstream, winter); B3B (downstream, winter); B1C (upstream, spring); B2C (midstream, spring) and B3C (downstream, spring). Buffalo River samples: F1A (upstream, autumn); F3A (downstream, autumn); F1B (upstream, winter); F2B (midstream, winter); F3B (downstream, winter); F1C (upstream, spring); F2C (midstream, spring) and F3C (downstream, spring). Swartkops River samples: S1A (upstream, autumn); S3A (downstream, autumn), S1B (upstream, winter); S2B (midstream, winter); S3B (downstream, winter); S1C (upstream, spring); S2C (midstream, spring) and S3C (downstream, spring). Tyhume River samples: T1A (upstream, autumn); T3A (downstream, autumn); T1B (upstream, winter); T2B (midstream, winter); T3B (downstream, winter); T1C (upstream, spring); T2C (midstream, spring) and T3C (downstream, spring). Grahamstown samples: G1A (influent, autumn), G2A effluent, autumn), G1B (influent, winter), G2B (effluent, winter), G1C (influent, spring), G2C (effluent, spring). King Williams Town samples: K1B (influent, winter), K2B (effluent, winter), K1C (influent, spring), K2C (effluent, spring). Alice samples: A1B (influent, winter), A2B (effluent, winter), A1C (influent, spring), A2C (effluent, spring). Uitenhage samples: U1B (effluent, winter), U1C (effluent, spring).

The dendrogram shows that some wastewater influents (G1B, G1C, K1B, and A1B) clustered together, and most river samples appeared on the same cluster. Some river samples like B2B clustered with wastewater, indicating the similarity of features.

5.3 Conclusion

The results of NMR and FT-IR analyses show that there are many chemical compounds in the water samples. Both reports highlighted the differences in the water samples from different geographical backgrounds. These differences exist within samples from the same geographic location and among samples from different geographical areas. Some functional groups like aliphatic aldehydes, disubstituted alkenes, substituted aromatic compounds, alkynes, halogen and sulphur compounds reported in the midstream and downstream samples of the rivers were not present in the upstream samples. Both analyses revealed the presence of pollutants, especially functional groups of pesticides and emerging contaminants in the environmental water samples. The presence of functional groups of compounds such as alkyl halides in the treated effluent samples confirmed the inability of the treatment plants to remove them effectively from wastewater influents. Certain primary amides, aryl aldehydes, organophosphates, fluorinated hydrocarbons, urethanes, and substituted aromatic compounds were not effectively removed from the wastewater at the WWTPs. They were present in the treated effluents, thereby contributing to river pollution. This study has been able to establish that some compounds filtered into freshwater through improperly treated wastewater effluents. Some functional groups like cyclohexyl present in the wastewater influents were not present in the receiving rivers, which may signify that the WWTPs effectively removed some compounds. However, halogenated compounds, used in the treatment of some of the wastewaters were getting into the receiving rivers. Multivariate analyses show that little differences exist between most wastewaters and the receiving surface water, an indication that the rivers were polluted and the wastewaters poorly treated. The results indicate that both NMR and FT-IR are useful tools in the analysis of the water samples.

CHAPTER 6

DETERMINATION OF THE ENDOCRINE DISRUPTING COMPOUNDS IN THE RIVERS AND WASTEWATERS

6.0 Introduction

In this chapter, some chemical compounds that may disrupt the functions of the endocrine system and their hormones were analysed in the samples. These compounds include nonylphenol (NP), 2,4-dichlorophenol (DCP), 4-octylphenol (OP) oestrone (E1), 17β-oestradiol (E2), bisphenol A (BPA), imidazole (IM), triazole (TA), atrazine (AT) and triclosan (TC). Samples were analysed with liquid chromatography coupled to mass spectrometry (LC-MS/MS). The procedure, results, and discussions were presented in this chapter. The result obtained was statistically analysed, and conclusions are drawn based on the outcome.

6.1 Procedure

300 mL of each sample was frozen in liquid nitrogen placed in the water bath mounted on a rotary evaporator (Büchi Rotavapor R-210 with Büchi Bath B-491, Büchi Labotechnik, Switzerland). Frozen water samples were dried under a vacuum pump. The lyophilised samples were dissolved in acetone, and filtered to extract the organic compounds. The extraction was repeated by dissolving the precipitate in methanol, followed by filtration. The filtrates from both solvents were combined to a labelled vial. The samples were allowed to dry in an oven maintained at 37 ^{o}C. The dried filtrates were re-dissolved in deionised water before transferred to labelled SPE tubes for solid-phase extraction. The control experiment used deionised water instead of the sample water. Samples were analysed in the Stellenbosch University Central Laboratory.

6.1.1 Solid-phase extraction (SPE)

Cleaning of water sample extracts was with disposable LC-18 solid-phase extraction (SPE) columns (Olujimi et al., 2010; Neale et al., 2018). The SPE columns were conditioned with 5 mL methanol and rinsed with deionised water (Minh et al. 2016). The re-dissolved water extracts were purified through the SPE tubes mounted on Supelco vacuum manifold connected to a vacuum pump. The sample flow was regulated to 15 drops per minute. The tubes were rinsed with deionised water before elution. Methanol was used to elute the compounds from the SPE tubes (Lv et al., 2016). Elutes were collected into glass vials, dried and carefully labelled for LC-MS analysis.

6.1.2 Sample Analysis

The LC-MS analysis was according to the method described by Petrie et al. (2016) and Archer et al. (2017). The dried samples were reconstituted in 9 mL of 10% MeOH, together with 1 mL of 50 µg/L p-aminosalicylic acid (PAS) as the internal standard. The entire 10 mL sample was passed through HLB SPE cartridge (Waters, Milford, USA), cartridge, washed with water and the analytes eluted off using 1 mL methanol. Chromatography was performed on Waters Acquity ultra-high-performance liquid chromatography (UPLC). The sample injected into the UPLC at 40°C and the flow rate was 0.4 mL/min. The optimal mobile phase consisted of a linear gradient system of: (A) 0.1% formic acid in acetonitrile; and (B) 0.1% formic acid in water, 0–2 min, 98% A; 2–3 min, 98–80% A; 3–7 min, 80–5% A; 7–9 min, 5% A; 9–11 min, 5–98% A; 11–13 min, 98% A. The UPLC column was ethylene bridge hybrid (BEH) C18, 2.1x100mm, 1.7 µm. The UPLC was coupled to Xevo tandem quadrupole spectrometer (TQ-S), forming UPLC-MS/MS for quantitative and qualitative analyses of samples.

The instrument was operated in the positive electrospray ionisation (ESI) mode, with the reaction transitions monitored for each component for quantification and identification, respectively. The instrument pressure was maintained within 0-12000 psi.

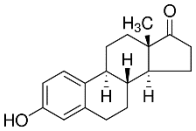
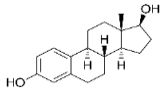
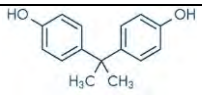
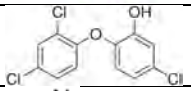
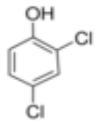
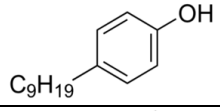
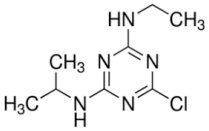
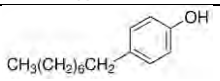
6.2 Method Development

For determination of the accuracy of the method, triplicates of three different concentration levels of the EDCs (10, 100 and 200 µg/L) were prepared and spiked in LC/MS grade ultra-pure water, evaporated and reconstituted in 10% MeOH and analysed. The analyte recoveries were determined by LC–electrospray ionization–tandem MS (LC–ESI–MS/MS). The relative standard deviation of the replicates provided the analysis variation and indicated the precision of the test method (Comerton et al., 2009). The mean of the replicates (in percentage), suggests the accuracy of the test method. The relative recoveries of the spiked samples (%) were calculated as measured concentration/spiked concentration × 100 (Table 6.1).

For the quantitative analysis of the reference compounds, LC/MS grade water was injected as calibration point zero (Dusza et al., 2019). Reference standards at 5, 10, 20, 50, 100 and 200 µg/L were prepared in the matrix to compensate for any matrix-induced ion suppression during the LC–

ESI–MS/MS analysis. The responses were recorded, and the regression line of each reference compound was constructed and forced through zero (Appendix V). The matrix-matched calibration graphs were prepared, and excellent linearity ($r^2 > 0.999$) was achieved over the concentration range tested. The limits of detection (LoD) of measured compounds were calculated as three times the standard deviation (SD) of the extraction blanks, that is, a full extraction procedure performed with a nonspiked HPLC grade water (Dusza et al., 2019). Appendix V shows the calibration curves for the analysed compounds. Table 6.1 shows the parameters for the method validation, with the limit of detection (LoD) of the samples in LC-MS/MS.

Table 6.1: Method development parameters for the analysed compounds

Standard	**Structure**	**Mol wt.**	**Empirical formula**	**Method Accuracy (%)**	**LoD in water samples (µg/L)**	**R^2**
Oestrone		270.37	$C_{18}H_{22}O_2$	98	0.0003	0.9964
17B-oestradiol		272.38	$C_{18}H_{24}O_2$	101	0.0003	0.9952
Bisphenol A		228.29	$(CH_3)_2C(C_6H_4OH)_2$	103	.0010	0.9948
Triclosan		289.54	$C_{12}H_7Cl_3O_2$	99	.0025	0.9933
Imidazole		68.08	$C_3H_4N_2$	97	0.0008	0.9895
Triazole		69.07	$C_2H_3N_3$	99	0.0005	0.9976
2,4-Dichlorophenol		163.00	$Cl_2C_6H_3OH$	102	0.001	0.9945
Nonylphenol		220.35	$C_{15}H_{24}O$	103	0.0005	0.9876
Atrazine		215.68	$C_8H_{14}ClN_5$	99	0.0004	0.9967
4-Octylphenol		206.32	$CH_3(CH_2)_7C_6H_4OH$	99	0.00025	0.9919

6.3 Results and Discussion

Table 6.2 shows the descriptive statistics of the compounds in the water samples. The results showed that nonylphenol, with a mean concentration of 1.297 µg/L in the water samples, had the highest level compared with other compounds analysed. Next to NP was dichlorophenol (DCP) with a mean concentration of 0.449 µg/L and bisphenol A (BPA) with 0.415 µg/L. The least being 17β-oestradiol (E2) with 0.0095 µg/L.

Table 6.2: Descriptive Statistics of the compounds in the samples (mean values are in µg/L)

Variable	Mean	Median	Minimum	Maximum	Variance	Std.Dev.	Coef.Var.	Standard Error
NP	1.296993	0.301700	0.026400	6.970000	3.342143	1.828153	140.9532	0.242145
DCP	0.449782	0.132000	0.000000	2.273000	0.375047	0.612411	136.1572	0.081116
E1	0.066058	0.002000	0.000000	1.100000	0.056184	0.237032	358.8253	0.031396
E2	0.009544	0.000000	0.000000	0.149000	0.000918	0.030305	317.5291	0.004014
BPA	0.414946	0.218700	0.005600	1.766700	0.270699	0.520287	125.3868	0.068914
OP	0.199032	0.000000	0.000000	1.727700	0.234064	0.483802	243.0779	0.064081
TC	0.349558	0.037000	0.000000	3.141300	0.721149	0.849205	242.9369	0.112480
AT	0.076060	0.000000	0.000000	0.853400	0.035208	0.187639	246.6996	0.024853
IM	0.153470	0.077000	0.000000	0.749800	0.037139	0.192716	125.5723	0.025526
TA	0.066088	0.026000	0.000000	0.451000	0.010109	0.100544	152.1370	0.013317

All the wastewater influent samples showed higher concentrations of nonylphenol (NP) but reduced in the corresponding effluents except for Grahamstown effluents (GE) that retained 58% of the NP.

Table 6.3 shows the concentrations of nonylphenol, dichlorophenol, oestrone, oestradiol and bisphenol A in the samples. King Williams Town wastewater influents (KW) had the highest mean level of nonylphenol with 6.72 µg/L, but only 5.7% was present in the effluents (KE). Alice wastewater (AW) has 3.131 µg/L, but 11.4% escaped to the effluents (AE). Among the freshwater samples, Bloukrans midstream (BM) had the highest mean concentration of NP (2.553 µg/L) with 2.456 µg/L in the downstream (BD). The presence of NP in the samples may be attributed to its applications in the production of household materials like

detergents, emulsifiers, antioxidants, paints, pesticides, personal care products, plastics, solubilisers and as lubricating oil additives (Soares et al., 2008).

Table 6.3: Concentrations of EDCs in the samples (values are means ± standard deviations).

Sample	Average concentration (µg/L ± SD)				
	Nonylphenol	**Dichlorophenol**	**Oestrone**	**Oestradiol**	**Bisphenol A**
BU	0.0627 ± 0.002	0.0107 ± 0.002	0.0017 ± 0.000	0.0163 ± 0.002	0.0173 ± 0.002
BM	2.5533 ± 0.084	0.7373 ± 0.076	0.0613 ± 0.004	< LoD	0.465 ± 0.084
BD	2.4560 ± 0.142	0.4920 ± 0.124	0.0403 ± 0.007	0.0075 ± 0.001	0.4770 ± 0.036
FU	0.1017 ± 0.009	0.0353 ± 0.003	0.0054 ± 0.000	< LoD	0.0465 ± 0.007
FM	0.1962 ± 0.021	0.0312 ± 0.003	< LoD	< LoD	0.0943 ± 0.012
FD	0.1454 ± 0.020	0.2971 ± 0.020	0.0013 ± 0.000	< LoD	0.0185 ± 0.003
SU	0.0312 ± 0.003	0.0067 ± 0.001	< LoD	< LoD	0.0067 ± 0.001
SM	0.164 ± 0.011	0.0963 ± 0.011	0.002 ± 0	< LoD	0.3417 ± 0.023
SD	0.3337 ± 0.021	0.1273 ± 0.007	0.002 ± 0	< LoD	0.3117 ± 0.015
TU	0.1293 ± 0.007	0.013 ± 0.001	< LoD	< LoD	0.0327 ± 0.005
TM	0.2141 ± 0.009	0.0263 ± 0.003	0.0009 ± 0.000	< LoD	0.1173 ± 0.011
TD	0.5867 ± 0.019	0.2613 ± 0.028	< LoD	< LoD	0.0177 ± 0.004
GW	4.3773 ± 0.287	0.854 ± 0.287	0.0263 ± 0.003	0.0113 ± 0.002	1.4683 ± 0.087
GE	2.5557 ± 0.081	< LoD	0.0151 ± 0.002	< LoD	0.3451 ± 0.035
KW	6.7197 ± 0.167	1.719 ± 0.036	0.0124 ± 0.002	0.0061 ± 0.001	1.011 ± 0.037
KE	0.384 ± 0.025	0.7113 ± 0.034	< LoD	< LoD	0.0181 ± 0.002
AW	3.1307 ± 0.081	2.2 ± 0.048	1.06 ± 0.027	0.135 ± 0.009	1.19267 ± 0.045
AE	0.358 ± 0.04	0.118 ± 0.009	0.0131 ± 0.004	0.0026 ± 0.000	0.2187 ± 0.014
UE	0.143 ± 0.008	0.809 ± 0.02	0.0134 ± 0.001	0.0025 ± 0.000	1.6837 ± 0.055
CTR	< LoD	< LoD	< LoD	< LoD	< LoD

Bloukrans River: upstream (BU), midstream (BM), downstream (BD). Buffalo River: upstream (FU), midstream (FM), downstream (FD). Swartkops River: upstream (SU), midstream (SM), downstream (SD). Tyhume River: upstream (TU), midstream (TM), downstream (TD). Grahamstown wastewater: influents (GW), effluents (GE). King Williams Town wastewater: influents (KW), effluents (KE). Alice wastewater: influents (AW), effluents (AE). Uitenhage treated effluents (UE); Control (CTR).

Nonylphenol may be formed from the anaerobic breakdown of the ethoxylated alkylphenols (Araujo et al., 2018). Chokwe et al. (2016) observed the NP concentration range of 20 - 127 ng/L

in the Vaal River. Manda et al. (2017) reported 98 ng/L mean concentrations of NP in Mpumalanga wastewater; 12.0 ng/L in Mkomazane River; a range of 1.35 – 28.72 ng/L in Roodeplat dam, Gauteng and 2.57 ng/L in Krokodil River, North West.

Dichlorophenol (DCP) has its highest mean concentration of 2.20 µg/L in Alice wastewater influents (AW), but only 5.36% was present in the effluents (AE). King Williams Town wastewater influents (KW) had 1.719 µg/L of DCP, but 41.38% escaped into the effluents. DCP was below the detection limit in Grahamstown wastewater effluent (GE) samples. The mean concentration of dichlorophenol in the midstream samples of Bloukrans River (BM) was 0.737 µg/L while it was 0.492 µg/L in the downstream samples (BD). In the downstream samples of Swartkops (SD) and Tyhume (TD) Rivers, the mean concentrations were 0.127 and 0.261 µg/L, respectively. On average, the levels of DCP were higher in the downstream samples than other reaches of the rivers. Olujimi et al. (2012) recorded 233 µg/L mean concentration of DCP in Bellville wastewater influents and 163 µg/L in the effluents. They recorded a DCP concentration of 39 µg/L in Stellenbosch wastewater influents and 27 µg/L in the effluents. In the same report, downstream Veldwatchers River receiving effluents from Stellenbosch had 156 µg/L (Olujimi et al., 2012). The concentration of DCP in Zandvliet wastewater influents was 100 µg/L, but 83 µg/L was present in the effluents, and the downstream of the receiving river (Kiuls) has 63 µg/L (Olujimi et al., 2012). The values recorded by these workers were higher than the result obtained in this study because they sampled more populated environments.

Oestrone (E1) and β-oestradiol (E2) hormones were present in most of the samples with E1 more common than E2 (Table 6.3). The mean concentrations of both compounds were not as high as other compounds in the samples (Table 6.2). Alice wastewater influents (AW) had the highest mean concentrations of both hormones, with 1.06 µg/L of E1 and 0.135 µg/L of E2. Fort Hare University owns the WWTP in Alice; the hostels might have contributed to the elevated level of both hormones in Alice wastewater. Bloukrans River midstream samples (BM) had the second-highest mean concentration of E1 with 0.062 µg/L. The observed value in sample BM may be due to wastes (urine) from grazing cattle around the river valley, or most of the wastewaters from Grahamstown are not passing through the sewers or both. The higher mean concentration of E1 over E2 in the samples might be because E1 is available in drugs (menopausal hormonal

supplement) and virginal creams for women (Friel et al., 2005; Searchlight, 2016) and also excreted with urine as oestrone sulphate (Kuhl, 2005). Truter et al. (2016) observed an E2 range of 0.72 – 30.8 ng/L in a study of upper Olivant River. Fatoki et al. (2018) observed the E2 concentration range of 870 – 1094 ng/L of E2 in a survey of some streams around Cape Town. The result obtained by Fatoki et al. (2018) showed that livestock wastes contribute to environmental oestrogen. Manickum and John (2014) observed mean concentrations of 84 ng/L of E1 and 119 ng/L E2 in wastewater influents study in Pietermaritzburg; the effluents were 32 and 20 ng/L of both hormones respectively. Van Zijl et al. (2017) recorded mean concentrations of 2.87 and 0.03 ng/L of E1 and E2 respectively around Pretoria Rivers; and 0.04 and 0.75 ng/L for rivers around Cape Town.

Bisphenol A (BPA) had its highest mean concentration in Uitenhage wastewater effluents (UE) with 1.684µ/L, followed by Grahamstown influents (GW) with 1.468 µg/L. Access was denied to Uitenhage WWTP; hence the analysis of the wastewater influents was not possible. BPA was present in all the samples at different concentrations (Table 6.3). Its levels were lower in the upstream samples than other reaches of the rivers. BPA is a constituent of plastics and their products. The presence of BPA in the river samples is the result of wastes reaching the water bodies. Wanda et al. (2017) reported a BPA range of 4.0 – 181 ng/L in Mpumalanga; 7.34 – 81.24 ng/L in Krokodil River, North West; 3.0 – 30.34 ng/L in Roodeplat dam, Gauteng.

Table 6.4 shows the mean concentration of triclosan (TC), 4-octylphenol (OP), atrazine (AT), imidazole (IM) and triazole (TA) in the samples. TC has the highest concentration in Alice wastewater influents (AW) with 2.856 µg/L, followed by sample SD (downstream Swartkops River) with 2.715 µg/L. It was below the detection limit in all the upstream samples (BU, FU, SU and TU). Its presence in treated effluents was an indication that WWTP cannot effectively remove it. Triclosan is present in pharmaceuticals, personal care and household products from where it gets into the wastewater (Dhillon et al., 2015). Madikizela et al. (2014) recorded TC concentrations range of 400 – 900 ng/L in Mbokodweni River (Kwazulu-Natal), 2100 – 9000 ng/L in wastewater influents and 1300 – 6400 ng/L in effluents. The mean concentration of TC in wastewater recorded by Amdany et al. (2014) in Gauteng was 78000 ng/L. The results obtained by these workers show higher concentration than those reported in this study.

Table 6.4 Concentrations of some EDCs in the samples (values are means ± standard deviations).

	Average concentration (µg/L)				
Sample	**Triclosan**	**4-Octylphenol**	**Atrazine**	**Imidazole**	**Triazole**
BU	< LoD	< LoD	0.0207 ± 0.002	< LoD	< LoD
BM	0.1469 ± 0.013	< LoD	< LoD	0.5737 ± 0.067	0.181±0.017
BD	0.0623 ± 0.009	0.0850 ± 0.009	< LoD	< LoD	0.0505 ± 0.007
FU	< LoD	< LoD	< LoD	< LoD	0.0392 ± 0.005
FM	< LoD	< LoD	< LoD	0.0213 ± 0.003	0.0061 ± 0.001
FD	< LoD	< LoD	< LoD	0.1788 ± 0.017	0.0129 ± 0.001
SU	< LoD	< LoD	< LoD	0.0119 ± 0.002	< LoD
SM	< LoD	1.4533 ± 0.025	0.0446 ± 0.004	0.248 ± 0.021	0.0187 ± 0.004
SD	2.7147 ± 0.037	0.4013 ± 0.014	0.0203 ± 0.004	< LoD	0.026 ± 0.003
TU	< LoD	< LoD	< LoD	0.015 ± 0.001	0.017 ± 0.001
TM	< LoD	< LoD	< LoD	0.0897 ± 0.008	0.0207 ± 0.005
TD	0.0097 ± 0.002	< LoD	< LoD	< LoD	< LoD
GW	0.2323 ± 0.027	0.0357 ± 0.003	0.8123 ± 0.027	0.6187 ± 0.087	0.149 ± 0.021
GE	0.0446 ± 0.008	< LoD	< LoD	0.0950 ± 0.014	0.0943 ± 0.008
KW	0.1767 ± 0.009	0.0572 ± 0.006	0.151 ± 0.009	0.2665 ± 0.014	0.429 ± 0.015
KE	0.041 ± 0.003	< LoD	0.0123 ± 0.000	0.0174 ± 0.003	0.0273 ± 0.005
AW	2.8563 ± 0.19	0.0193 ± 0.001	0.256 ± 0.015	0.3717 ± 0.021	0.1013 ± 0.009
AE	0.2743 ± 0.018	1.6827 ± 0.03	0.1184 ± 0.013	0.2027 ± 0.007	0.0153 ± 0.002
UE	0.0827 ± 0.005	0.047 ± 0.008	0.0095 ± 0.002	0.2055 ± 0.011	0.0673 ± 0.006
CTR	< LoD	< LoD	< LoD	< LoD	< LoD

Bloukrans River: upstream (BU), midstream (BM), downstream (BD). Buffalo River: upstream (FU), midstream (FM), downstream (FD). Swartkops River: upstream (SU), midstream (SM), downstream (SD). Tyhume River: upstream (TU), midstream (TM), downstream (TD). Grahamstown wastewater: influents (GW), effluents (GE). King Williams Town wastewater: influents (KW), effluents (KE). Alice wastewater: influents (AW), effluents (AE). Uitenhage treated effluents (UE).

4-octylphenol (OP) had its highest concentration in Alice wastewater (AW) with 1.683 µg/L, followed by Swartkops River midstream (SM) with 1.453 µg/L (Table 6.4). It was below the limit of detection in all the upstream samples. The presence OP in wastewaters may be due to its

presence in household consumer products such as detergents, emulsifiers, disinfectants, spermicides and contraceptives (Ripamonti et al., 2018). Chokwe et al. (2016) recorded 0-46 ng/L OP in Vaal River sediments. DWAF (1996) set a maximum limit of 30 µg/L for phenolic compounds in aquatic ecosystems.

Atrazine (AT) has its highest concentration in Grahamstown wastewater influents (GW) with 0.812 µg/L, but it was below the detection limit in effluents (GE). Alice wastewaters (AW) had 0.256 µg/L, with 0.122 µg/L retained in the effluents (AE) and King Williams Town wastewater effluent samples (KW) 0.141 µg/L. Atrazine was below the detection limit in upstream samples SU, TU, FU; midstream samples BM, FM, TM; downstream samples BD, TD, FD, and Grahamstown effluents, GE. There is a recreational resort located upstream of Bloukrans River (BU). The presence of atrazine in sample BU might be a result of the chemicals used in maintaining the lawns. Woodings et al. (2017) recorded 0.40 ng/L AT in Marais dam, Gauteng. Atrazine is one of the top 25 chemicals used as pesticides in South Africa, being the active ingredient in glyphosate and other herbicides; it has high mobility the environment, this might account for its presence in the samples (Dabrowski et al., 2014). Being a pesticide, DWAF (1996) set the limit of 10 µg/L for AT in the environment.

Imidazole (IM) was present in all the wastewater influent and effluent samples. Its concentration was highest in Grahamstown wastewater influents (GW) with 0.619 µg/L, and the effluents had 15.24% of the compound retained after treatments. Alice wastewater influents (AW) had 0.372 µg/L of IM, but the effluents (AE) had 0.203 µg/L or 54.53% retained. King Williams Town wastewater influents (KW) had IM concentration of 0.267 µg/L, with 6.64% retained in the effluents (KE). Midstream samples of Bloukrans (BM) and Swartikops (SM) Rivers had IM concentrations of 0.574 and 0.248 µg/L, respectively, an indication of sources other than WWTPs. Imidazole is commonly used in the manufacturing of drugs, dye, photographic chemicals, polyurethanes, and corrosion inhibitors and may enter the environment through these products (Spasiano et al. 2016). Imidazole based drugs had been reported to be present in wastewater and receiving rivers in several countries (Mirzaei et al. 2019; Wang et al. 2018).

Triazole (TA) was present in almost all the samples. It has its highest mean concentration in King Williams Town wastewater (KW) with 0.429 µg/L, but 6.29% of it escaped the WWTP. Grahamstown wastewater (GW) had a mean concentration of 0.149 µg/L of TA and 0.094 µg/L or 63.29% in its treated effluents. Alice wastewater contains 0.101 µg/L of TA, with 15.15% of that concentration present in its treated effluents (AE). Among the freshwater samples, Bloukrans midstream ranked highest with TA concentration of 0.181 µg/L, followed by its downstream sample (BD) with 0.051 µg/L. Triazoles are components of drugs, light stabilizers, chemosensors, and corrosion retarding agents (Rani 2014; Ceesay et al. 2016). They have been reported as pollutants in wastewaters and receiving rivers (Huang et al. 2013; Vimalkumar et al. 2018).

Table 6.5 shows the effectiveness of the WWTPs at removing EDCs from the wastewater influents. WWTPs achieved 100% removal of dichlorophenol and octylphenol except for Alice, where the level of OP in the effluents was higher than influents. A higher level of OP in the effluents might suggest that it is a component of the disinfectants used in the treatment of the wastewater at Alice WWTPs.

Table 6.5: The WWTPs efficiency at removal of EDCs from the wastewater influents

	Removal efficiency (%)		
Compound	Grahamstown WWTP	King Williams Town WWTP	Alice WWTP
Nonylphenol	41.61	94.29	88.56
Dichlorophenol	100.00	58.62	94.64
Oestrone	42.71	100.00	98.77
Oestradiol	100.00	100.00	98.10
Bisphenol A	76.50	98.21	81.66
Octylphenol	100.00	100.00	0.00
Triclosan	80.79	76.80	90.40
Atrazine	100.00	91.88	53.75
Imidazole	84.64	93.47	45.47
Triazole	36.71	93.63	84.90

Lesser than half of nonylphenol, oestrone and triazole in Grahamstown wastewater influents were removed at the WWTP. Alice WWTP was unable to remove half the quantity of imidazole in the wastewater influents. The performances of the WWTPs may look good by the percentage of EDCs

removed, but the activeness and the cumulative ability of EDCs in living things at minute range require that WWTPs be 100% effective.

Table 6.6 shows the correlation between the environmental concentrations of the compounds within the sampled area. It is an expression of the relative abundance of the samples in the environments investigated. A positive value of 0.5 or above shows a strong correlation of concentrations while -0.5 or below indicates a strong negative correlation. The colour box indicates related correlation values. The values of NP and TA show strong correlation while E1 and TC were negatively correlated.

Table 6.6: Correlations coefficient of the compounds in the samples. Correlations are significant at $p < .01000$

Compound	AT	IM	TC	E2	BPA	DCP	NP	TA	OP	E1
AT	1.000000	0.636009	0.173760	0.291686	0.541188	0.359658	0.421405	0.058146	0.015784	0.071339
IM	0.636009	1.000000	0.132318	0.300888	0.657630	0.561609	0.588409	0.459607	0.102937	0.104296
TC	0.173760	0.132318	1.000000	0.685608	0.284220	0.461524	0.145917	0.041668	0.040815	-0.023408
E2	0.291686	0.300888	0.685608	1.000000	0.402683	0.710985	0.291900	0.107310	-0.101261	-0.050721
BPA	0.541188	0.657630	0.284220	0.402683	1.000000	0.707456	0.596034	0.484694	-0.069815	-0.063525
DCP	0.359658	0.561609	0.461524	0.710985	0.707456	1.000000	0.722827	0.638858	-0.194283	-0.108847
NP	0.421405	0.588409	0.145917	0.291900	0.596034	0.722827	1.000000	0.881323	-0.189161	-0.088262
TA	0.058146	0.459607	0.041668	0.107310	0.484694	0.638858	0.881323	1.000000	-0.165548	-0.092728
OP	0.015784	0.102937	0.040815	-0.101261	-0.069815	-0.194283	-0.189161	-0.165548	1.000000	0.721569
E1	0.071339	0.104296	-0.023408	-0.050721	-0.063525	-0.108847	-0.088262	-0.092728	0.721569	1.000000

Table 6.7 shows the linear correlation statistics of the sites regarding EDCs concentrations in their samples. Correlation statistics made use of the mean concentrations of the endocrine-disrupting compounds in each sample. Positively correlated sites are in blue while the negative red. The deepness of both colours is a reflection of the strength of the correlation. Samples in ash colour show near-zero correlations. Some river samples, like Bloukrans downstream (BD), buffalo midstream (FM), show a strong correlation with wastewater influents such as King Williams Town influent (KW). Grahamstown wastewater effluents (GE) strongly correlated with KW. A high

correlation coefficient between two samples showed that their composition, regarding EDCs, were similar.

Table 6.7: Correlation statistics of the samples (correlation is significant at $p < 0.0100$). The samples in blue are positively correlated and red negative

Sample	BU	BM	BL	GW	GE	FU	FM	FL	KW	KE	SU	SM	SL	UE	TU	TM	TL	AW	AE
BU	1.000000	0.850165	0.911002	0.931938	0.907421	0.790868	0.878342	0.230250	0.901843	0.400077	0.821184	-0.161974	-0.157920	0.078301	0.887364	0.770215	0.848703	0.498133	-0.101413
BM	0.850165	1.000000	0.970063	0.955305	0.949726	0.882308	0.934738	0.539675	0.981816	0.578161	0.979285	-0.104280	-0.062565	0.116894	0.968127	0.901073	0.951769	0.665433	-0.044567
BD	0.911002	0.970063	1.000000	0.967846	0.977005	0.908306	0.953342	0.383798	0.994570	0.530369	0.924718	-0.043612	-0.036972	0.119609	0.976479	0.861435	0.953543	0.666458	0.029148
GW	0.931938	0.955305	0.967846	1.000000	0.957984	0.884971	0.971161	0.376230	0.963560	0.461425	0.940344	-0.092210	-0.069350	0.205658	0.970055	0.916524	0.897285	0.617062	-0.040500
GE	0.907421	0.949726	0.977005	0.957984	1.000000	0.867598	0.936831	0.255095	0.967661	0.359837	0.924317	-0.057947	-0.026306	0.002959	0.984980	0.873759	0.890291	0.591465	0.021453
FU	0.790868	0.882308	0.908306	0.884971	0.867598	1.000000	0.920649	0.371482	0.910712	0.532356	0.815262	-0.128981	-0.142676	0.333265	0.924642	0.840859	0.851505	0.563568	-0.113511
FM	0.878342	0.934738	0.953342	0.971161	0.936831	0.920649	1.000000	0.346398	0.936370	0.422638	0.924357	-0.039703	-0.081003	0.348863	0.968579	0.955386	0.852541	0.598207	-0.020936
FD	0.230250	0.539675	0.383798	0.376230	0.255095	0.371482	0.346398	1.000000	0.449020	0.857466	0.540041	-0.123673	-0.209624	0.256006	0.338448	0.392553	0.584006	0.412017	-0.146158
KW	0.901843	0.981816	0.994570	0.963560	0.967661	0.910712	0.936370	0.449020	1.000000	0.581264	0.933640	-0.087744	-0.055682	0.092168	0.972790	0.851894	0.971659	0.668577	-0.012769
KE	0.400077	0.578161	0.530369	0.461425	0.359837	0.532356	0.422638	0.857466	0.581264	1.000000	0.483180	-0.151424	-0.078765	0.258351	0.413263	0.313491	0.738611	0.606489	-0.113973
SU	0.821184	0.979285	0.924718	0.940344	0.924317	0.815262	0.924357	0.540041	0.933640	0.483180	1.000000	-0.060315	-0.118686	0.130502	0.946909	0.941339	0.885026	0.574996	-0.025130
SM	-0.161974	-0.104280	-0.043612	-0.092210	-0.057947	-0.128981	-0.039703	-0.123673	-0.087744	-0.151424	-0.060315	1.000000	-0.052594	0.033409	-0.071758	-0.037138	-0.103664	-0.297656	0.970009
SD	-0.157920	-0.062565	-0.036972	-0.069350	-0.026306	-0.142676	-0.081003	-0.209624	-0.055682	-0.078765	-0.118686	-0.052594	1.000000	-0.070583	-0.087748	-0.134801	-0.057908	0.563049	0.118330
UE	0.078301	0.116894	0.119609	0.205658	0.002959	0.333265	0.348863	0.256006	0.092168	0.258351	0.130502	0.033409	-0.070583	1.000000	0.128848	0.358463	0.061384	0.190667	-0.107119
TU	0.887364	0.968127	0.976479	0.970055	0.984980	0.924642	0.968579	0.338448	0.972790	0.413263	0.946909	-0.071758	-0.087748	0.128848	1.000000	0.925817	0.893553	0.576647	-0.022153
TM	0.770215	0.901073	0.861435	0.916524	0.873759	0.840859	0.955386	0.392553	0.851894	0.313491	0.941339	-0.037138	-0.134801	0.358463	0.925817	1.000000	0.745468	0.483233	-0.052528
TD	0.848703	0.951769	0.953543	0.897285	0.890291	0.851505	0.852541	0.584006	0.971659	0.738611	0.885026	-0.103664	-0.057908	0.061384	0.893553	0.745468	1.000000	0.709364	-0.018141
AW	0.498133	0.665433	0.666458	0.617062	0.591465	0.563568	0.598207	0.412017	0.668577	0.606489	0.574996	-0.297656	0.563049	0.190667	0.576647	0.483233	0.709364	1.000000	-0.154111
AE	-0.101413	-0.044567	0.029148	-0.040500	0.021453	-0.113511	-0.020936	-0.146158	-0.012769	-0.113973	-0.025130	0.970009	0.118330	-0.107119	-0.022153	-0.052528	-0.018141	-0.154111	1.000000

Bloukrans River: upstream (BU), midstream (BM), downstream (BD). Buffalo River: upstream (FU), midstream (FM), downstream (FD). Swartkops River: upstream (SU), midstream (SM), downstream (SD). Tyhume River: upstream (TU), midstream (TM), downstream (TD). Grahamstown wastewater: influents (GW), effluents (GE). King Williams Town wastewater: influents (KW), effluents (KE). Alice wastewater: influents (AW), effluents (AE). Uitenhage treated effluents (UE); Control (CTR).

Figure 6.1 shows the result of hierarchical cluster analysis (HCA) of the samples as a dendrogram. The cluster analysis made use of Euclidean distance between the samples, using the Ward D algorithm. There are three main clusters and many sub-clusters. Samples SD and AW occupied cluster 1a while BM, BL and GE were on 1b. Sample GW and KW occupy Cluster 2. Samples AE and SM show close relations and occupied cluster 3b. The rest samples clustered on 3a. Cluster analysis further reinforced the fact that the concentrations of EDCs in some freshwater samples correlate with wastewater.

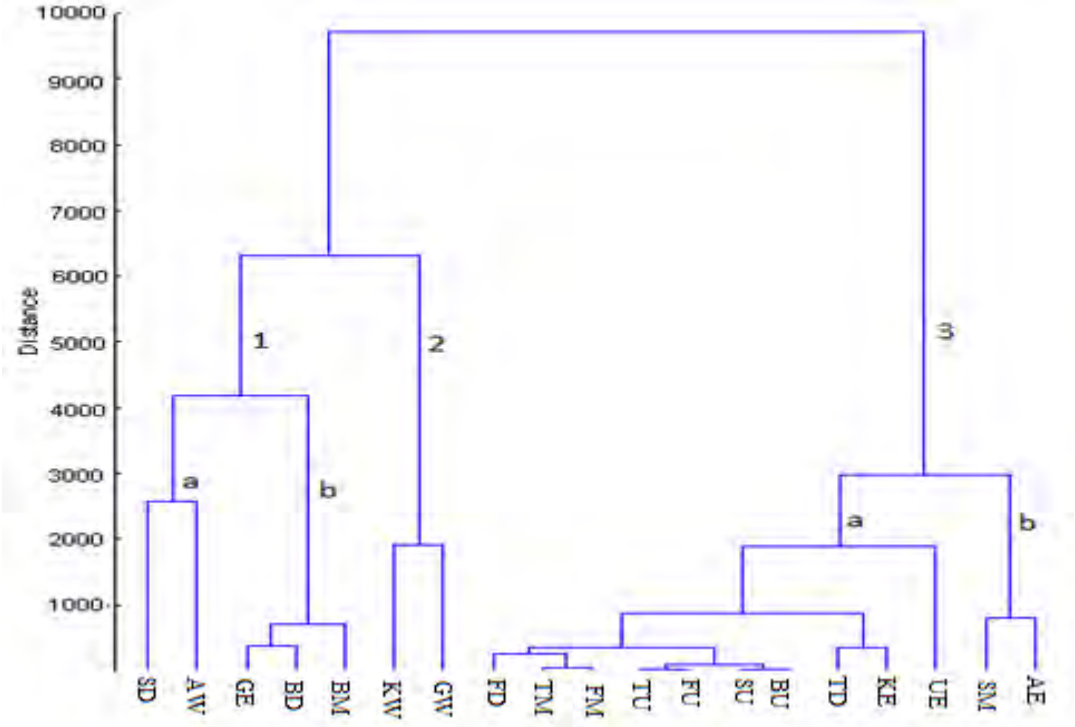


Figure 6.1: clustering result shown as a dendrogram, distances between the samples are measured using Euclidean and clustering algorithm using Ward D.

6.5 Conclusion

The LC-MS analyses confirmed the presence of ten EDCs in the samples. Nonylphenol had the highest mean concentration, followed by dichlorophenol and bisphenol A. Octylphenol, triclosan, and atrazine concentrations were below the detection limits in some samples. The upstream samples of the rivers showed the least concentrations of the compounds, and in most cases, they were below detection limits. The midstream samples had the highest concentrations of the compounds. This means that EDCs entered the rivers through sources other than the WWTPs. The result also showed that EDCs present in the wastewater samples were not effectively removed during treatment, thereby making the effluents another source of EDCs in the rivers. Statistical

analysis showed that the EDC composition of some water samples correlates with wastewater. Wastewater treatment needs to be improved to achieve the total removal of these compounds. The communities need environmental education for proper waste disposal.

CHAPTER 7
DETERMINATION OF HEAVY METALS IN THE RIVERS AND WASTEWATERS

7.1 Procedure

The sampling method was as described in chapter 3 of this thesis. Water samples were acidified to 2% ultra-pure HNO_3 solutions. The samples were filtered to remove particulates before analysis. A fraction of the sample was introduced into the Agilent 7900 through an autosampler by a peristaltic pump. The sample passes through the nebuliser to become a fine aerosol. The spray chamber removes large droplets for small droplets to pass through to the plasma. The plasma torch generated positively charged ions. Prevention of photons from reaching the detector reduced the signal noise. Ions produced in the plasma pass into the mass spectrometer through the interface region maintained at a vacuum of 1 - 2 torrs with a mechanical pump. The ions go through a series of processes in the equipment before been passed to the mass spectrometer. The mass separation devices in this analysis made use of quadrupole to allow analyte ions of a particular mass-to-charge ratio (m/z) through to the detector and to filter out all the non-analytes, interfering, and matrix ions. In the final process, an ion detector converts the ions into an electrical signal. Masshunter software picks the signals and expresses it as counts for calculating results.

7.2 Instrumentation

The Agilent 7900 can measure trace elements as low as one part per trillion (ppt) and quickly scan more than seventy elements to determine the composition of an unknown sample (Al-Rimawi et al., 2013) The MassHunter Workstation software automates the analysis and accurately interprets the resulting data. The ICP/MS instrument consists of an on-board peristaltic pump that controls the flow of sample solution into and waste (drain). It has a nebulizer (Micro Mist nebulizer) that uses a stream of argon to disperse the sample. It also has an ICP Argon plasma torch that uses Argon as plasma gas. There is also auxiliary gas and nebuliser (carrier) gas, two pumps for evacuation, quadrupole mass analyser with 0.8 amu resolution at 10% height, an octapole reaction system (ORS), and an electron multiplier detector (Sakai et al., 2014). The samples were analysed against the United States National Institute of Standards and Technology (NIST) traceable standards and independent quality control.

Table 7.1: The Agilent 7900 ICPMS instrument Conditions:

RF Power (W)	1600
Carrier gas (L/min) (Argon)	0,83
Sample depth (mm)	10
Make-up gas (L/min)	0,15
He flow (mL/min)	5
H2 flow (mL/min)	6
Nebuliser	0.4 mL/min Micro mist

7.3 Preparation of solutions

A stock multi-standard solution of the metals was made by dilution in 0.5% ultrapure nitric acid as diluent. From the stock solution, six solutions of the nine metals at: 1.0, 5.0, 100.0, 300.0, 500.0, and 1000.0 µg/L concentrations were prepared. These solutions were used for linearity and range study. For the determination of metal recovery from water, three concentrations of the metals (1.0, 100.0, and 1000.0 µg/L) were prepared by spiking of the metals in Milli-Q water. Each sample was analysed three times and the results expressed as mean ± SD (SD: standard deviation). The solutions prepared for linearity with concentrations of 1.0, 100.0, and 1000.0 µg/L were used for the precision study. An internal standard (ISTD) mix containing ^{45}Sc, ^{72}Ge, ^{89}Y, ^{103}Rh, and ^{175}Lu was prepared using 2% HNO_3 and 1% HCl. HCl was included to ensure the stability of Hg in solution (Schultz et al., 2017).

7.4 Method validation

For evaluation of linearity of the method, six calibration standards of the metals with concentrations of 1, 5, 100, 300, 500, and 1000 µg/L were analysed by ICP-MS and the responses recorded. A plot of the ratio of response (cps) of the metal analyte divided by the response of the internal standard (Y) versus concentration of the metal (in µg/L) was linear in the range of 1-1000 ppb, and with a correlation coefficient greater than 0.999 (Table 7.2).

Accuracy is measured as the per cent of analyte recovered after spiking samples in a blank. For accuracy, three different concentration levels were made in three replicates covering the specified range. At each level analysed, replicate samples were evaluated. The relative standard deviation (RSD) of the replicates provides the analysis variation and indicates the precision of the test method. The mean of the replicates (in percentage), suggests the accuracy of the test method.

Evaluation of the accuracy of the process, three spiked solutions of the metals at three levels of concentrations (1.0, 100.0, and 1000.0 µg/L) were prepared by spiking specific volume of the metals stocks solution into a blank (Mili-Q water). The solutions were analysed with three runs performed for every concentration, and the responses recorded. The recovery of the metals was calculated as a percentage of the spiked solution. The average recovery and the RSD for each level were calculated. The ICP-MS method used in this study showed an acceptable performance for the analysis of the metals, with a high correlation coefficient value >0.999. Table 7.2 shows the mean % recovery of the metals from water at the three concentration levels (1.0, 100.0, and 1000.0) ranged from 97 to 109%.

Table 7.2: Validation data for the analysed heavy metals

Element	Isotope mass	Integration time (sec)	Method Accuracy %	LoD (µg/L)	R^2
Cr	52	0.3	102	0.26	0.9995
Mn	55	0.3	103	0.23	0.9995
Ni	60	0.3	105	0.07	0.9995
Cu	63	0.3	107	0.16	0.9995
Zn	66	0.3	106	0.11	0.9995
As	75	0.5	97	0.03	0.9995
Cd	111	0.3	101	0.01	0.9995
Hg	201	0.3	109	0.02	0.9995
Pb	208	0.3	103	0.01	0.9995

The limit of detection (LoD) is the lowest concentration of an analyte in a sample, which can be detected under the stated experimental conditions. To determine the LoD, blank (Mili-Q ultra-pure water) was aspired, and signal intensities for the blank recorded. After that, a solution of 5.0 µg/L of all the metals was aspired, and their signal intensities recorded. The limit of detection was calculated from the result as:

$LoD = \frac{3.SD(blank).Conc(sample)}{I(sample)-I(blank)}$ Where SD (blank) is the standard deviation for the signal recorded on the blank, Conc (sample) is the concentration (µg/L) of the analyte in the sample, I (sample), I

(blank) are the signal intensities recorded for the sample and blank respectively. Table 7.2 shows the LoD of the metals analysed in this study.

7.5 Results and Discussion

Table 7.3 shows the descriptive statistics of the metals in the samples. Chromium had the highest concentration in the samples with an overall mean of 432.81 µg/L. Nickel ranked second with a mean concentration of 318.01 µg/L, followed by manganese with 296.89 µg/L. The range of concentration of Cr in the samples was 0.2 µg/L to 9529.9 µg/L and nickel 3.6 µg/L to 3564.5 µg/L. Zinc had the least mean concentration of 80.85 µg/L in the samples. The results of the metal analysis for the individual sample were shown in Appendix VI (A and B). All the heavy metals were present in the samples at different concentrations, except for cadmium and lead that were below the detection limit in samples of Tyhume River midstream (T2B) and upstream (T1C) (Appendix V). The upstream samples of the rivers have far lesser concentrations of metals than other reaches; a trend observed by Silambarasan et al. (2012). The presence of heavy metals in some of the upstream samples may be related to weathering. Rain and erosion encourage leaching of these metals from the rocks, soil and farmlands (Pan, 2013).

Table 7.3: Descriptive statistics of the heavy metals in the water samples (values are µg/L)

	Cr	**Mn**	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Pb**	**Hg**
N	47	47	47	47	47	47	47	47	47
Mean	432.81	296.89	318.01	290.19	80.85	247.33	212.35	237.01	87.79
SD	1456.79	754.08	675.83	777.54	46.94	596.09	661.66	695.19	267.68
Min	0.28	0.7	3.6	4.2	29.2	0.2	<LOD	0.01	0.06
Max	9529.9	4187.3	3564.5	4352.7	299	2905.8	4279.3	3909.2	1187.01

Table 7.4 and 7.5 shows the concentration of some of the metals analysed in this work. Generally, the midstream and downstream samples of the rivers had higher metal concentrations than the upstream, as remarked under chapter 6, open waste dump characterised the rivers sampled and might have contributed to the high level of pollutants in them.

Among the wastewater, chromium concentration was highest in Alice wastewater influents samples (AW) with a mean value of 888.15 µg/L, but the treated effluents (AE) had 14.5 µg/L

(Table 7.4). Grahamstown wastewater influents (GW) had a mean value of 724.06 µg/L Cr, while the effluents (GE) had 23.23 µg/L or 3.21% of the metal released to the environment. On the average, Cr concentration is higher in Swartkops River samples (mean 1202.49 µg/L) and least in Bloukrans River samples (mean 164.04 µg/L). The motor vehicle assembly plant and other metal industries close to the downstream of the Swartkops River (SD) might have contributed to the metal load in these samples. Chromium in the river samples, especially at midstream and downstream reaches, might be due to its extensive usage in industries for electroplating of metals, chemical manufacturing, textile industries, petroleum refinery, automobile manufacturing, wood preservatives, alloy preparations and tanning industries (Faisal and Hasnain, 2006). The various uses of chromium made it a pollutant of the air, soil and water bodies. UNEP (2016) set 0.08 µg/L of Cr as a limit for high integrity water, and at 1.0 µg/L, the water is extremely impaired. South African directorate of water, environment and forestry, DWAF (1996) recommended a maximum of 0.01 µg/L of Cr in agricultural waters. Binning and Baird (2001) reported a range of 5.6-20.1 µg/L Cr concentration in Swartkops River samples, Watling et al. (1985) reported a range of 0.1-5.4 µg/L in Buffalo River and Sibanyoni (2011) reported a range of 2000-18000 µg/L in a five-year study of groundwater in Mpumalanga Province. Apart from four samples that show concentrations in the range of thousands, chromium in other samples are comparable to the observation of the above workers.

Manganese had its highest concentration, among the freshwater samples, in Swartkops River downstream samples (SD) with a mean value of 824.33 µg/L (Table 7.4). The presence of Mn in sample SD may be due to the same reason Cr was high in that sample. Bloukrans midstream samples (BM) with a mean concentration of 429.1 µg/L had the second-highest concentration of Mn. The least mean concentration of Mn was in Tyhume upstream samples (FU) with 1.9 µg/L. In the wastewater category, Alice wastewater influents samples (AW) had the highest mean concentration of Mn (2226.8 µg/L) while the effluents had 11.3 µg/L. Jackson et al. (2009) recorded a range of 100-300 µg/L while studying Plakenburg River and 100-200 µg/L in Diep River. Gilbert and Avenant-Oldewage (2014) observed 15.0 µg/L in their studies of Vaal River; Watling et al. (1985) observed a range of 1.0-610.0 µg/L in Buffalo River samples and Binning and Baird (2001) observed a range of 36.8-134.4 µg/L in Swartkops River. The target of the DWAF limit of Mn in agricultural water is 20 µg/L and not greater than 180 µg/L for aquatic life.

Table: 7.4 Concentrations of selected metals in the samples (values are means ± standard deviations).

Sample	Concentration (µg/L ± SD)				
	Cr	Mn	Ni	Cu	Zn
BU	2.92 ± 0.73	4.56 ± 2.22	24.42 ± 17.86	14.76 ± 9.22	58.00 ± 4.2
BM	425.15 ± 62.37	429.09 ± 58.34	356.29 ± 28.86	259.08 ± 32.2	124.87 ± 27.9
BD	64.09 ± 26.66	50.35 ±24.4	81.80 ± 29.4	80.42 ± 28.44	55.98 ± 11.62
FU	2.09 ± 1.4	1.92 ± 0.7	6.59 ± 2.6	5.66 ± 1.45	39.28 2.05
FM	685.12 ± 83.3	420.96 ± 41.8	698.62 ± 71.9	771.92 ± 57.57	80.45 ± 29.45
FD	130.99 ± 25.6	123.86 ± 37.51	557.85 ± 134.65	154.32 ± 75.32	60.93 ± 8.0
SU	33.55 ± 4.88	25.81 ± 13.06	47.45 ± 26.42	43.31 ± 26.37	60.67 ± 27.15
SM	6.40 ± 3.7	10.21 ± 6.22	21.67 ± 15.5	21.96 ± 10.15	72.27 ± 11.4
SD	3179.45 ± 423.62	824.31 ± 95.11	467.77 ± 75.82	224.64 ± 76.02	97.74 ± 16.64
TU	21.79 ± 9.35	24.51 ± 13.4	35.02 ± 19.6	30.51 ± 9.42	41.76 ± 18.44
TM	9.65 ± 4.6	6.15 ± 4.55	23.3 ± 10.46	20.53 ± 6.5	48.99 ± 19.8
TD	573.46 ± 42.6	367.44 ± 47.15	626.33 ± 79.48	711.54 ± 91.13	73.84 ± 6.09
GW	724.43 ± 29.6	601.78 ± 70.89	692.59 ± 83.54	788.67 ± 97.14	126.20 ± 42.11
GE	22.86 ± 9.08	46.89 ± 19.31	40.41 ± 10.46	43.99 ± 18.28	72.71 ± 10.93
KW	800.91 ± 91.74	510.47 ± 56.31	1804.83 ± 177.85	2194.53 ± 264.85	184.22 ± 81.51
KE	7.87 ± 1.6	11.96 ± 7.8	19.43 ± 7.5	25.44 ± 4.25	78.32 ± 2.25
AW	888.18 ± 67.05	2226.79 ± 960.5	417.94 ± 205.8	201.34 ± 46.7	89.98 ± 18.0
AE	14.50 ± 6.31	11.32 ± 9.6	222.21 ± 133.15	44.36 ± 12.65	83.21 ± 1.55
UE	53.85 ± 25.5	91.09 ± 44.05	142.77 ± 93.45	83.75 ± 29.15	94.69 ± 16.6

Bloukrans River: upstream (BU), midstream (BM), downstream (BD). Buffalo River: upstream (FU), midstream (FM), downstream (FD). Swartkops River: upstream (SU), midstream (SM), downstream (SD). Tyhume River: upstream (TU), midstream (TM), downstream (TD). Grahamstown wastewater: influents (GW), effluents (GE). King Williams Town wastewater: influents (KW), effluents (KE). Alice wastewater: influents (AW), effluents (AE). Uitenhage treated effluents (UE).

Nickel concentration in the freshwater was highest in Buffalo midstream (FM) samples with a mean value of 698.6 µg/L, and least in the upstream samples (FU) with 6.6 µg/L (Table 7.3). In the wastewater category, Ni has its highest mean concentration in King Williams Town influent samples (KW) with 1786.65 µg/L and 37.6 µg/L in the effluents. The Ni concentrations in Bloukrans River samples ranged from 74.0 to 849.6 µg/L, Buffalo 4.0 to 1370.5 µg/L, Swartkops 6.2 to 1331.5 µg/L and Tyhume 3.6 to 1839.0 µg/L. Nickel is naturally available in the soil and

the fifth most common element on the earth (Harasim and Filipek, 2015). It may also get into the environment through industrial wastewater, and air pollutants washed into rivers. Nickel can be mobilised from the soil by acid rain. Watling et al. (1985) recorded a Ni concentration range of 1.0 to 132 µg/L in Buffalo River. Awofolu et al. (2004) observed a mean concentration of 1777.0 µg/L Ni in Tyhume River, while Jackson et al. (2009) recorded a range of 100 to 500 µg/L and 100 to 400 µg/L in Plakenburg and Diep Rivers respectively. Gilbert and Avenant-Oldewage (2014) recorded Ni level of 6.3 µg/L in the Vaal River. UNEP (2016) proposed 20 µg/L concentration of Ni for freshwater, while DWAF set a limit of 0.2 ng/L for nickel in agricultural waters.

The highest mean concentration of copper (Cu) among the freshwater samples was observed in Buffalo midstream (FM) samples with a mean value of 771.9 µg/L and least in the upstream samples (FU) with 5.66 µg/L. Among the wastewater samples, King Williams Town influents (KW) had the highest concentration of Cu with 2194.53 µg/L and least in the effluents (KE) with 32.5 µg/L. The mean concentration of copper in Bloukrans, Buffalo, Swartkops and Tyhume Rivers for all the seasons were 118.1 µg/L, 310.63 µg/L, 105.99 µg/L 283.41 µg/L respectively. Binning and Baird (2001) observed the Cu concentration range of 4.3 to 21.1 µg/L in the Swartkops River. Watling et al. (1985) recorded a concentration range of 0.2-29.0 µg/L in Buffalo River, and Awofolu et al. (2004) recorded 383.0 µg/L in the Tyhume River. The maximum limit of copper recommended by UNEP was 1.0 µg/L and DWAF 0.0 µg/L for the aquatic ecosystem, while DWAF allowed 200 µg/L in agricultural water. The result of these analyses shows that copper is present in the sampled rivers above the recommended levels.

The midstream samples of Bloukrans River (BM) had the highest mean concentration of Zn among the rivers with 124.9 µg/L, followed by SL with 97.73 µg/L (Table 7.3). The least concentration in that category was recorded in the upstream samples of Buffalo River (FU) with 39.28 µg/L. The mean concentration of zinc in Bloukrans River samples was 79.62 µg/L, Buffalo 60.2 µg/L, Swartkops 77.47 µg/L and Tyhume 55.59 µg/L. In the wastewater category, King Williams Town effluent samples (KW) ranked highest with a mean value of 184.22 µg/L Zn while the effluents had 78.32 µg/L. Alice wastewater influent samples (AW) had a mean concentration of 89.98 µg/L Zn while it was 83.21 µg/L in the effluents. Awofolu et al. (2004) recorded 18.0 µg/L of Zn in

Tyhume River, Watling et al. (1985) reported 0.1-62.0 µg/L in Buffalo River and Binning and Baird (2001) reported a range of 9.0 to 173.0 µg/L in Swartkops River. Jackson et al. (2009) recorded 100 - 1100 µg/L of zinc in Plakenburg River, and 100 - 2500 µg/L in Diep River and Gilbert and Avenant-Oldewage (2014) recorded 0.24 µg/L in Vaal River. UNEP (2016) recommended 8.0 µg/L of Zn and DWAF (1996) 2.0 µg/L for a healthy water ecosystem.

The downstream samples of the Swartkops River (SD) had a mean concentration of 741.63 µg/L arsenic, the highest among the freshwater samples (Table 7.5). Next were the midstream samples of Buffalo River (FM) with a mean concentration of 518.4 µg/L. In Buffalo River, the level ranges from 0.4 to 1025.8 µg/L, 0.8 to 2205.8 µg/L in Swartkops, and Tyhume 0.2 to 1347.9 µg/L. Among the wastewater samples, King Williams Town influents (KW) had the highest mean concentration of As with 1462.66 µg/L and least in its treated effluent with 9.25 µg/L. Appendices V A and B show the concentration of arsenic in each sample. The mean concentration of arsenic in Bloukrans River was 122.51 µg/L; Buffalo 214.43 µg/L, Swartkops 288.86 µg/L and Tyhume 182.61 µg/L. Arsenic concentrations of 0.98 - 1.23 µg/L and 0.43 - 0.44 µg/L were observed for Guguletu and Langa Rivers in Cape Town, respectively (Akinsoji et al., 2013). Gilbert and Avenant-Oldewage (2014) reported 28.97 µg/L in the Vaal River and Ahoule et al. (2015) reported various concentrations of arsenic in African waters. Arsenic is naturally present in the soil from where it gets into surface water and groundwater; this might account for its presence in the water samples. Other sources of arsenic in rivers include herbicides, fungicides, foundry works and combustion of fossil fuels. Arsenic may be excreted through the body fluids such as urine, sweat, and breast-milk (Georgescu et al., 2011), and hence makes human and animal milk sources of contamination to young human and animals. The environmental standard set by UNEP (2016) for As in water was 10 µg/L, while DWAF set 100 µg/L for agricultural waters. Arsenic concentrations in the river samples were higher than these recommended values.

Table 7.5: Concentrations of some of the selected metals in the samples (values are means ± standard deviations).

Sample	Concentration (µg/L ± SD)			
	As	**Cd**	**Pb**	**Hg**
BU	3.38 ± 1.33	2.67 ± 1.08	2.90 ± 1.53	2.53 ± 1.53
BM	301.50 ± 75.73	279.88 ± 31.76	194.93 ± 37.68	18.93 ± 16.22

BD	62.65 ± 24.64	32.36 ± 12.62	57.46 ± 26.22	2.15 ± 2.04
FU	3.61 ± 1.2	0.72 ± 0.68	1.16 ± 0.68	0.22 ± 0.035
FM	518.41 ± 57.4	330.34 ± 72.05	598.05 ± 89.52	411.01 ± 410.8
FD	121.31 ± 61.1	76.26 ± 28.95	119.65 ± 42.05	12.49 ± 11.97
SU	22.91 ± 19.33	15.69 ± 9.93	25.14 ± 13.33	7.75 ± 6.95
SM	8.67 ± 5.50	5.54 ± 2.45	5.52 ± 2.45	0.31 ± 0.16
SD	741.63 ± 97.61	1480.60 ± 367.8	181.77 ± 65.80	71.68 ± 63.94
TU	22.58 ± 10.35	46.92 ± 7.45	50.30 ± 4.99	3.07 ± 1.03
TM	10.67 ± 5.55	57.94 ± 4.62	55.52 ± 6.51	1.190 ± 0.82
TD	457.30 ± 58.42	286.45 ± 62.22	596.01 ± 77.54	0.18 ± 0.082
GW	574.61 ± 63.93	356.22 ± 47.04	719.92 ± 91.90	356.95 ± 272.04
GE	26.82 ± 13.33	12.87 ± 6.84	21.50 ± 7.84	3.69 ± 1.55
KW	1462.66 ± 452.45	372.53 ± 65.90	1962.58 ± 453.9	312.45 ± 211.58
KE	9.25 ± 0.95	4.84 ± 1.85	6.82 ± 1.85	1.04 ± 0.17
AW	247.42 ± 50.10	426.79 ± 107.5	95.33 ± 12.35	22.55 ± 9.77
AE	30.74 ± 12.05	8.11 ± 5.7	13.88 ± 3.95	3.27 ± 2.11
UE	79.55 ± 35.47	37.80 ± 11.58	37.49 ± 13.67	4.89 ± 4.22

Bloukrans River: upstream (BU), midstream (BM), downstream (BD). Buffalo River: upstream (FU), midstream (FM), downstream (FD). Swartkops River: upstream (SU), midstream (SM), downstream (SD). Tyhume River: upstream (TU), midstream (TM), downstream (TD). Grahamstown wastewater: influents (GW), effluents (GE). King Williams Town wastewater: influents (KW), effluents (KE). Alice wastewater: influents (AW), effluents (AE). Uitenhage treated effluents (UE).

Cadmium has its highest mean concentration in the downstream samples of Swartkops River (SD) with 1480.6 µg/L. The least observed mean concentration of Cd was 0.72 µg/L in upstream samples of Buffalo River (FU). The ranges of concentrations observed for Cd in the river samples are Bloukrans 0.1 to 756.4 µg/L, Buffalo 0.04 to 659.3 µg/L, Swartkops 0.1 to 540.9 µg/L and Tyhume 0.0 to 847.3 µg/L (Table 7.5). Some Tyhume samples had Cd concentrations below the detection limit (Appendix 5). The highest concentration of Cd among the wastewater samples was in Alice influent samples (AW) with 426.79 µg/L, and least in the treated effluents (AE) with 8.11 µg/L. The mean concentration of cadmium per annum in Bloukrans River was 104.97 µg/L, in Buffalo it was 135.77 µg/L, Swartkops 562.5 µg/L and Tyhume 152.32 µg/L. The presence of Cd in the surface waters may be due to natural and industrial activities such as smelting and refining,

mining, manufacturing of batteries, paints, and pigments released to the environment (Friberg et al., 2018). Awofolu et al. (2004) observed cadmium level in Tyhume River to be 30 µg/L, and Watling et al. (1985) recorded 0.04-1.12 µg/L in Buffalo River. UNEP (2016) recommended 0.08 µg/L of Cd for environmental water while in South Africa, DWAF (1996) limit it to 10 ng/L. The concentrations of Cd recorded in this study were higher than the recommended values.

The highest mean concentrations of Lead (Pb) in the freshwater samples was 598.05 µg/L in the midstream samples of Buffalo River (FM), followed by downstream samples of Tyhume River (TD) with 596.01 µg/L and least in FU with 1.16 µg/L. Among wastewater samples, Alice influents (AW) had 426.8 µg/L of Pb while it was 8.1 µg/L in the effluents (AE). Table 7.5 shows the mean concentration of Pb in the samples. Some samples from the Tyhume River, such as T2B and T1C, had concentrations below the detection limit of 0.01 µg/L (Appendix V). Bloukrans River samples had a mean concentration of 85.1 µg/L Pb, Buffalo 239.62 µg/L, Swartkops 78.98 µg/L and Tyhume 307.98 µg/L. Lead is present cosmetic and children products, industrial wastewater and released during the combustion of fossil fuels [Fuad and Al-Momani, 2018; Gao et al., 2018) from where it enters wastewater and surface waters. Awofolu (2004) observed 24.0 µg/L Pb concentration in the Tyhume River. Binning and Baird (2001) recorded 4.1 - 62.0 µg/L for Swartkops River. Watling et al. (1985) observed 1.0 - 207.0 µg/L in Buffalo River and 0.032 µg/L in the Vaal River (Gilbert and Oldewage, 2014). DWAF recommended a maximum of 200 µg/L and 0.02 µg/L of lead for agricultural and aquatic life, respectively. UNEP (2016) recommended 2.0 µg/L of Pb in the aquatic environment.

Mercury (Hg) has low concentrations in both the freshwater and wastewater samples with most of the individual sample having concentrations close to the limit of detection. Midstream samples of Buffalo River (FM) had the highest mean concentration of 411.01 µg/L and least in the downstream samples of Tyhume River (TD) with 0.18 µg/L (Table 7.5). The mean concentrations of Hg in the rivers were Bloukrans 7.78 µg/L, Buffalo 141.24 µg/L, Swartkops 29.86 µg/L and Tyhume 149.86 µg/L. The presence of Hg in the samples may be due to its usage in manufacturing industries, smelting and mining. It is present as a component of many electrical instruments, medical products and appliances (Iavicoli et al., 2009). Mercury may spread through air pollution and runoff from mining industries (Obrist et al., 2018). Watling et al. (1985) reported a range of

0.12 to 8.12 µg/L Hg in Buffalo River, and Walters et al. (2011) observed a concentration of 5.00 ng/L in Vaal River. DWAF recommended a maximum of 0.04 ng/L Hg in agricultural waters. UNEP (2016) recommended 0.05 µg/L of Hg for water ecosystem.

Generally, downstream samples of Swartkops River (SD) had higher concentrations of metals than other freshwater samples, which may be due to the presence of Volkswagen motor assembly plant and foundry industries close to the source of these samples. Alice WWTP is operated by Fort Hare University, which might have contributed to the heavy metal contents of Alice wastewater through laboratory runoff. The WWTPs were able to reduce the concentrations of the heavy metals during treatment since their concentrations were less in the effluents (Table 7.6). The removal of Zn from the three WWTPs was not as effective as other metals. Crane et al. (2010) observed that membrane filtration was unable to reduce the concentration of Zn in wastewater effluent significantly and concluded that its removal in biological wastewater treatment processes might be due to factors influencing metal solubility. The WWTPs in Grahamstown and King Williams Town appeared to perform better than the one in Alice concerning the removal of metals from wastewater.

Table 7.6: The efficiency of the WWTPs at removing the metals from wastewater

	Removal efficiency (%)		
Metal	**Grahamstown WWTP**	**King Williams Town WWTP**	**Alice WWTP**
Cr	96.84	99.02	98.36
Mn	92.21	97.66	99.49
Ni	94.16	98.92	46.83
Cu	94.42	98.84	77.97
Zn	42.38	57.48	7.52
As	95.33	99.37	87.57
Cd	96.38	98.70	98.09
Pb	97.01	99.65	85.44
Hg	98.96	99.67	85.49

A comparison of these results with previous analyses shows that environmental concentrations of some of these metals were increasing in Swartikops, Buffalo, and Tyhume Rivers. Zhang et al. (2018) attributed this type of increase in environmental heavy metals to the rise in the population and economic development. In the year 2000, South Africa population was 45.7 million but increased to 57.8 million in 2018 (The World Bank, 2019). The increase in population will also create pressure on the environment and cause an expansion in agricultural activities with the application of more agrochemicals to boost agricultural productivity. More agrochemical input might have also contributed to the observed levels of the heavy metals in this study. Increase in the human population will necessitate upgrading WWTPs facilities to handle the increased inflow of wastewaters for their efficiency to be sustained. Li et al. (2019) observed a general increase in the concentrations of Cd, Cr, Cu, Ni, and Mn in the global water system over the period between 1970 and 2017, which were attributed mainly to wastes discharge and weathering in the African continent. The results of this research tend to support their observations. The effects of seasons were not noticeable on the levels of metals in the water samples. Rain is scanty in South Africa; the spring may not witness more rainfall than the winter. The upstream samples had far lesser

concentrations of metals than other reaches, a trend observed by Xu et al. (2018) and Silambarsan et al. (2012).

Table 7.7 shows the correlation statistics of the samples based on the metal composition. It expresses the degree of variability of one sample from the other. Pearson's correlation coefficient was used in this computation (Shroff et al., 2015). Samples vary according to their metallic composition and concentrations. Correlation of 0.5 and above are strong positive correlation while -0.5 or below are strong negative correlation. From the table, BU/BU (Bloukrans upstream) has a coefficient of 1. At the same time, BU/BM (Bloukrans upstream and midstream) showed a negative correlation (-0.27315). Samples BU/FU (Bloukrans and Buffalo upstream) showed a correlation coefficient of 0.9635, indicating that the two samples are almost similar regarding metallic composition. The table also indicates that some treated effluents correlate with wastewaters (GE/KW; UE/GW).

Table 7.7: Coefficients of correlation of the heavy metals at the sampling sites

0	BU	BM	BL	FU	FM	FL	SU	SM	SL	TU	TM	TL	GW	GE	KW	KE	AW	AE	UE
BU	1																		
BM	-0.27315	1																	
BL	0.2829	0.5852	1																
FU	0.9635	-0.32057	0.17438	1															
FM	-0.49235	0.44603	0.53495	-0.62658	1														
FL	0.16359	0.44181	0.62664	-0.072883	0.50598	1													
SU	0.85359	0.13508	0.7137	0.76864	-0.034771	0.42402	1												
SM	0.98317	-0.2395	0.29804	0.98407	-0.52876	0.040001	0.84935	1											
SL	-0.36004	0.63318	0.09423	-0.29394	0.22983	-0.040189	-0.12665	-0.32258	1										
TU	-0.49067	0.3568	0.50554	-0.62791	0.99141	0.4914	-0.057076	-0.5315	0.12701	1									
TM	0.97757	-0.15772	0.42563	0.9429	-0.38979	0.19285	0.90922	0.97814	-0.26749	-0.40111	1								
TL	0.91669	-0.092195	0.31546	0.93102	-0.53999	0.0076827	0.8166	0.95724	-0.31267	-0.55009	0.9056	1							
GW	0.53268	0.34946	0.43382	0.51103	-0.2279	0.14806	0.62248	0.58994	-0.19555	-0.25663	0.53506	0.78691	1						
GE	-0.50875	0.5654	0.60679	-0.61336	0.9603	0.4595	-0.01524	-0.51411	0.24233	0.95327	-0.40391	-0.46367	-0.068706	1					
KW	-0.16605	0.20339	0.69916	-0.29452	0.79945	0.51564	0.2091	-0.1852	-0.22867	0.84348	-0.065375	-0.22044	-0.069133	0.81189	1				
KE	0.96982	-0.10998	0.4902	0.92975	-0.3493	0.23614	0.93392	0.97234	-0.28379	-0.35684	0.99417	0.91517	0.57949	-0.34095	-0.00014	1			
AW	0.54111	0.15496	0.54731	0.32318	0.18618	0.90239	0.64139	0.41368	-0.23915	0.18054	0.54269	0.33215	0.24419	0.10697	0.32975	0.56532	1		
AE	-0.25398	0.70617	0.068405	-0.241	0.016796	0.045071	-0.092948	-0.20324	0.36327	-0.050346	-0.26118	0.060968	0.59136	0.18029	-0.26387	-0.22652	-0.15854	1	
UE	0.53182	0.50125	0.78028	0.37837	0.16192	0.76225	0.75311	0.49634	-0.13022	0.12321	0.5951	0.55071	0.66689	0.2047	0.33426	0.64095	0.80929	0.23969	1

Bloukrans River: upstream (BU), midstream (BM), downstream (BD). Buffalo River: upstream (FU), midstream (FM), downstream (FD). Swartkops River: upstream (SU), midstream (SM), downstream (SD). Tyhume River: upstream (TU), midstream (TM), downstream (TD). Grahamstown wastewater: influents (GW), effluents (GE). King Williams Town wastewater: influents (KW), effluents (KE). Alice wastewater: influents (AW), effluents (AE). Uitenhage treated effluents (UE).

Figure 7.1 shows the partial least square-discriminant analysis (PLS-DA) variable importance projection (VIP) scores for the metals. The metals were ranked according to their concentrations and distribution in the water samples. Chromium and cadmium were of high priority in Swartkops River, manganese in Alice wastewater, lead, copper, arsenic and zinc in King Williams Town wastewater. Reducing the levels of these heavy metals in the rivers should be a matter of priority.

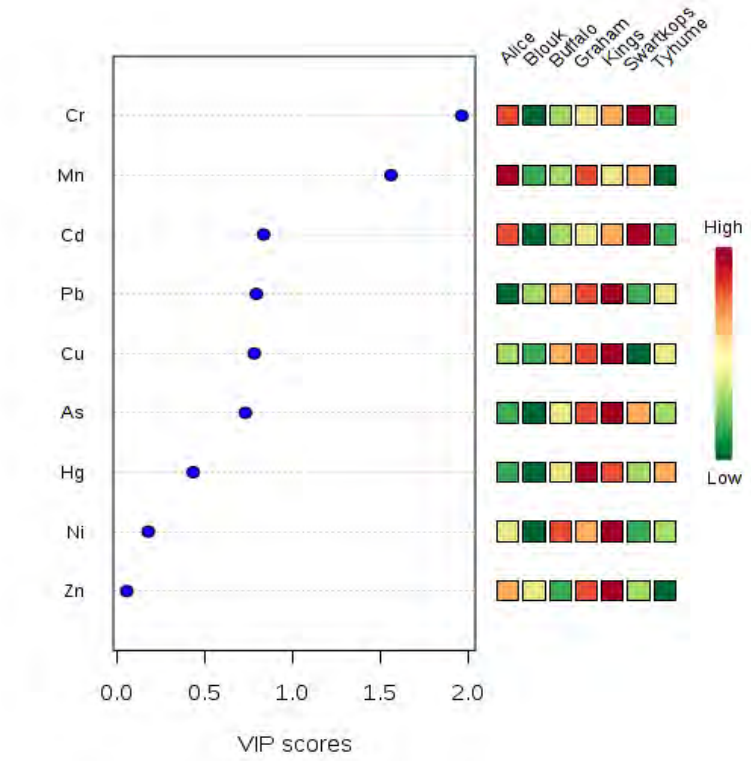


Figure 7.1: Important features identified by PLS-DA. The coloured boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study.

7.6 Conclusion

This study shows that heavy metals were present in all the rivers and wastewaters analysed. The concentrations of the heavy metals vary along the river reaches, with midstream and downstream having higher levels than the upstream. Sources of heavy metals in the rivers include wastewater effluents from WWTPs. Heavy metals concentrations, in this study, were higher in the rivers than the recommended values for the aquatic environment. These high values were observed at the midstream and downstream reaches of the rivers. Comparison with previous records shows that

the concentrations of the heavy metals in the river were increasing. It can be concluded that the waters were polluted with heavy metals.

CHAPTER 8

GENERAL DISCUSSIONS, CONCLUSION AND RECOMMENDATIONS

8.0 Introduction

This chapter generally discusses the overall importance of this research. The outcomes of various analyses of water samples form the basis of the general discussion and conclusion in this chapter. The promotion of better water quality of rivers in the Eastern Cape Province of South Africa is the focus of the recommendations for future research.

8.1 General Discussion

Water bodies are always the recipient of wastes released to the environment, whether in the air or land. Pollutants in the air and soils are always washed into water bodies by rain and leaching, making the pollutants in them available for humans, animals and plants uptake. The problems facing global freshwater bodies made them targets of sustainable development goals (SDG). The biodiversity of freshwater ecosystems has been degraded more than any other ecosystem because of pollution, habitat degradation, change in land use and climate (Weiss et al., 2018; Bogardi et al., 2012; Reid et al., 2013; UN-Water, 2016). Poverty and an increase in population size, especially among developing countries, had worsened the problem of freshwater. Increased human activities have aggravated the problem of nutrient reaching water bodies, which encourage eutrophication (Human et al., 2018). Untreated and poorly treated municipal wastewater encouraged the growth of pathogens and introduced organic and micro-pollutants into freshwater bodies. The mining industries introduce mine wastes rich in heavy metals and acids from mine runoff into water bodies (Mason et al., 2019). Chemical, pharmaceutical and agricultural industries pollute water bodies with chemicals compounds with far-reaching effects, not only on the aquatic ecosystems but also on all lives that depend on these water bodies. Some of these pollutants can modify the physiological processes of man and other animals when taken up (Huang et al., 2019; Chen et al., 2018).

The physicochemical characteristics of the rivers, wastewaters, and their treated effluents analysed in this study showed that much improvement is needed to bring the effluents to environmentally sustainable standards. The result of this study and those of other researchers, mentioned in Chapter

4, showed that chemical oxygen demands (COD) of the treated effluents were above the recommended level (DWAF, 1996). The high concentration of phosphate ions observed in treated effluents and the lower reaches of the rivers will promote eutrophication, which is another problem in water resources management (Paddeda et al., 2017). Chlorides and sulphates were reported to be a long-standing problem in South Africa freshwater because of the chemical weathering of the rocks and salinisation (Huizenga, 2011). This research shows that runoffs and wastewater effluents also contribute to ion contamination of the rivers. Despite the high permissible levels set for chloride and sulphate ions, their effluents and concentrations were higher than the environmental standards. Salinity was higher than 1.0 mg/L in the lower reaches of Bloukrans and Swartkops Rivers, an indication of salinisation. These observed physicochemical characteristics of the waters were enough to cause distress to the aquatic wildlife, loss of biological integrity of aquatic ecosystems, gradual decline in the volume of water available, progressive worsening of water quality, and continually rising costs associated with treating water for reuse (Ashton 2010). If the current trend in water quality deterioration continues, it may prevent the achievement of social and economic growth and the elimination of poverty (Maree et al., 2016).

Analyses of the water samples for chemical functional groups with FT-IR and NMR spectroscopies showed that there are more pollutants in the water samples than imagination. The chemical functional groups encountered in this study were indications that there is a need for more research to achieve better water quality management. The untargeted spectroscopies (FT-IR and ^{1}H-NMR) used in this research, showed the presence of groups related to volatile organic compounds (VOCs) and persistent organic pollutants (POPs) in the samples. VOCs such as methylene chloride and fluorocarbons, organochlorides, and substituted aromatic compounds, are toxic to humans and wildlife (USEPA, 2005; Loganathan and Masunaga, 2015). Chemical functional groups of some drugs such as antibiotics, analgesic and anticancer agents were present in the water samples indicating the possibility of these drugs reaching the surface waters from domestic and industrial sources, including agricultural farms. These are groups of emerging contaminants in surface waters, which required in-depth analysis. The presence of drugs-related compounds in the environment will aid the adaptation of microorganisms to such compounds and produce resistant strains (Kraemer et al., 2019).

Emerging contaminants pose a threat to water treatment and reuse because conventional water treatment plants were not designed to eliminate them from wastewater (Krzeminski et al., 2019). The burden of emerging contaminants all over the world started with industrial development and has become a global problem (Adeel et al., 2017). The concentrations of emerging contaminants posed a significant threat to water management since some of them, like the EDCs, are active at low levels (Bai and Acharya, 2019). The accurate measurement of these compounds is a problem in both industrial and scientific laboratories all over the world (Armstrong, 2017). Developments in analytical chemistry and equipment had helped track such chemical compounds in the environment, especially in water samples. Recently various chromatographic techniques coupled with mass spectrometry had been developed and employed to monitor these minute pollutants in water (Scognamiglio et al., 2016). The low concentrations of EDCs in water samples pose significant problems to analysts because not all analytical instruments can accurately measure them at the nanogram concentration range. Some earlier works on EDCs analysis using gas chromatography coupled with mass spectrometry (GC-MS) (Moreira et al., 2015; Winnike et al., 2015; Ronderos-Lara et al., 2018). GC-MS is useful in monitoring highly hydrophobic and volatile compounds but may not be appropriate for non-volatile compounds, except its volatility is enhance through a time-consuming procedure (He and Aga, 2019). The second method commonly and widely used for EDCs analysis is liquid chromatography coupled to mass spectrometry (LC-MS). LC-MS is highly selective, sensitive, and capable of determining a wide range of polar and amphiphilic compounds without derivatisation (Lindholm et al., 2014). It can analyse thermally labile compounds that are not suitable for gas chromatography (Kanda and Glendinning, 2011). This research used LC-MS with positive ion electrospray ionisation (ESI-MS/MS) for the analysis of ten EDCs in the water samples. The EDCs are moderately polar and would not ionise in solution (He and Aga, 2019). Also, LC-MS has a lower limit of detection (LoD) than GC-MS, and that makes it a better detector, if combined with solid-phase extraction (SPE) of samples, better results are achieved (Tsakelidou et al., 2017). For better results, this research combines SPE with LC-MS to monitor ten endocrine-disrupting compounds in water samples in this work. The categories of EDCs analysed in this work include the mammalian hormones (oestrone and 17β-oestradiol); plasticiser (bisphenol A); pesticides (atrazine, 2,4-dichlorophenol and triclosan); food preservative (4-octylphenol); pharmaceuticals and personal care products (nonylphenol, imidazole and triazole). Some of these compounds have many applications and fall into different categories.

Among all these compounds, the control of personal care products as represented by nonylphenol, pesticides (atrazine, 2,4-D and 4-octylphenol) and plasticisers should be a matter of priority, because their concentrations are increasing in the environment, as compared to earlier observations. The increase of these contaminants in the environment may be due to population increase. Zhang et al (2018) attributed increase of environmental pollutants to increase in the population and economic development. In the year 2000, South Africa population was 45.7 million but increased to 57.8 million in 2018 (World Bank, 2019). This increase will also create pressure on the environment and cause expansion in agricultural activities. Application of agrochemicals to boost agricultural productivity might have also contributed to the observed increase in the concentrations of pollutants. Imidazoles and triazoles encountered in the samples are common in the production of drugs, especially antimicrobials (Binh et al., 2018; Buil et al., 2019), apart from being EDCs, their presence in the environment may promote mutation and resistance in target organisms.

Endocrine-disrupting compounds (EDCs) have gotten the attention of governmental, non-governmental organisations and researchers all over the world for the problems associated with them (Attina et al., 2019; Cao et al., 2019). Researchers draw their knowledge of EDCs through experiments and observations obtained from organisms and ecosystems they live in, which had been the basis for decisions at the governmental levels (Street et al., 2018). Biomonitoring has severally indicated the presence of EDCs in humans and the most vulnerable groups are children because various products such as baby feeding bottles and toys contain EDC products (Szczepanska et al., 2016). Their presence in pregnant women, including breast milk, makes the unborn babies and neonates vulnerable (Park et al., 2019; Chemek and Nevoral, 2019). The concern is the increasing trends in EDCs related disorders in man and wildlife, as observed in:

-low sperm count and semen quality among young men in some countries;
-increased in the incidence in genital malformations, especially among male babies;
-increase rate of thyroid hormone-related neuro-behavioural disorders among children;
-incidence of adverse pregnancy outcomes;
-increase in endocrine-related cancers all over the world;
-increase in the global incidence of Type 2 diabetes and obesity (UNEP, 2019).

This study uses modern spectroscopy to monitor the presence of heavy metals in environmental water samples as a means of contributing to resource direct measurement (RDM) in South Africa. Conventional analysis of metals in water samples may involve atomic absorption spectrometry (AAS), inductively coupled plasma with optical emission spectrometry (ICP-OES) and inductively coupled plasma with mass spectrometry (ICP-MS). The advantages of ICP-MS used in this research include high element sensitivity and selectivity with excellent detection limits (Schone and Ottleben, 2017; Bolea-Fernandez et al., 2017; Ammann, 2007), which aided the achievement of 0.01 µg/L limit of detection (LoD). All the nine heavy metals analysed were present in the samples at concentrations higher than the specified environmental limits, especially at midstream and downstream reaches of the rivers, indicating that inputs were possibly from the waste-dump and runoffs. The higher values observed in this study over previous studies highlighted in Chapter 7 might be due to better equipment and or an increase in the population pressure on water resources. Local economic activities such as the presence of metal industries, reflected in the concentrations of these heavy metals in the water samples. Inappropriate disposal of electronics products is also contributing to the presence of these heavy metals in the environment (He et al., 2017). The health effects of these metals were discussed in chapter 2.

South Africa is among the countries with limited water resources and projected to have a water crisis by the year 2025 (Rijsberman, 2006). About 77% of the needed water resources in South Africa are from surface water, which is under threat due to relatively low rainfall (about 450 mm annually) with little runoffs to boost the surface water (Binns et al., 2001). The country is developing with an increasing population, and so is the demand for use and access to water resources. Expansion of industrial and agricultural activities and the population pressure had impacts on the water quality, availability and the state of aquatic ecosystems (Maree et al., 2016). Reports showed that 50 000 litres of untreated wastewaters flow into South Africa rivers every second because most municipalities are unable to meet the cost of maintenance of the WWTPs (Kings, 2017). A report claimed that only 60 out of 824 WWTPs in South Africa release adequately treated effluents to the environment; the rest are releasing partially or untreated wastewater to the receiving rivers (Khumalo, 2017). This report supports the observation in the South Africa Green- and Blue Drop reports that some municipalities cannot effectively treat their municipal and industrial effluents to environmental standards without further compromising water quality (Maree

et al., 2016). The results of various analytical techniques in this study support the views raised in these reports.

Some of the endocrine disruptors banned in European countries were observed in this research, indicating that they are still in use in the country. Industrial uses of nonylphenol and bisphenol A are prohibited in the European Union (EU) (EUC, 2019). All pesticides with endocrine disruptive abilities, such as atrazine, had been prohibited by the EU parliament (Oziel, 2019). Some compounds that are under prohibition in developed countries are produced and exported to African countries that have no legislation or enforcement of appropriate legislation against them (Flynn, 2015). Contributing to the African load of EDCs are electronics wastes such as television, computers and refrigerators dumped in the continent as second-hand goods by advanced countries (Akbar, 2015). These have contributed toxic heavy metals, flame-retardants and plastics to the environment, thereby increasing the EDCs load in the continent (Bornman et al., 2017; Asante et al., 2016). The inflow of prohibited compounds into Africa and many other developing countries is due mainly to the lack of the needed infrastructure for comprehensive research into environmental issues that are necessary to form sound environmental management policies (Bornman et al., 2017). The inflow of these toxic products will increase the incidence of non-communicable diseases in poor and developing nations. While still battling with communicable diseases, African nations need to brace up for the non-communicable diseases (NCDs) that are currently prevailing on the continent, arising from these environmental pollutants. These NCDs are common among women, children and the poor in developing countries (Atiim and Elliott, 2016). There is a knowledge gap between EDCs and the prevalence of diseases such as diabetes, obesity and organ dysfunctions in the continent of Africa (Mbanya et al., 2014). It has been reported that type 2 diabetes (an endocrine-related disease) is increasing faster in Africa than in other parts of the world (Bornman et al., 2017). Africa has not been able to quantify the cost of diseases attributed to EDCs as done in the developed nations (Trasande et al., 2015). The advanced countries conduct researches into the effects of EDCs, but since EDCs are global problems, their effects apply to Africans. Evidence has shown that Africans and Asians may be at risk due to their exposure to some EDCs, such as pesticides, commonly used in the continents (Fang et al., 2015). In order to address the issues of EDCs in Africa, the University of Pretoria organised the first African conference on health effects of endocrine disruptors, 2 – 6 November 2015. The

conference aimed to identify the exposure and health effects of EDCs among African human and wildlife populations (Bornman et al., 2017).

UNEP (2019) acknowledged that close to 800 chemicals are capable of interfering with hormonal functions, but only a few were investigated and their mechanism of endocrine disruption described. Various governmental bodies had launched efforts to contain EDCs and minimise exposure. The European Commission (EU) proposed a strategy that targets the harmonisation of various legislations relating to EDCs to generate a coherent policy that will combat the menace (Oziel, 2018). Part of the EU strategy is to pay attention to areas and chemicals inputs into household consumer products that are not covered by specific legislation such as toys, food-packaging materials, cosmetics, among others. In the latest attempt to control EDCs' menace, the EU plans to minimise the overall exposure to EDCs by paying attention to vulnerable periods of life, such as pregnancy and puberty (Oziel, 2018). It also proposed the development of a robust research base, built on existing knowledge about EDCs with a focus on areas where there are gaps and promote active regular dialogue with stakeholders to build an all-inclusive and interactive knowledge base that will be made available to all citizens in a one-stop website on EDCs. South Africa can copy these initiatives to curb the increasing concentrations and spreading of endocrine-disruptive compounds in the environment.

All the four rivers sampled in this work were polluted and may not be able to support diversities of organisms necessary to keep a balanced aquatic ecosystem. The chemical oxygen demand and other physicochemical factors of the rivers will encourage the survival of bacteria and algae to the exclusion of other organisms. It was apparent that the WWTPs sampled in this work were not able of process the wastewater to environmentally acceptable standard and may need facilities upgrade. The analyses also show that toxic disinfectants used in some WWTPs were reaching the receiving rivers and may affect the aquatic organisms.

8.2 General Conclusion

This work investigated some water quality criteria in Bloukrans, Buffalo, Swartkops and Tyhume Rivers in the Eastern Cape Province of South Africa. It also investigated the municipal wastewater influents and effluents from WWTPs in the cities around these freshwater bodies. Findings from this research show that the surface waters and the treated wastewater effluents released into them

have physicochemical parameters higher than the prescribed standards. Results of chemical functional groups analysed showed the functional groups of various emerging contaminants in the rivers and wastewater effluents. Ten endocrine-disrupting compounds and nine heavy metals were present in the water samples including treated effluents with concentrations higher than recommended levels of South Africa DWAF and UNEP. Generally, the upstream river samples have lesser concentrations of all the parameters analysed in this research work, and the pollution status of the rivers follows the same pattern with midstream > downstream > upstream. Point sources (WWTPs) and non-point sources were the contributors to the pollution of the studied rivers.

8.3 Recommendations

- Water pollution is a global issue that needs a unified and internationally coordinated approach to minimise the pollutants and their effects in the environment. The unified approach should encompass the government, industries and consumers. The chemical industries should be able to formulate alternatives to toxicants, especially in agrochemicals and household consumer products.
- There is the need to upgrade WWTPs to handle the pollutants. The engineering industries also have a role to play here.
- There must be strict legislation and adequate monitoring to control the composition of industrial wastewater.
- Expired chemical products should not be allowed to find its way to the environment; the producers must be made to take back and safely dispose of the unused and expired products.
- Chemical industries must be made to develop environment friendly compounds as alternatives to the present situation, and all chemical products must carry eco-labels on the packaging to instruct handlers and end-users on the toxicity, handling and safe disposal of such compounds.
- There is a need for consumers' information through education on various products, especially household products with toxic compounds. It is not uncommon for the people to microwave their foods in plastic products due to ignorance of the implication to their health.

- There should be environmental education to discourage the people from using rivers to carry away their household wastes.
- End-users of water, especially for livestock and domestic, should be encouraged to do additional processing to make the water from rivers fit for use.
- Further research is necessary to develop microorganisms that could break down chemical compounds in WWTPs.
- The knowledge on chemical pollutants is presently scanty despite the substantial advances made in understanding it. This knowledge gap needs to be filled for better protection of the public and wildlife. More products and compounds need to undergo toxicity screening to understand their roles in human and ecological problems.
- Future studies may further explore the presence of specific toxic compounds whose functional groups were identified with NMR and FTIR.

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APPENDICES

Appendix I

Appendix I-A: Some chemical parameters of individual freshwater samples with their limit of detection (LOD)

Sample	COD (mg/L)	PO_4^- (mg/L)	Cl^- (mg/L)	NH_4^+ (mg/L)	SO_4^- (mg/L)	NO_3^- (mg/L)	pH
B1A	ND	ND	ND	22.52±1.33	ND	ND	6.76±0.08
B2A	476.61±48.7	4.57±1.21	ND	110.22±6.34	226.32±21.30	5.0±0	7.65±0.03
B3A	176.87±14.8	4.94±0.11	ND	149.60±15.25	373.13±51.25	6.67±2.22	8.13±0.05
B1B	ND	ND	14.79±1.3	18.42±2.18	ND	ND	7.56±0.07
B2B	156.80±29.5	11.01±0.39	57.68±11.0	114.66±44.85	239.97±150.77	6.67±2.22	8.17±0.03
B3B	70.41±14.4	11.44±0.11	52.68±3.1	89.84±21.39	139.92±71.92	5.0±0	6.80±0.32
B1C	18.91±4.0	<LOD	66.59±12.2	20.20±0.23	ND	ND	7.85±0.09
B2C	360.51±46.5	24.60±0.00	588.79±4.0	60.17±0.09	57.84±0.31	ND	8.36±0.00
B3C	178.16±7.8	11.61±0.03	537.37±7.2	25.06±3.00	ND	ND	7.91±0.02
F1A	ND	ND	ND	19.77±0.76	ND	ND	7.26±0.01
F3A	120.55±39.3	4.39±0.12	297.71±52.4	17.96±3.82	ND	ND	6.77±0.34
F1B	ND	ND	45.07±9.3	20.05±1.03	ND	5.0±3.33	7.43±0.06
F2B	129.61±44.3	ND	362.66±7.0	21.46±3.34	ND	15.0±3.33	8.24±0.03
F3B	ND	10.10±0.70	398.94±48.3	18.97±0.35	ND	ND	8.29±0.02
F1C	ND	ND	ND	14.39±0.05	ND	ND	9.04±0.08
F2C	383.39±0.0	ND	340.06±0.7	15.92±0.42	ND	6.67±2.22	9.82±0.09
F3C	170.40±1.1	8.77±0.01	445.90±1.9	14.27±0.09	ND	6.67±2.22	7.94±0.02
S1A	ND	ND	ND	20.60±0.90	ND	ND	6.10±0.02
S3A	ND	10.69±0.65	ND	43.78±7.83	ND	5.0±0	7.32±0.03
S1B	32.46±9.2	0.11±0.15	ND	29.62±3.16	ND	ND	9.92±0.07
S2B	237.72±150.2	2.40±0.45	64.13±0.9	64.28±3.98	72.90±13.38	6.67±2.22	10.61±0.07
S3B	104.36±49.2	22.14±1.18	51.07±2.8	37.89±8.23	ND	ND	10.73±0.01
S1C	18.91±4.0	0.14±0.16	120.01±0.5	34.94±0.25	ND	ND	10.16±0.07
S2C	102.00±16.5	8.16±2.17	484.33±2.8	33.13±0.65	ND	ND	8.68±0.01
S3C	178.16±7.8	24.98±0.26	486.02±5.0	32.47±0.05	ND	8.33±2.22	8.72±0.00
T1A	ND	ND	11.10±5.0	27.40±6.65	ND	ND	10.70±0.08
T3A	20.20±14.7	ND	130.54±23.9	23.84±2.01	ND	ND	7.73±0.01
T1B	ND	ND	ND	20.68±4.66	ND	ND	7.18±0.05
T2B	10.49±5.1	0.90±0.61	53.06±11.2	44.08±24.74	7.11±83.17	5.0±0	8.32±0.06
T3B	ND	1.59±0.61	41.61±20.0	30.54±7.04	ND	8.33±2.22	7.98±0.10
T1C	59.69±2.2	ND	106.10±0.3	20.52±0.16	ND	ND	8.22±0.01
T2C	234.23±5.9	2.89±0.00	222.08±0.1	21.34±0.00	ND	ND	10.37±0.18
T3C	122.24±1.9	0.99±0.04	268.28±0.0	94.60±0.18	173.65±0.61	5.0±0	8.68±0.02

Appendix I-A continue

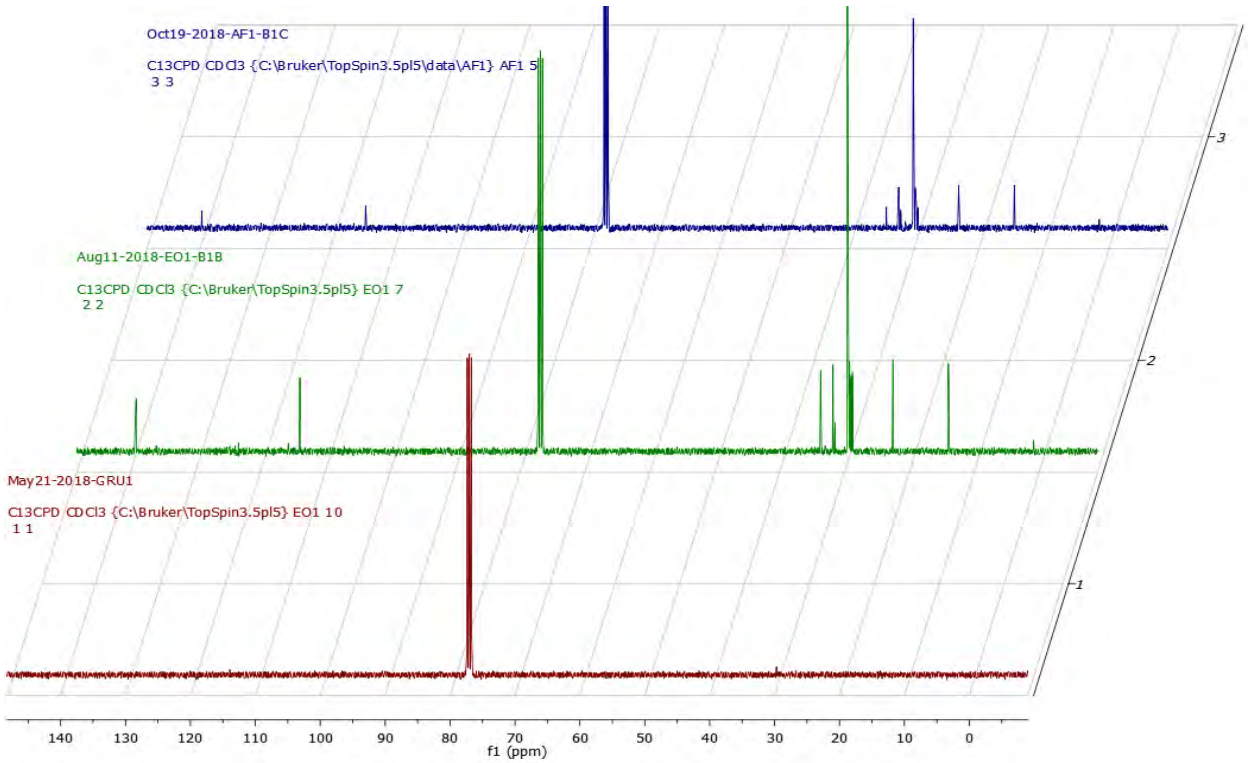
Sample	ORP (mV)	DO(mg/L)	Conductivity (µS/cm)	TDS (mg/L)	Turbidity (NTU)	Temp (^{0}C)	Flow Rate (m/s)	Salinity (PSU)
B1A	52.57±1.29	4.31±0.03	227.67±0.44	114.00±0.00	6.76±0.08	18.01±0.01	0.99±0.02	0.11±0.00
B2A	65.93±0.75	3.20±0.31	1427.67±7.56	712.67±1.56	118.38±3.08	19.13±0.04	0.18±0.02	0.72±0.00
B3A	61.57±0.45	1.98±0.10	1195.67±0.44	597.67±1.11	31.48±1.12	19.63±0.04	0.03±0.00	0.60±0.00
B1B	53.93±3.89	6.86±0.20	288.33±0.44	144.00±0.00	0.00	10.54±0.04	0.52±0.01	0.14±0.00
B2B	49.80±1.00	5.49±0.21	1540.33±1.78	770.33±0.44	122.00±2.00	11.23±0.00	0.18±0.02	0.78±0.00
B3B	32.33±1.44	5.33±0.30	1453.67±0.89	727.00±0.67	54.33±2.22	11.14±0.02	0.11±0.00	0.74±0.00
B1C	158.73±7.38	1.92±0.02	399.67±1.11	199.67±0.44	0.00	15.12±0.24	0.07±0.00	0.19±0.00
B2C	94.87±0.42	3.21±0.01	1731.33±0.44	865.67±0.44	402.33±16.89	15.43±0.31	0.68±0.00	0.88±0.00
B3C	104.50±2.67	4.47±0.02	1510.33±0.44	755.00±0.00	34.72±1.42	13.36±0.00	0.04±0.01	0.77±0.00
F1A	58.80±0.07	3.31±0.03	352.33±0.44	192.67±3.11	8.53±0.04	15.54±0.00	0.09±0.00	0.17±0.00
F3A	58.80±0.07	2.90±0.01	931.67±23.11	289.00±0.00	800.00±0.00	15.51±0.00	0.04±0.00	0.28±0.00
F1B	72.67±2.22	1.54±0.00	438.67±0.44	219.00±0.00	0.00	9.69±0.07	0.02±0.01	0.21±0.00
F2B	68.57±1.11	2.96±0.07	454.67±2.22	227.00±1.33	5.27±0.51	10.23±0.08	0.03±0.00	0.22±0.00
F3B	60.70±2.60	3.82±0.05	599.33±0.44	299.67±0.44	45.33±1.78	11.14±0.06	0.01±0.00	0.29±0.00
F1C	77.83±3.96	3.03±0.17	831.67±2.44	416.00±1.33	0.00	17.01±0.20	0.11±0.01	0.41±0.00
F2C	86.07±3.91	4.82±0.01	551.67±0.89	275.67±0.44	39.70±1.38	18.06±0.00	0.06±0.00	0.27±0.00
F3C	86.60±0.67	5.05±0.06	740.33±0.44	370.00±0.00	23.32±1.17	18.06±0.14	0.02±0.00	0.36±0.00
S1A	95.00±1.67	6.25±0.00	198.33±5.11	100.00±6.67	0.00	19.99±0.00	0.24±0.00	0.09±0.00
S3A	68.53±0.64	2.32±0.01	2855.67±8.89	1424.33±3.56	24.35±0.71	22.83±0.00	0.03±0.00	1.48±0.00
S1B	100.43±3.56	1.89±0.01	193.67±0.44	97.00±0.00	0.00	12.45±0.00	0.32±0.01	0.09±0.00
S2B	84.47±0.29	4.10±0.02	3688.33±2.89	1844.00±2.00	0.00	15.78±0.01	0.11±0.00	1.95±0.00
S3B	84.13±0.11	4.41±0.06	3076.33±1.78	1540.00±2.67	24.10±1.20	16.55±0.04	0.05±0.01	1.62±0.00
S1C	175.53±1.82	1.56±0.03	204.00±0.00	102.00±0.00	0.00	15.90±0.09	0.34±0.00	0.10±0.00
S2C	113.93±0.38	3.08±0.03	2026.67±3.11	1013.33±1.56	22.67±0.48	19.39±0.01	0.07±0.00	1.04±0.00
S3C	102.63±0.18	4.71±0.18	2487.67±1.11	1243.33±0.44	23.50±0.77	20.46±0.00	0.03±0.00	1.29±0.00
T1A	78.53±2.96	7.6±7.86	61.00±0.00	31.00±0.00	24.10±0.41	12.83±0.00	0.93±0.12	0.03±0.00
T2A	89.67±0.96	10.08±10.08	176.00±0.00	88.00±0.00	83.20±1.33	15.78±0.00	0.59±0.04	0.08±0.00
T3A	78.70±0.47	9.57±9.95	211.00±0.00	106.00±0.00	49.32±0.50	16.87±0.00	0.30±0.01	0.10±0.00
T1B	73.03±1.38	1.92±1.87	68.00±0.00	34.00±0.00	0.00	8.73±0.00	0.15±0.01	0.03±0.00
T2B	63.77±1.91	6.59±6.49	382.00±25.33	191.00±12.67	0.00	12.09±0.00	0.14±0.00	0.18±0.01
T3B	89.07±3.24	7.21±6.96	576.33±0.44	289.00±0.00	0.00	12.60±0.02	0.04±0.00	0.28±0.00
T1C	124.43±3.09	2.1±2.10	61.00±0.00	31.00±0.00	43.66±0.14	7.11±0.00	0.28±0.03	0.03±0.00
T2C	97.90±2.47	4.37±4.30	319.33±2.22	160.00±1.33	445.67±3.56	11.98±0.47	0.20±0.08	0.15±0.00
T3C	94.00±2.60	3.42±3.49	339.00±0.00	169.33±0.44	325.67±0.89	14.92±0.04	0.09±0.01	0.16±0.00

Appendix I-B: Some physicochemical parameters of wastewater samples with their limit of

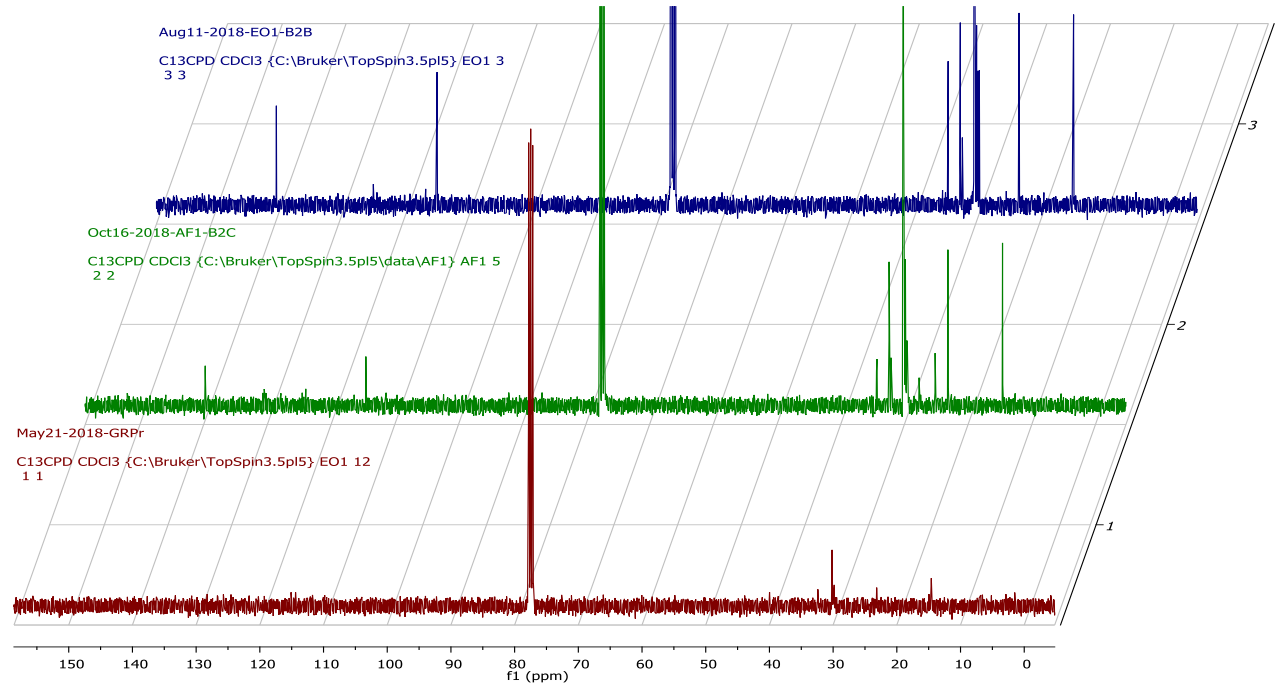
Sample	ORP (mV)	DO(ppm)	Conductivity (µS/cm)	Total Dissol Solids (ppm)	Turbidity (NTU)	Salinity (PSU)	Temp (^{0}C)
G1A	-102.40±0.0	0.37±0.01	1160.67±0.44	584.67±3.11	577.67±26.44	0.58±0.00	22.62±0.16
G2A	67.48±0.26	7.78±0.15	6.00±0.00	3.00±0.67	30.53±0.49	0.00±0.00	21.80±0.00
G1B	-83.67±1.58	3.70±0.18	1013.00±3.33	506.67±1.78	290.67±38.89	0.50±0.00	18.06±0.00
G2B	63.23±3.58	6.68±0.23	952.00±9.33	475.67±4.44	38.28±0.44	0.48±0.00	13.50±0.00
G1C	-71.23±3.56	4.22±0.01	1437.00±0.00	718.33±0.44	750.00±12.67	0.73±0.00	17.25±0.00
G2C	130.70±1.07	4.58±0.07	1026.00±0.00	513.00±0.00	24.67±0.99	0.51±0.00	17.05±0.00
K1B	-57.20±2.33	2.19±0.01	767.00±5.33	384.00±2.67	435.67±16.89	0.38±0.00	16.77±0.00
K2B	70.53±1.11	3.08±0.02	478.00±0.00	239.00±0.00	21.58±0.25	0.23±0.00	14.75±0.01
K1C	-10.17±2.69	2.36±0.09	874.33±1.56	437.00±0.67	849.00±12.67	0.43±0.00	17.38±0.00
K2C	171.47±7.58	4.02±0.09	489.00±0.00	244.67±0.44	0.00	0.24±0.00	17.45±0.01
U2B	88.97±1.09	4.43±0.08	16.54±0.02	828.33±0.89	32.23±1.04	0.84±0.00	18.23±0.00
U2C	121.40±0.07	3.64±0.01	2169.67±2.44	1084.67±1.11	23.76±0.46	1.12±0.00	19.48±0.00
A1B	-59.83±4.18	4.36±0.16	697.00±9.33	348.67±4.44	603.00±26.00	0.34±0.01	17.28±0.00
A2B	171.03±4.69	5.99±0.14	478.00±0.00	239.00±0.00	27.58±2.52	0.23±0.00	13.65±0.13
A1C	-91.60±0.60	3.32±0.13	660.33±1.78	330.00±0.67	381.33±15.11	0.32±0.00	16.72±0.01
A2C	88.73±1.62	4.96±0.12	471.33±15.78	229.33±0.44	0.00	0.22±0.00	14.84±0.0

Sample	COD (mg/L)	PO_4 (mg/L)	CL^- (mg/L)	NH_4 (mg/L)	SO_4 (mg/L)	NO_3^- (mg/L)	pH
G1A	80.41±10.3	26.11±2.66	<LOD	35.55±1.43	7.17±2.52	5.0±0	8.34±0.11
G2A	4.02±18.5	15.59±0.31	<LOD	85.62±7.77	12.93±3.95	15.0±3.33	7.33±0.04
G1B	83.65±25.3	20.12±0.69	50.45±2.1	32.43±12.70	2.38±1.50	5.0±0	7.19±0.00
G2B	ND	21.67±0.15	19.40±2.2	71.10±8.99	119.22±12.30	ND	7.77±0.06
G1C	734.92±109.3	38.00±0.88	594.94±20.1	121.64±1.00	3.37±1.97	ND	7.37±0.00
G2C	5.31±2.2	24.33±0.06	584.18±0.0	44.47±0.16	13.92±3.65	8.33±2.22	7.86±0.00
K1B	3508.33±249.7	21.66±0.43	454.13±0.0	154.65±19.79	33.05±7.48	ND	7.92±0.00
K2B	ND	16.61±5.66	382.65±21.5	28.75±13.47	2.85±1.73	ND	7.34±0.07
K1C	1166.72±2.2	31.74±0.48	433.30±1.7	74.00±0.09	2.14±1.39	5.0±3.33	9.06±0.01
K2C	ND	15.75±0.05	325.77±0.5	14.62±0.05	1.48±0.89	8.33±2.22	8.98±0.02
U2B	140.62±76.9	27.01±3.60	30.01±4.5	145.53±32.71	16.70±5.31	5.0±0	10.23±0.02
U2C	ND	24.64±0.14	479.57±2.7	43.90±0.09	2.59±1.50	6.67±2.22	8.26±0.00
A1B	46.10±11.9	23.26±2.65	6.49±35.8	125.17±23.46	7.98±2.52	20.0±3.33	7.39±0.05
A2B	ND	14.86±0.10	48.07±13.3	26.51±5.58	13.38±5.59	21.67±2.22	7.83±0.08
A1C	527.58±419.7	22.75±0.10	304.17±0.7	149.13±0.20	2.09±1.27	43.33±4.44	9.12±0.04
A2C	251.97±3.0	10.00±0.01	248.37±0.8	77.19±0.77	0.25±0.20	40.0±0	8.27±0.04

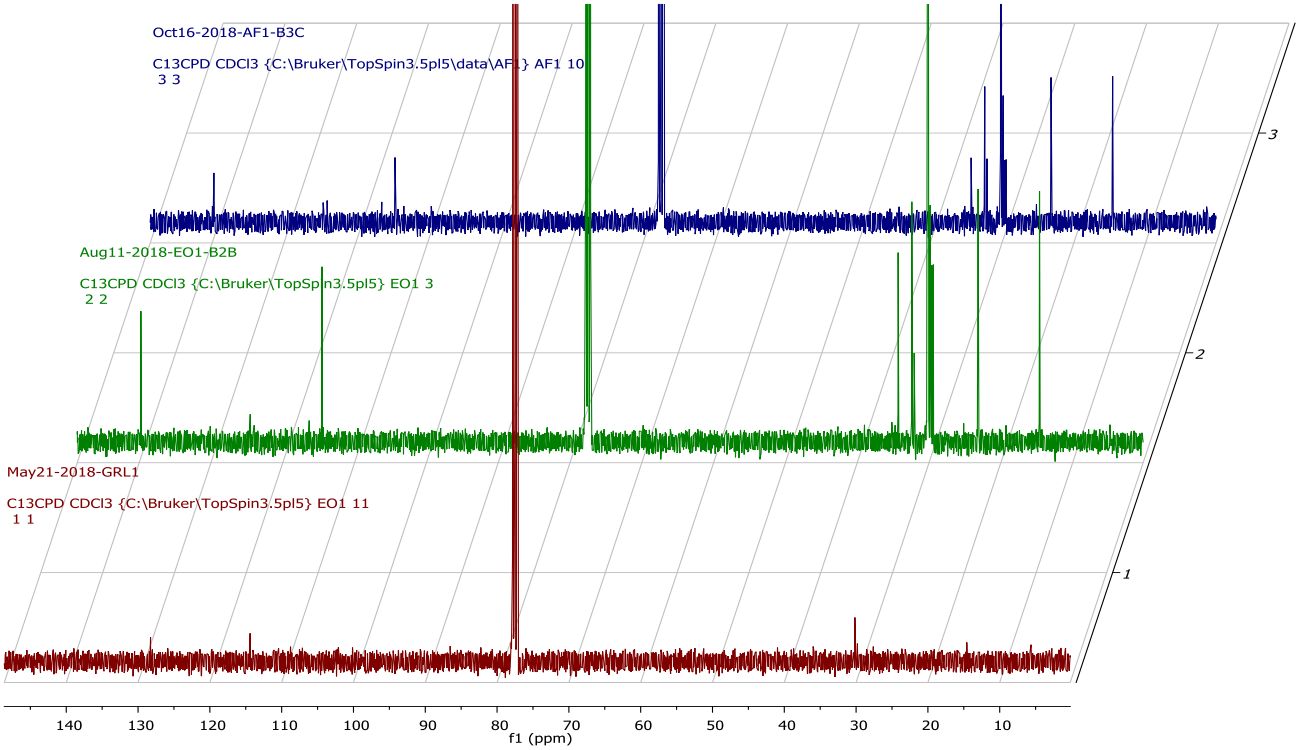
Appendix II



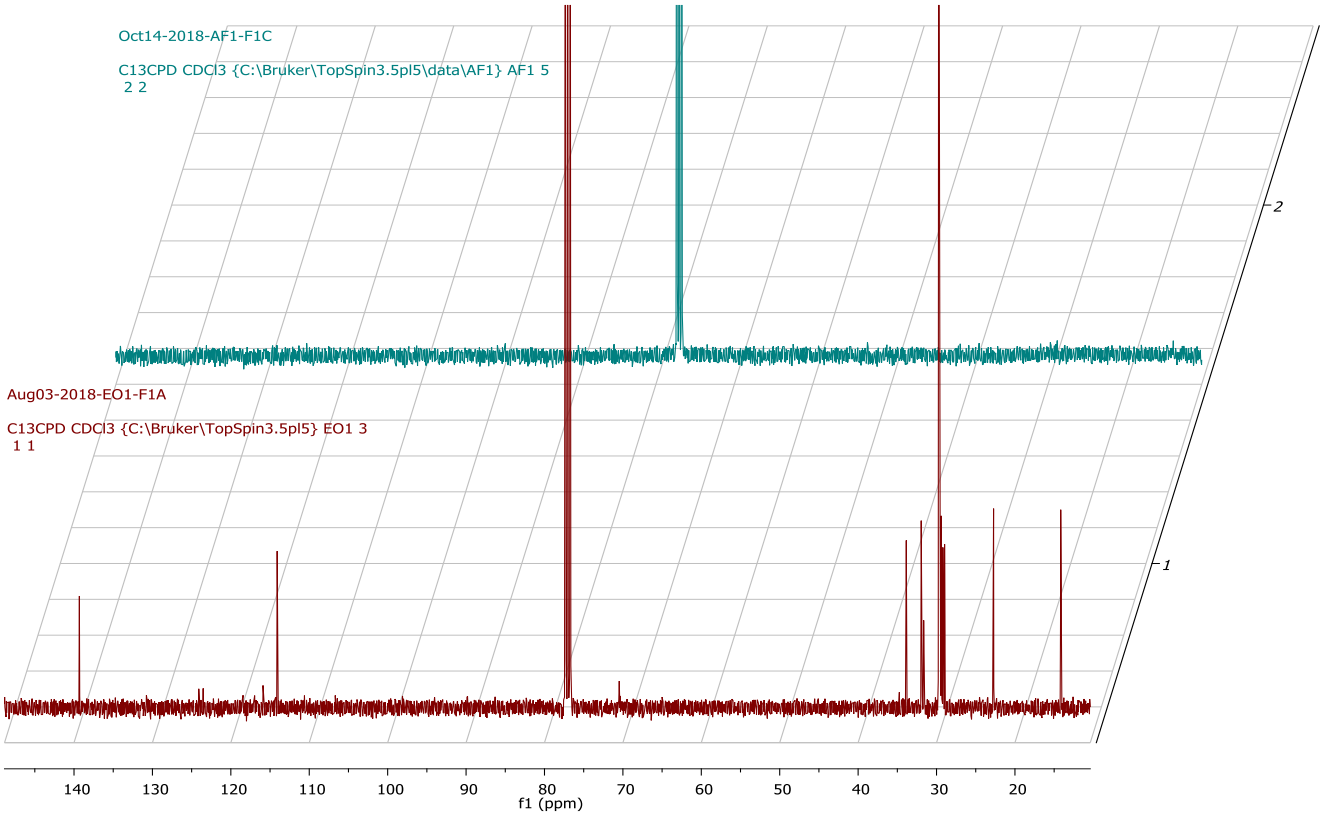
Appendix II-A: ^{13}C-NMR chemical shifts of Bloukrans River upstream samples.



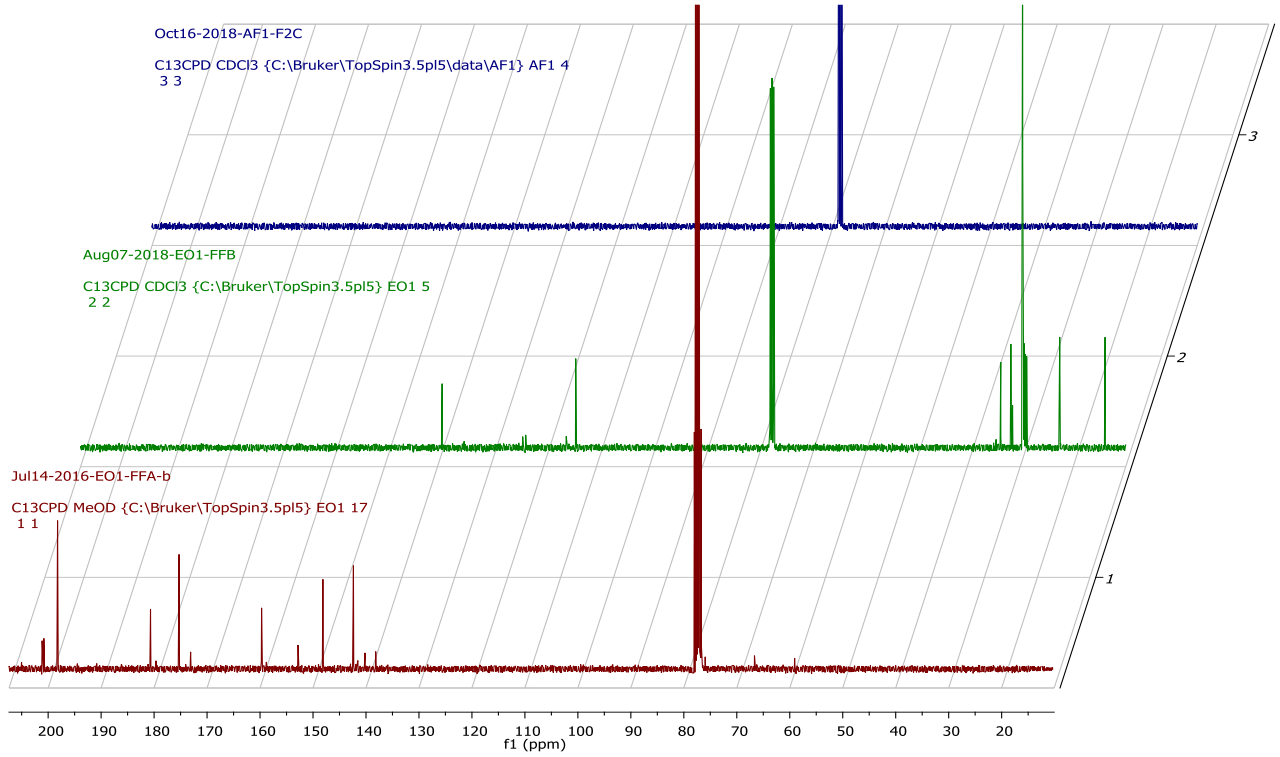
Appendix II-B: ^{13}C-NMR chemical shifts of Bloukrans River midstream samples.



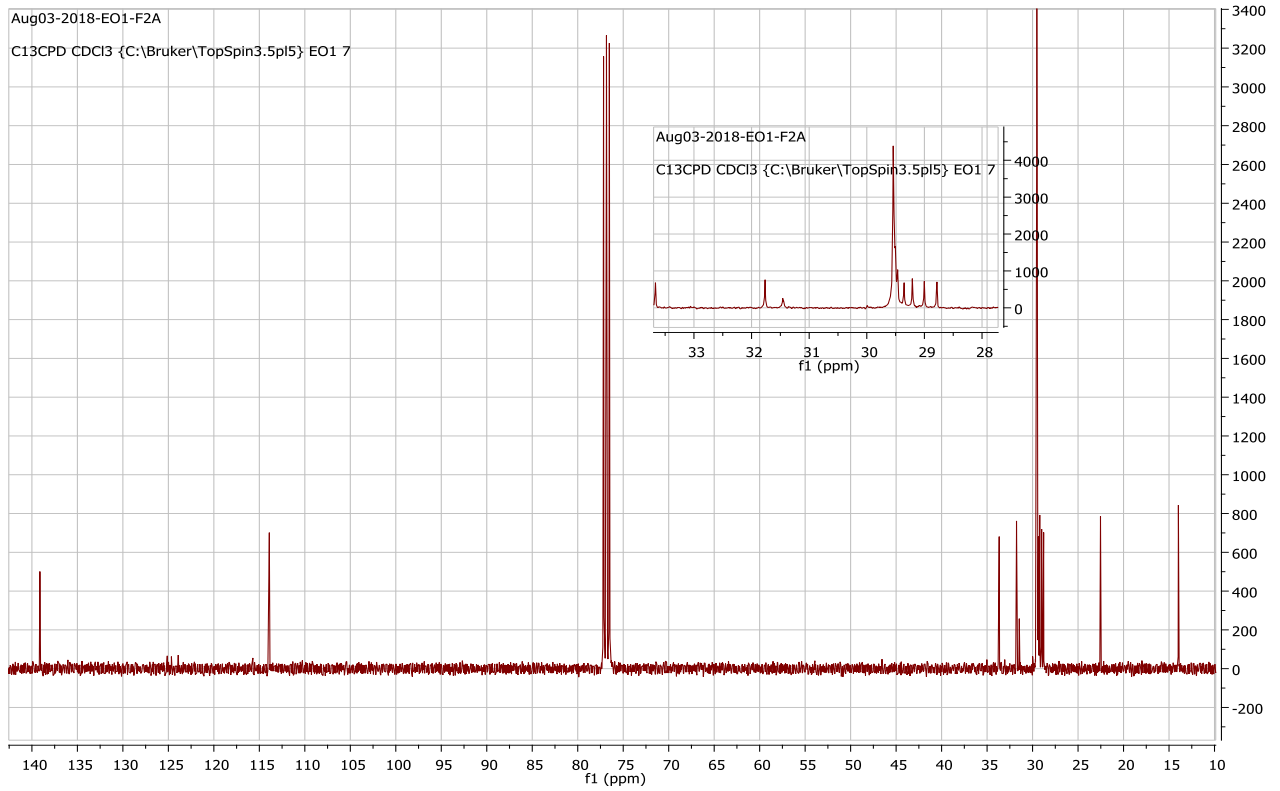
Appendix II-C: ^{13}C-NMR chemical shifts of Bloukrans River midstream samples.



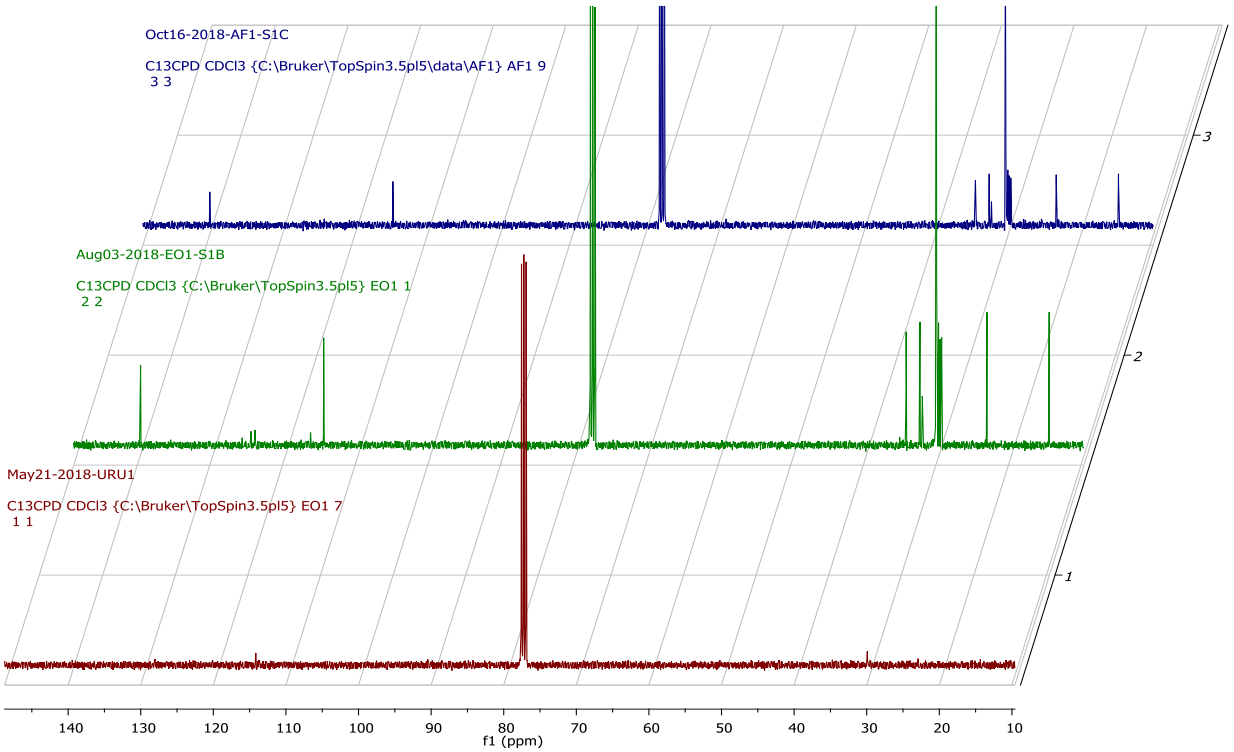
Appendix II-D: ^{13}C-NMR chemical shifts of Buffalo River upstream samples.



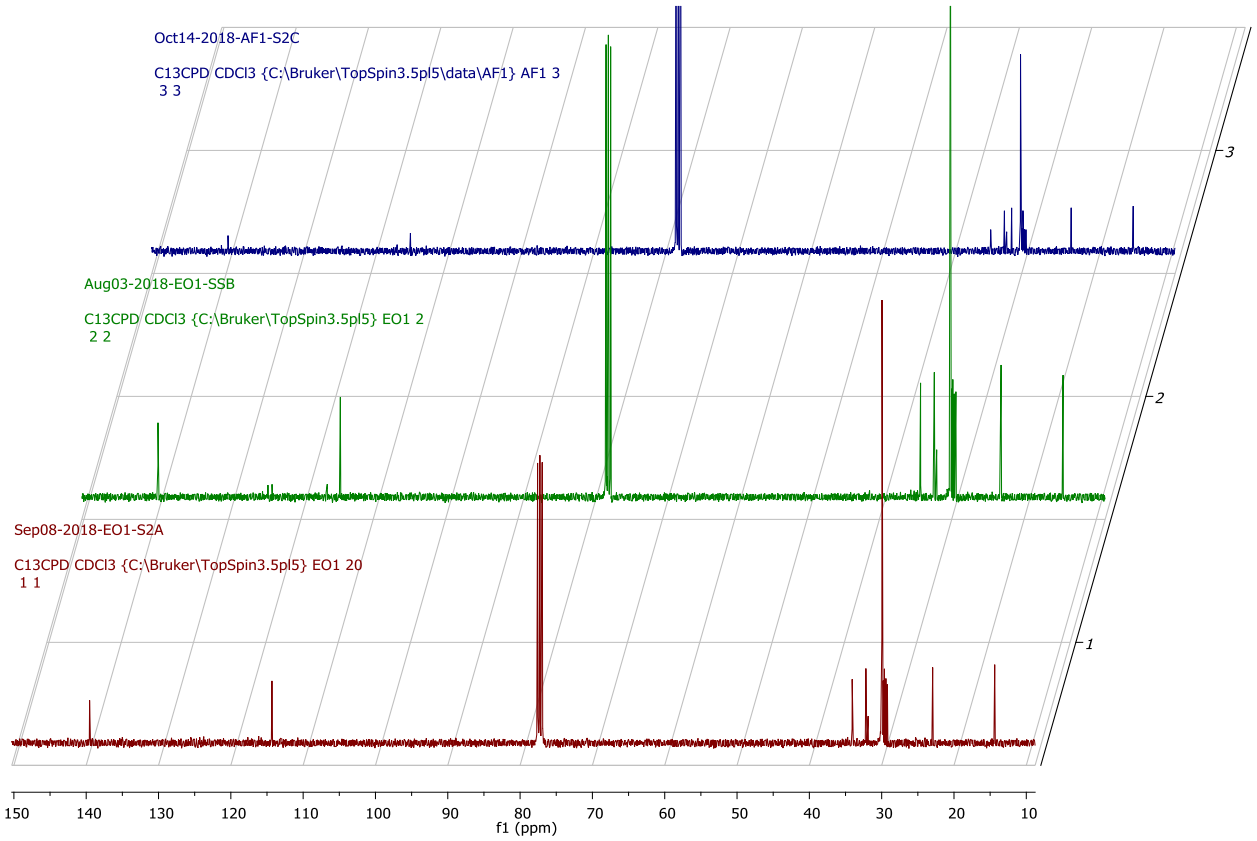
Appendix II-E: ^{13}C-NMR chemical shifts of Buffalo River midstream samples.



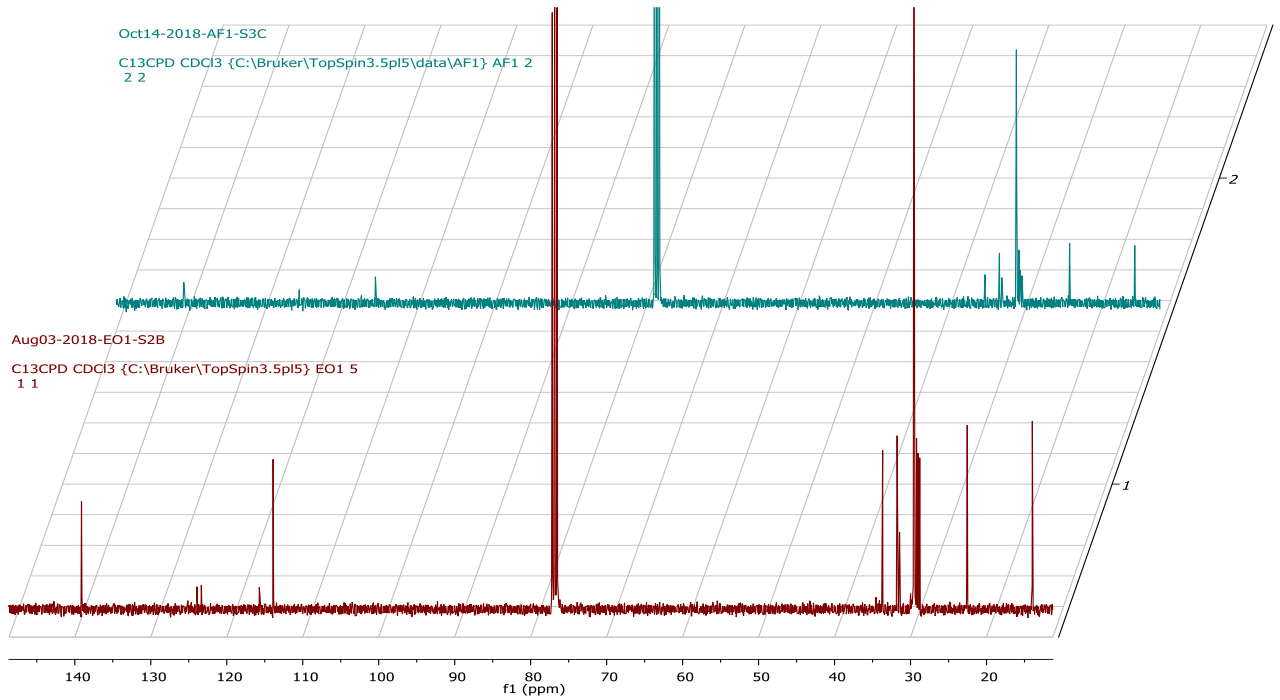
Appendix II-F: ^{13}C-NMR chemical shifts of Buffalo River downstream sample.



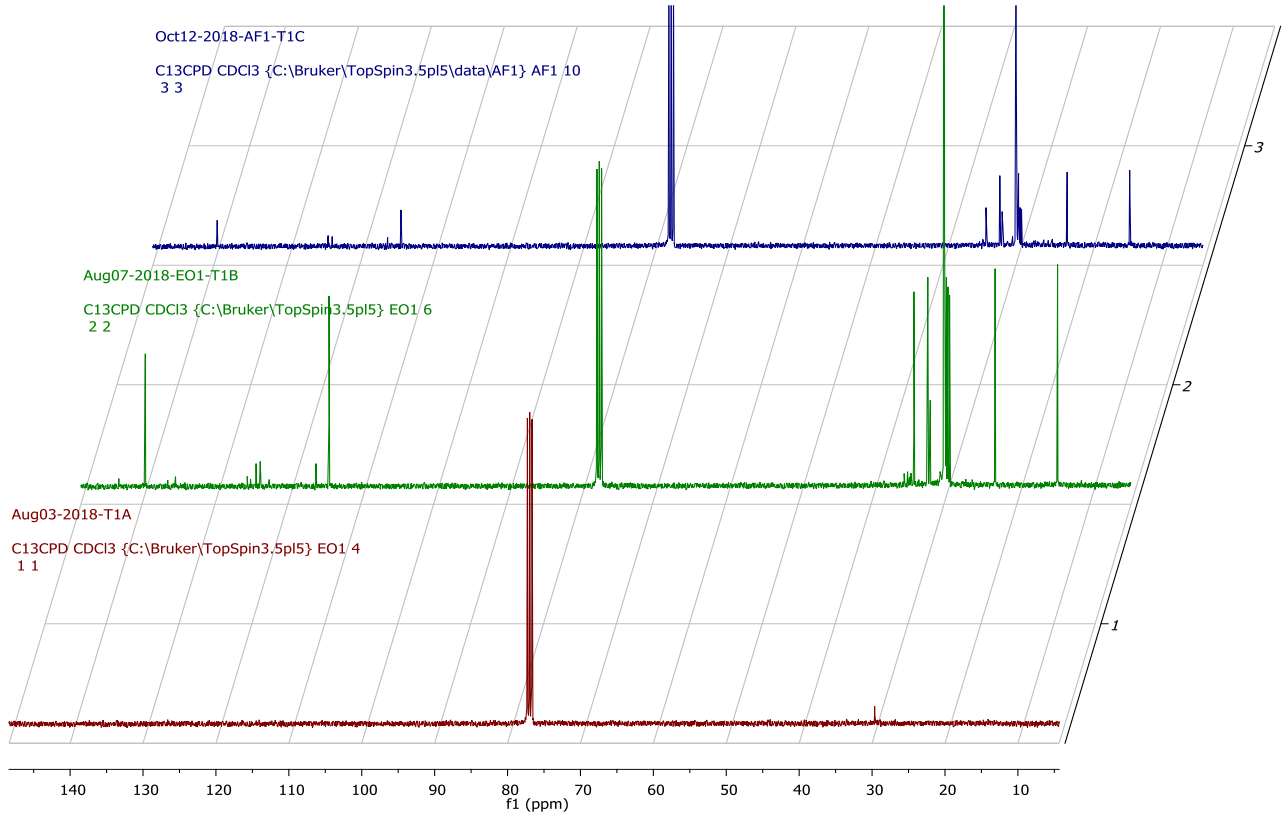
Appendix II-G: ^{13}C-NMR chemical shifts of Swartkops River upstream samples.



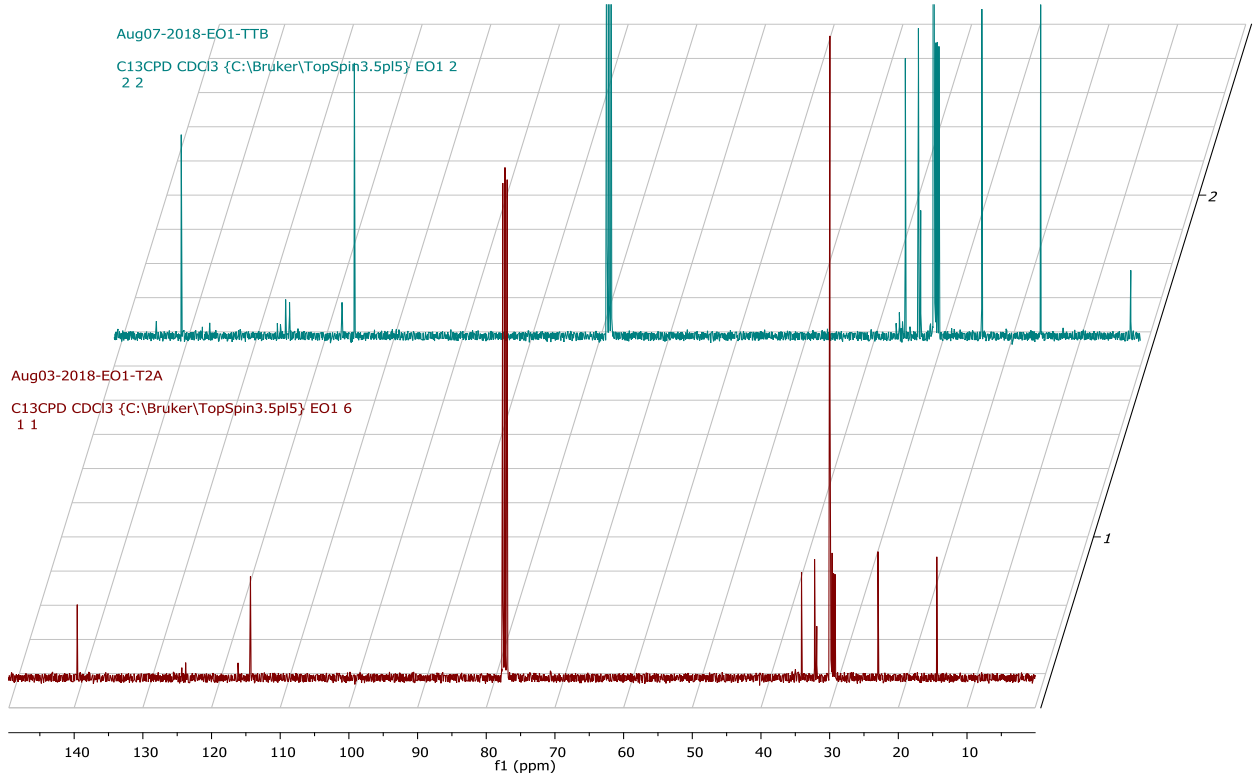
Appendix II-H: ^{13}C-NMR chemical shifts of Swartkops River midstream samples.



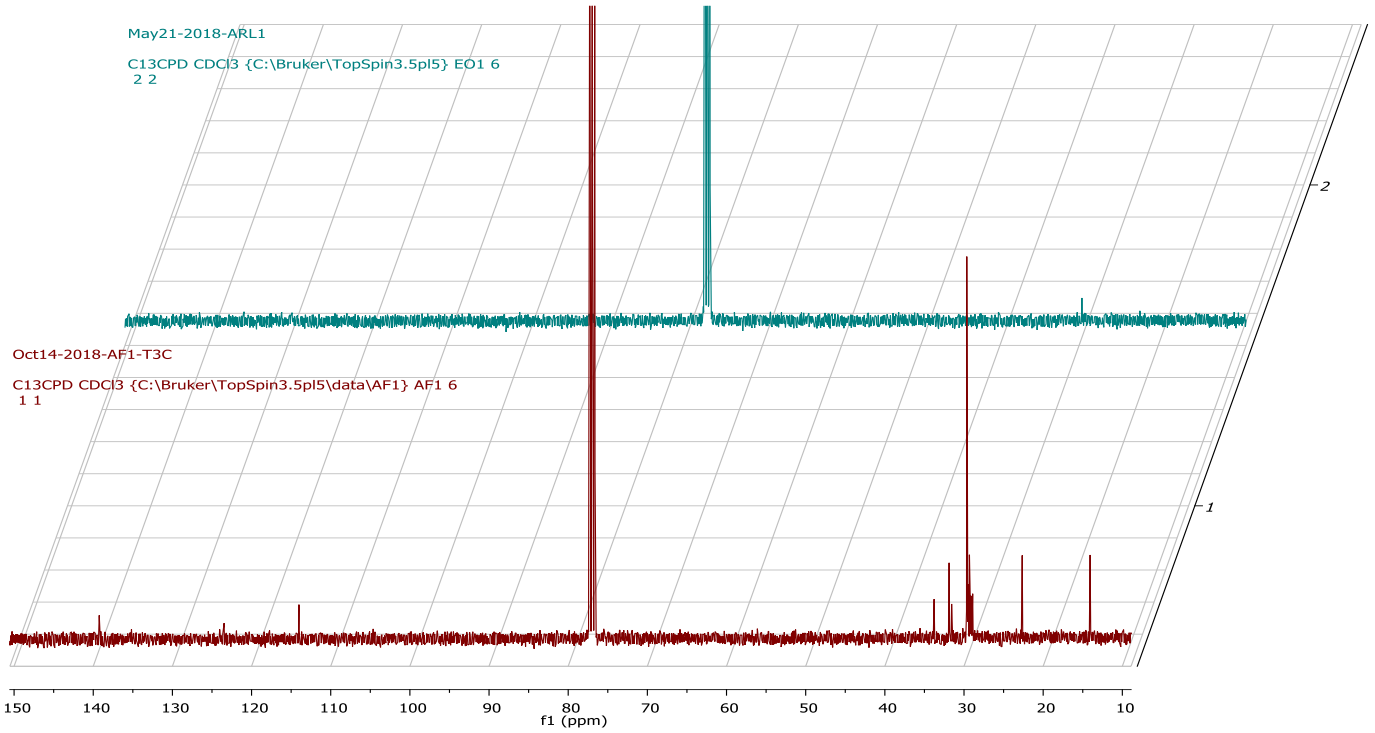
Appendix II-I: ^{13}C-NMR chemical shifts of Swartkops River downstream samples.



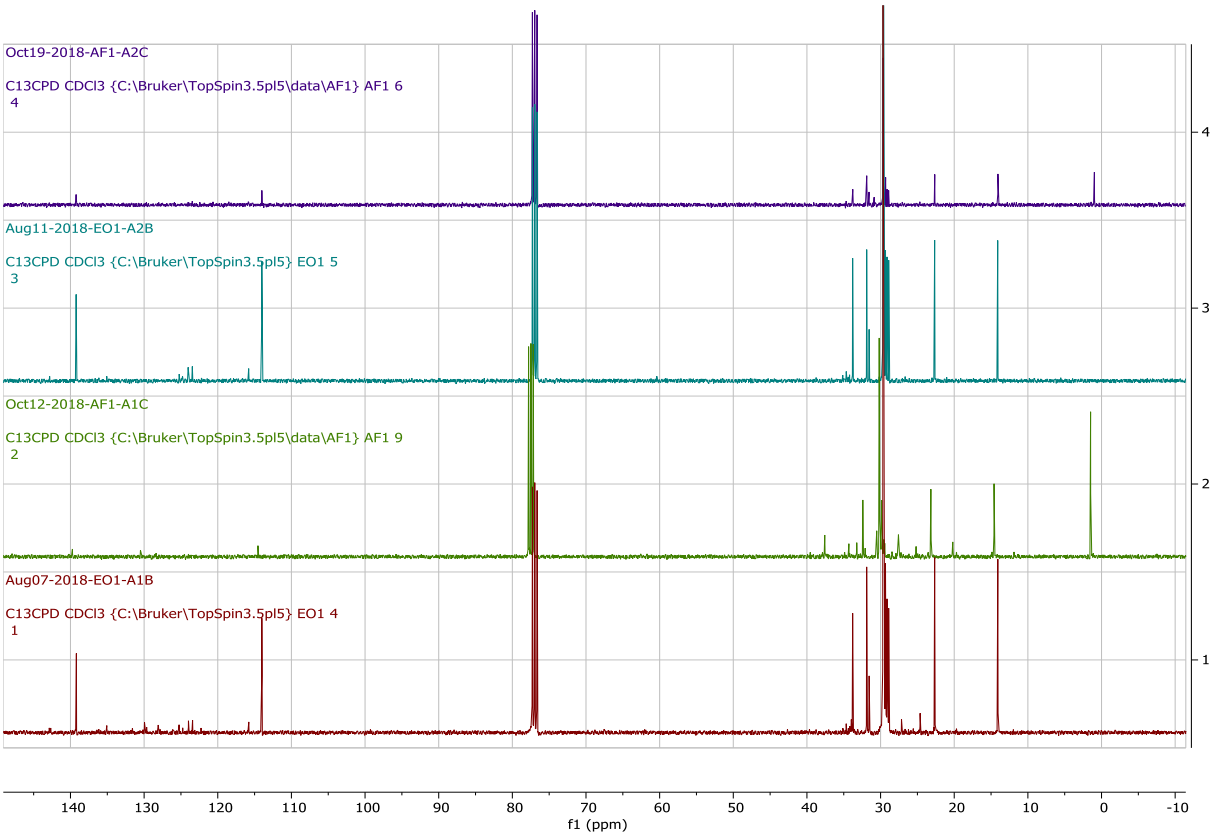
Appendix: II-J ^{13}C-NMR chemical shifts of Tyhume River upstream samples.



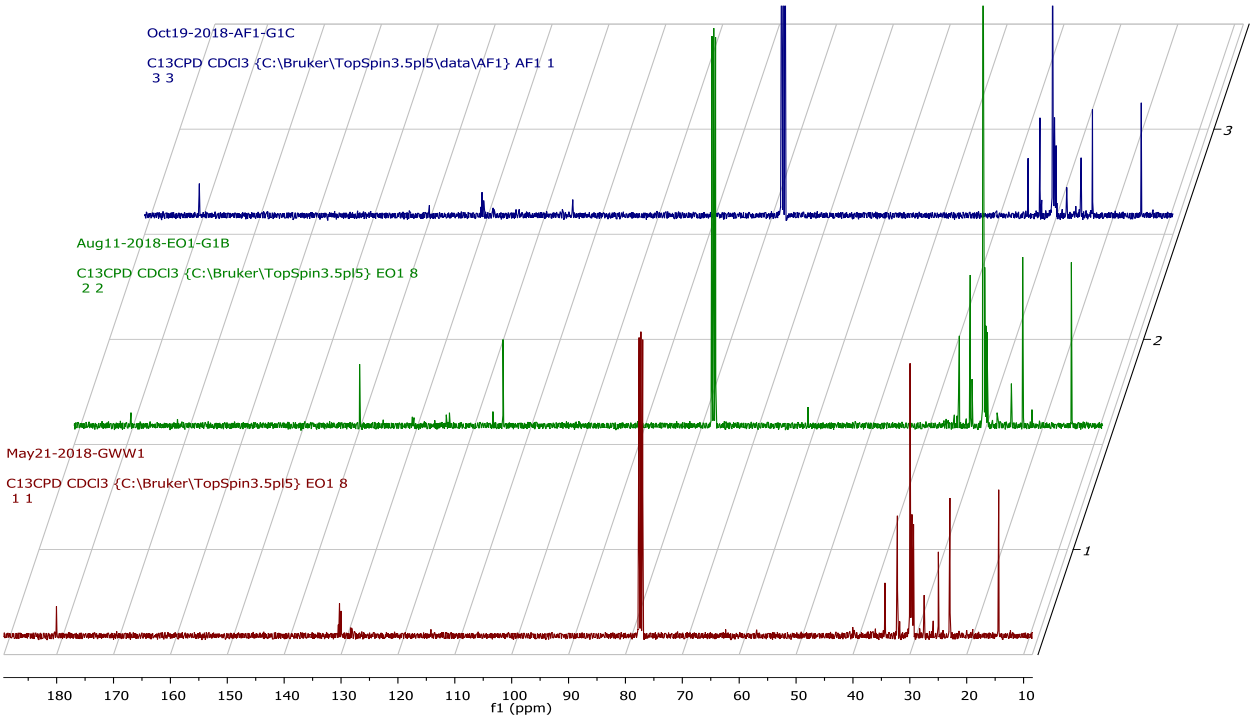
Appendix II-K: ^{13}C-NMR chemical shifts of Tyhume River midstream samples.



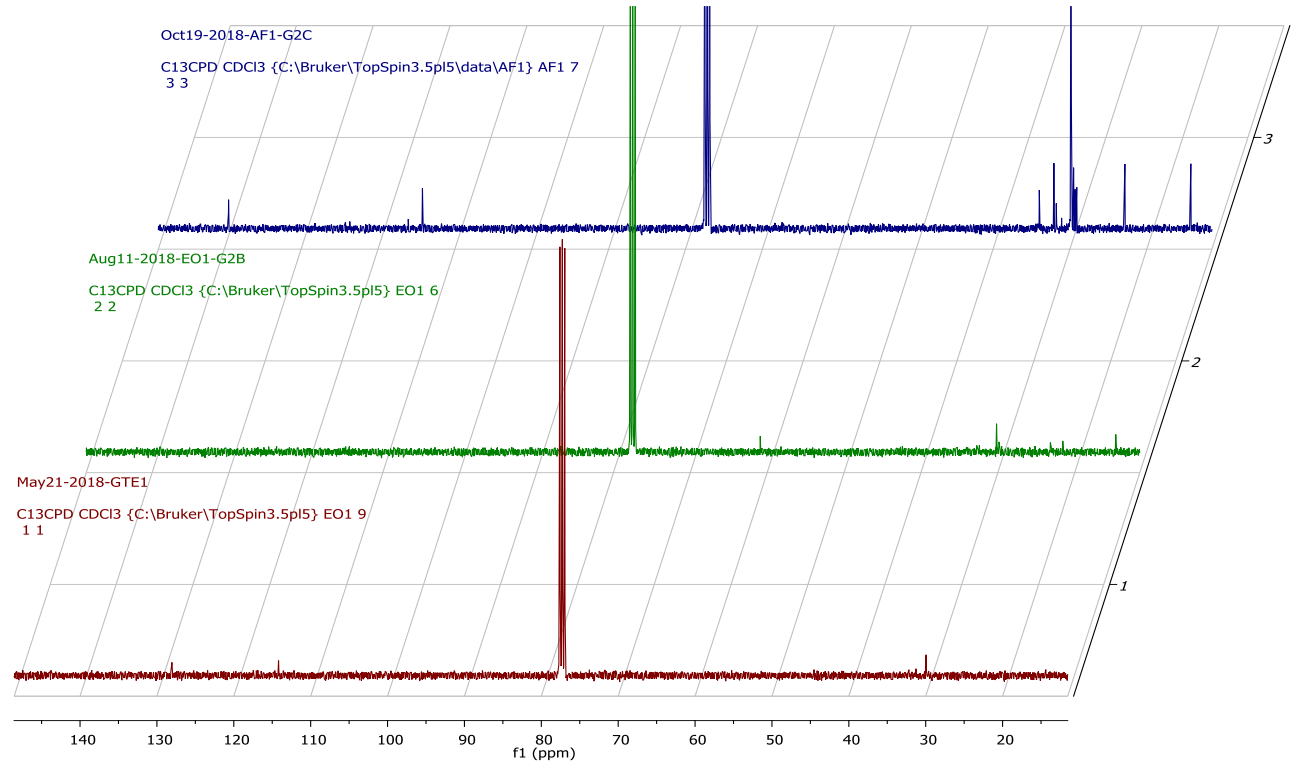
Appendix II-L: ^{13}C chemical shifts of Tyhume River downstream samples.



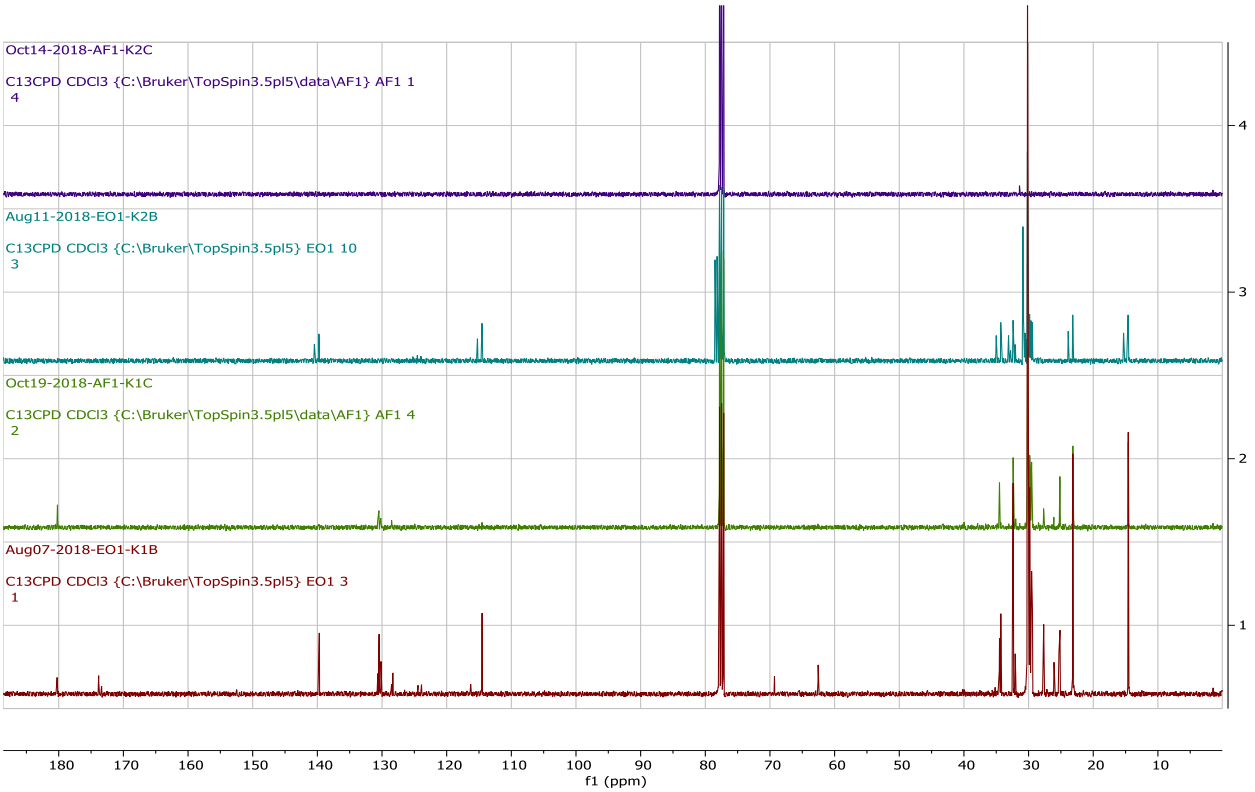
Appendix II-M: ^{13}C-NMR chemical shifts of Alice wastewater and treated effluent samples.



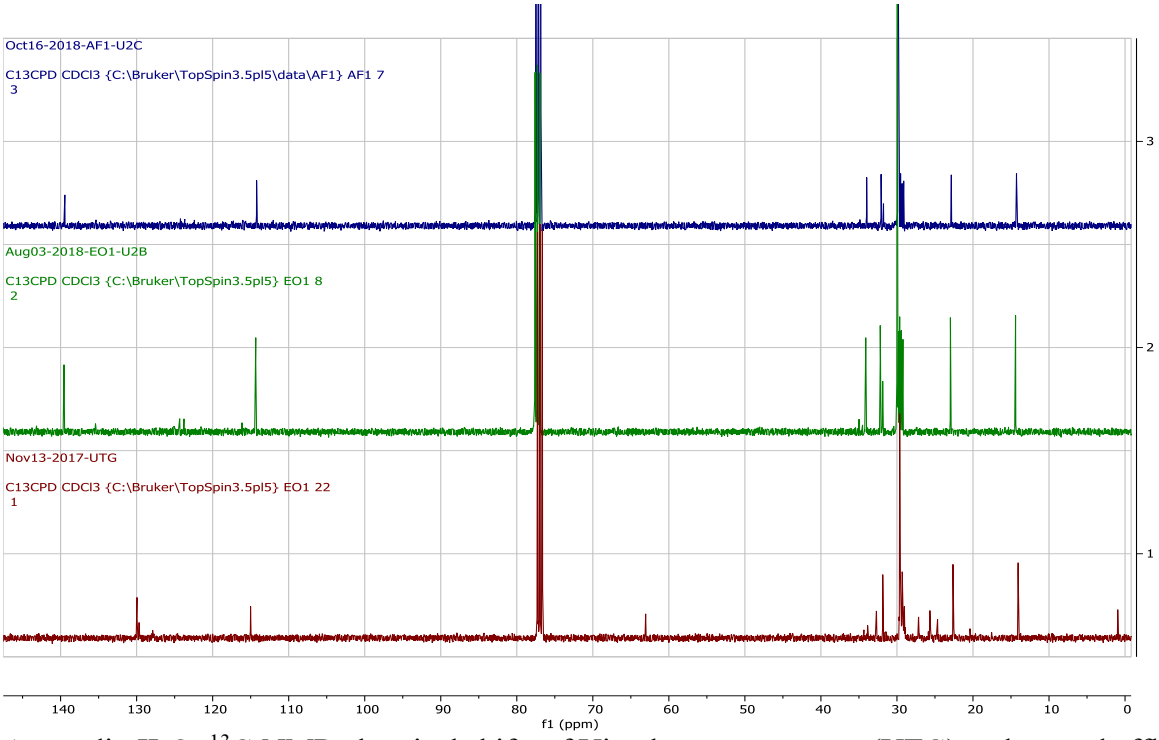
Appendix II-N: ^{13}C-NMR chemical shifts of Grahamstown wastewater samples.



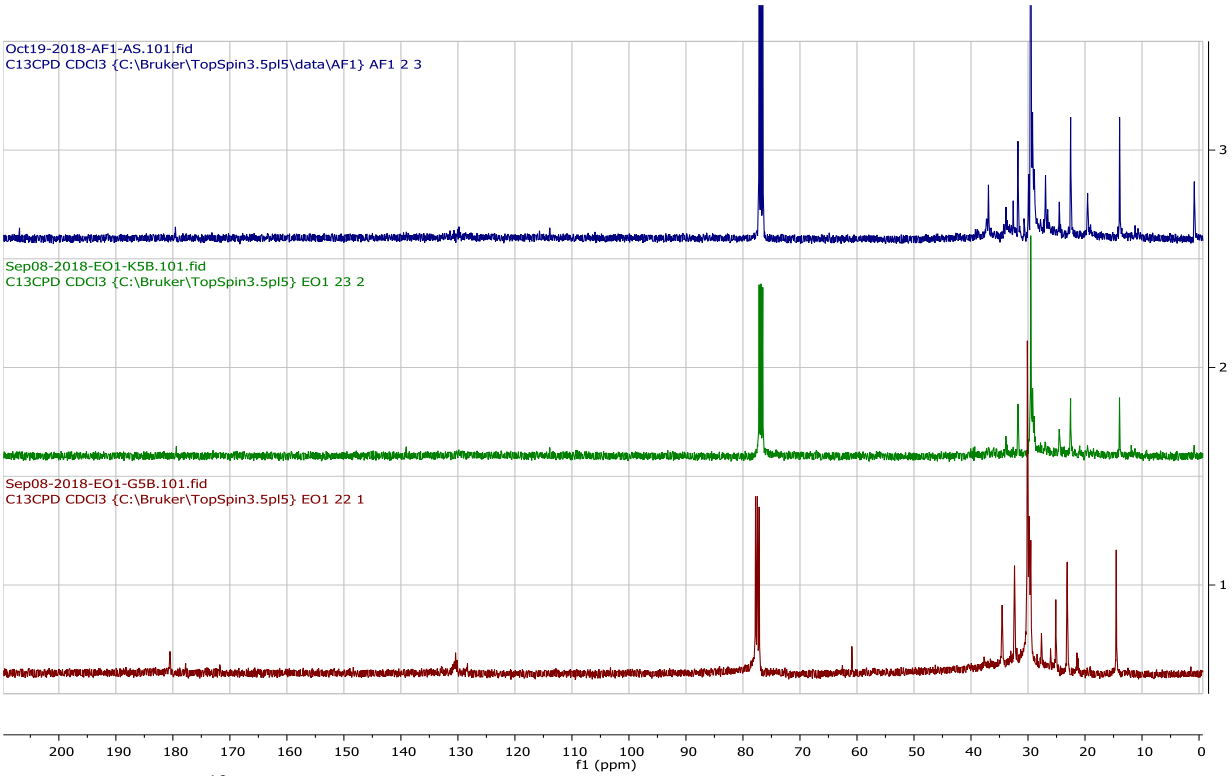
Appendix II-O: ^{13}C-NMR chemical shifts of Grahamstown treated effluent samples.



Appendix II-P: ^{13}C-NMR chemical shifts of King Williams Town wastewater (K1B, K1C) and treated effluent (K2B, K2C) samples.

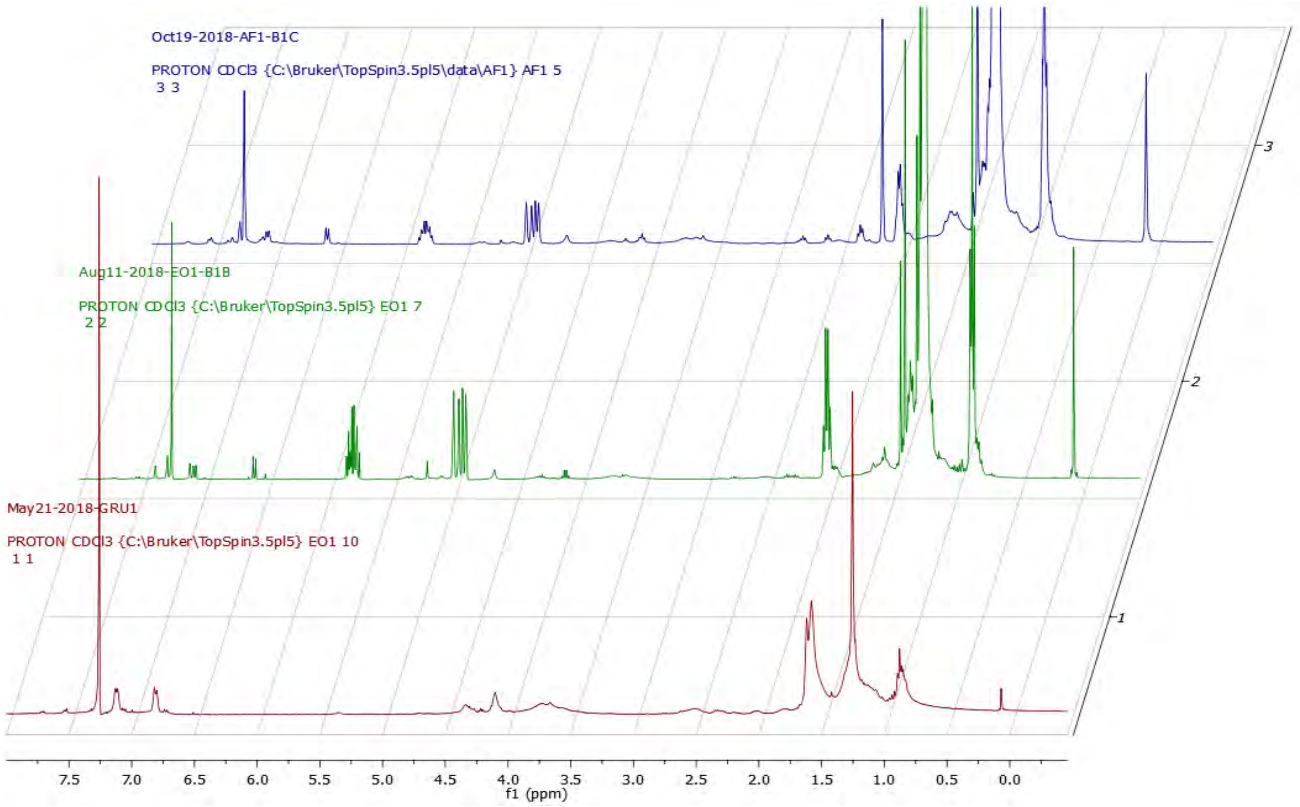


Appendix II-Q: ^{13}C-NMR chemical shifts of Uitenhage wastewater (UTG) and treated effluent (U2B, U2C) samples.

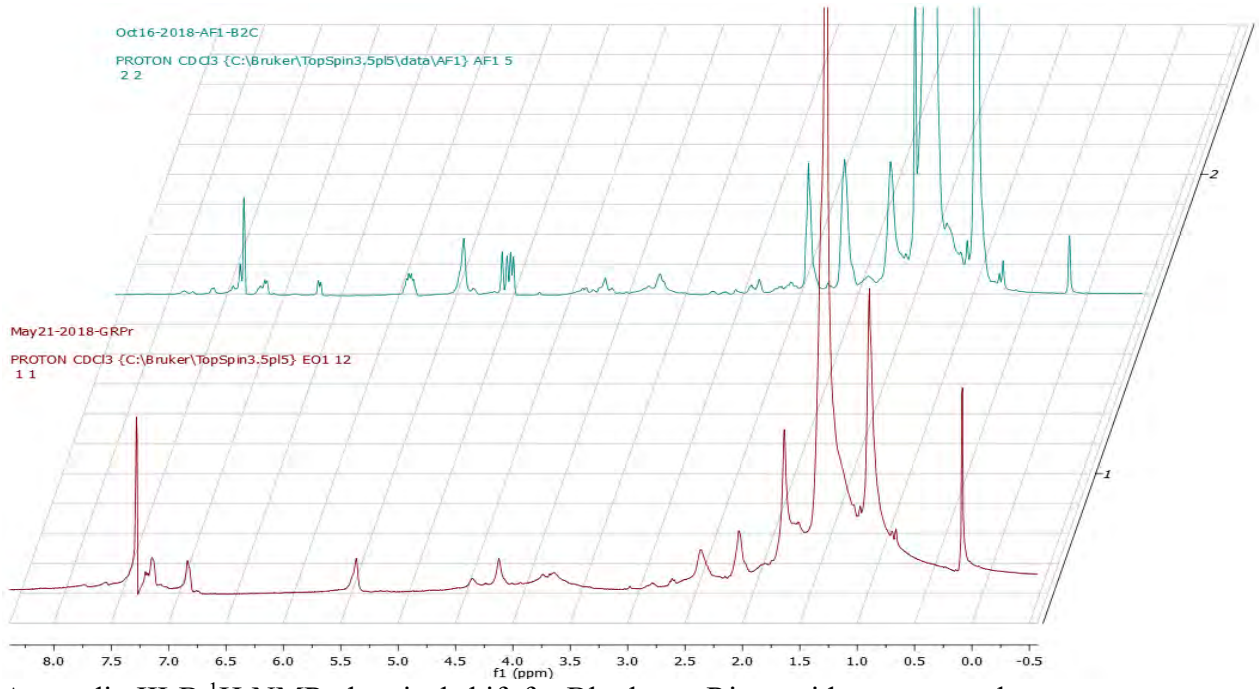


Appendix II-R: ^{13}C-NMR chemical shifts of Alice (AS), King Williams Town (KSB) and Grahamstown (GSB) sludge samples.

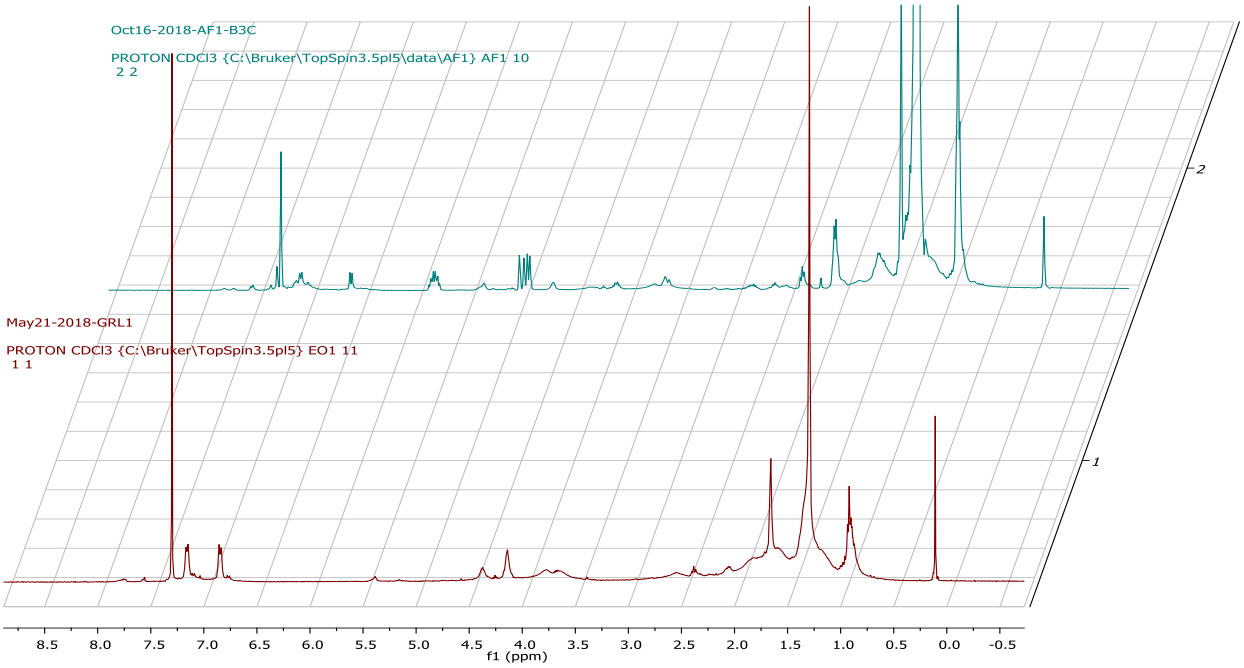
Appendix III



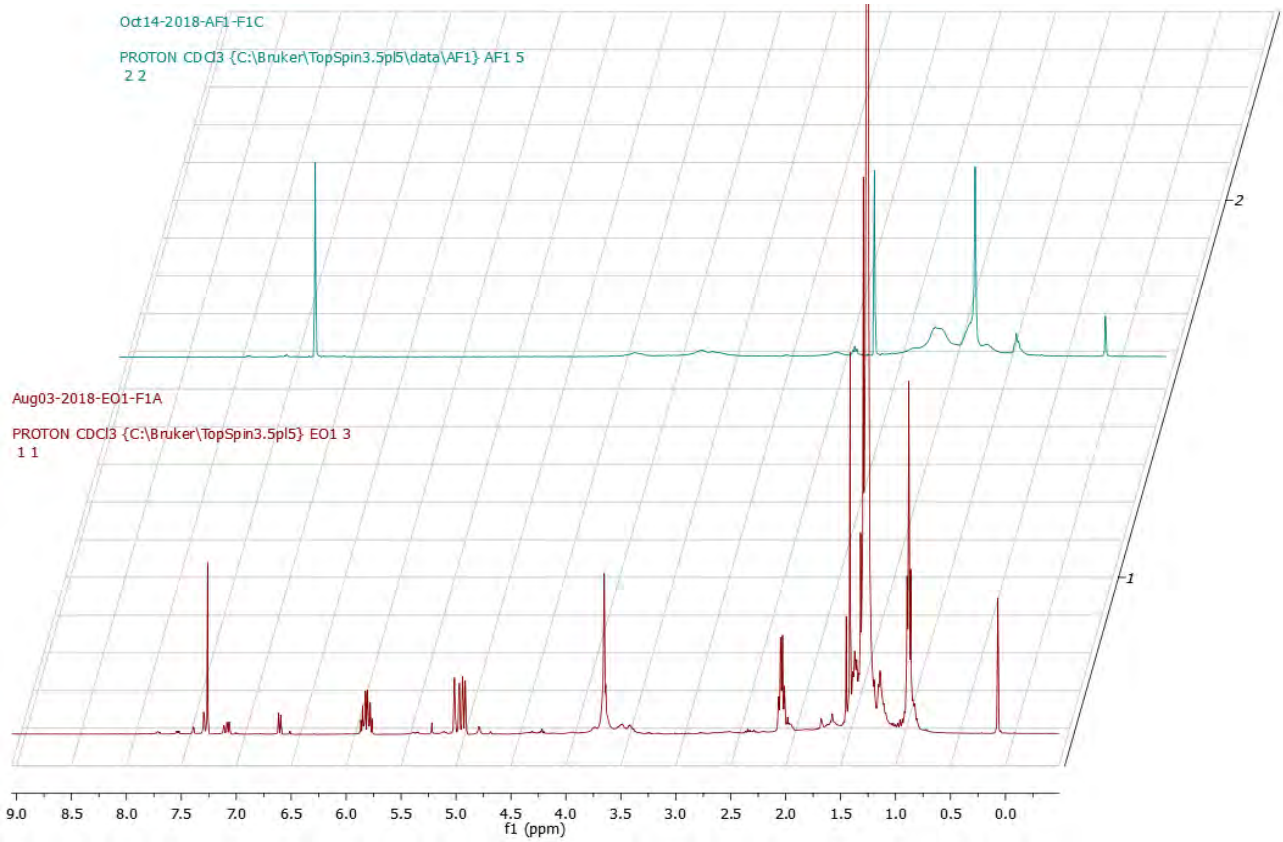
Appendix III-A: ^{1}H-NMR chemical shift for Bloukrans River upstream samples



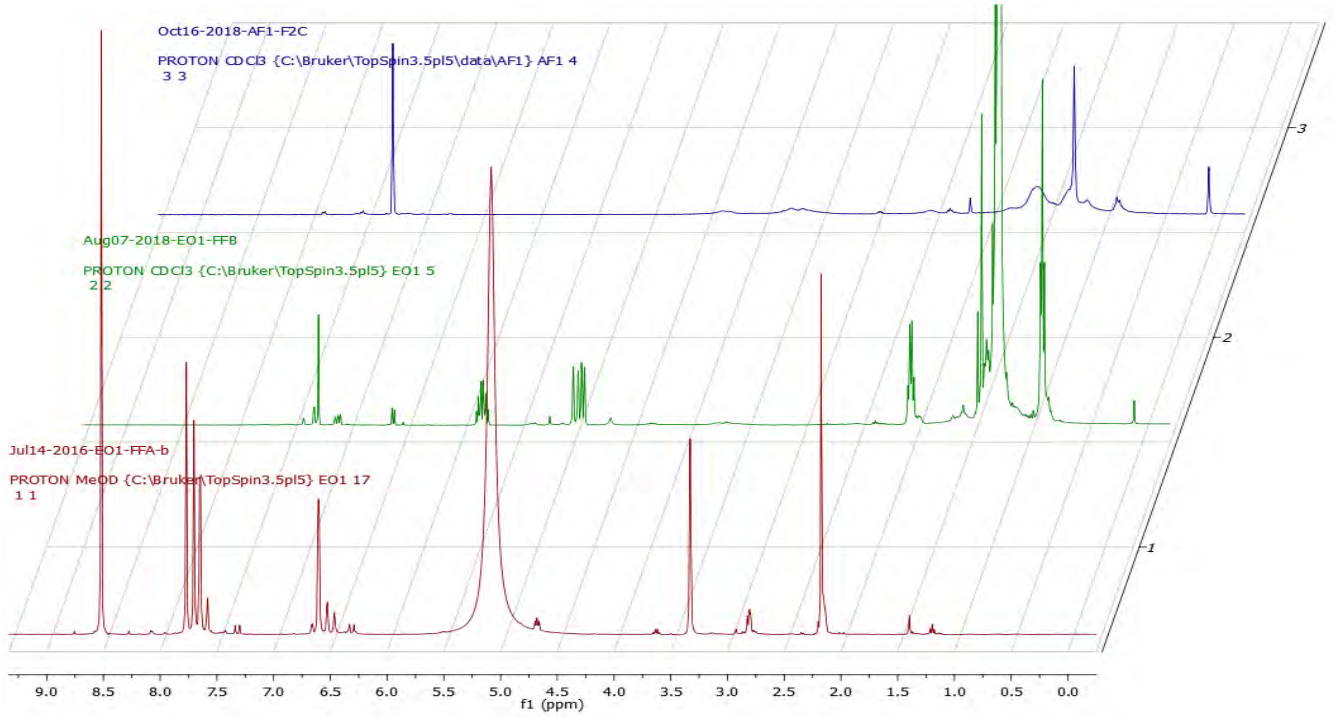
Appendix III-B ^{1}H-NMR chemical shift for Bloukrans River midstream samples



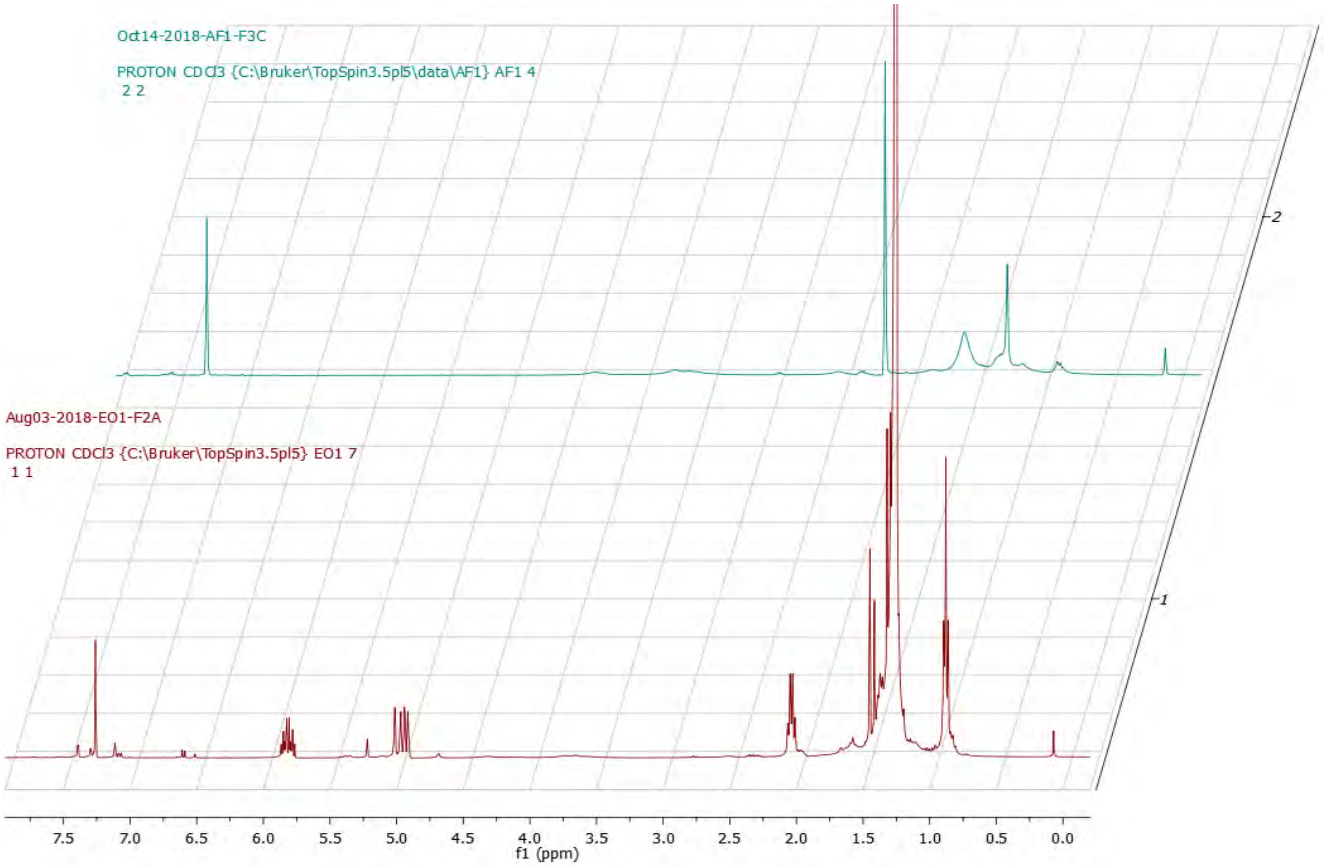
Appendix III-C ^{1}H-NMR chemical shift for Bloukrans River downstream samples



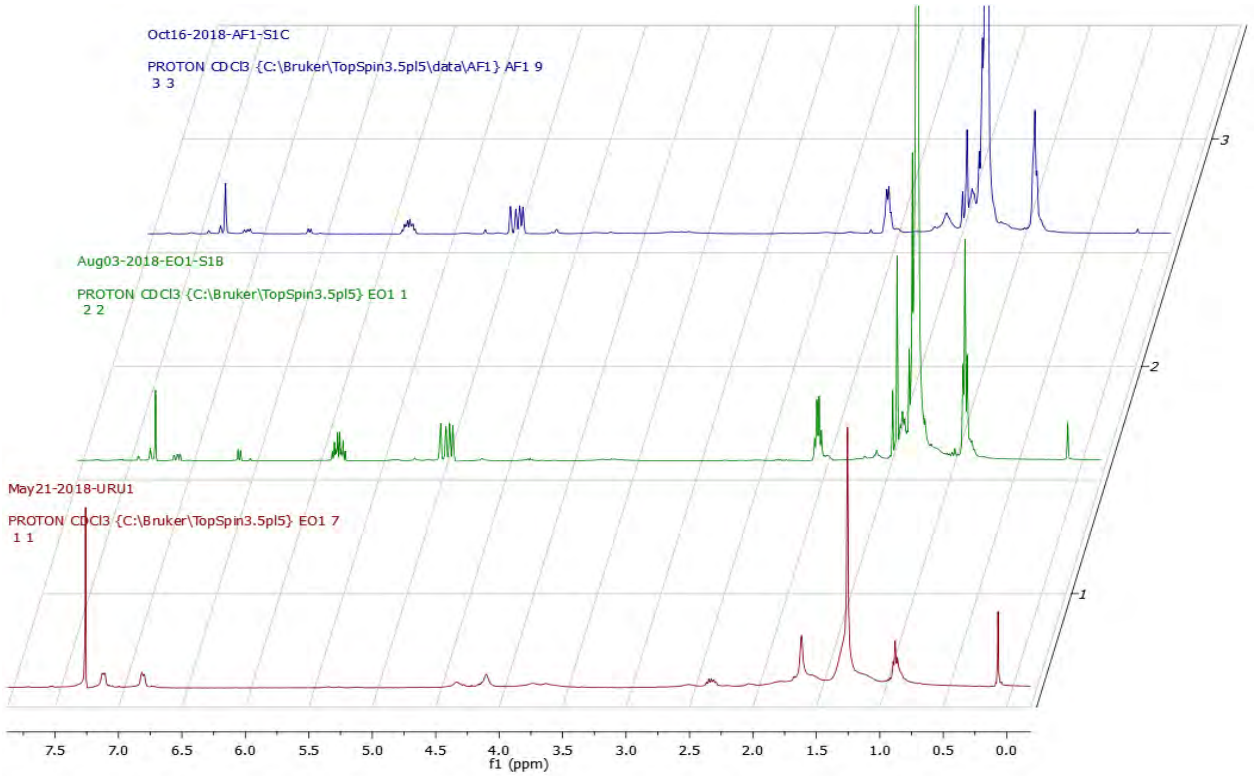
Appendix III-D: ^{1}H-NMR chemical shift for Buffalo River upstream samples



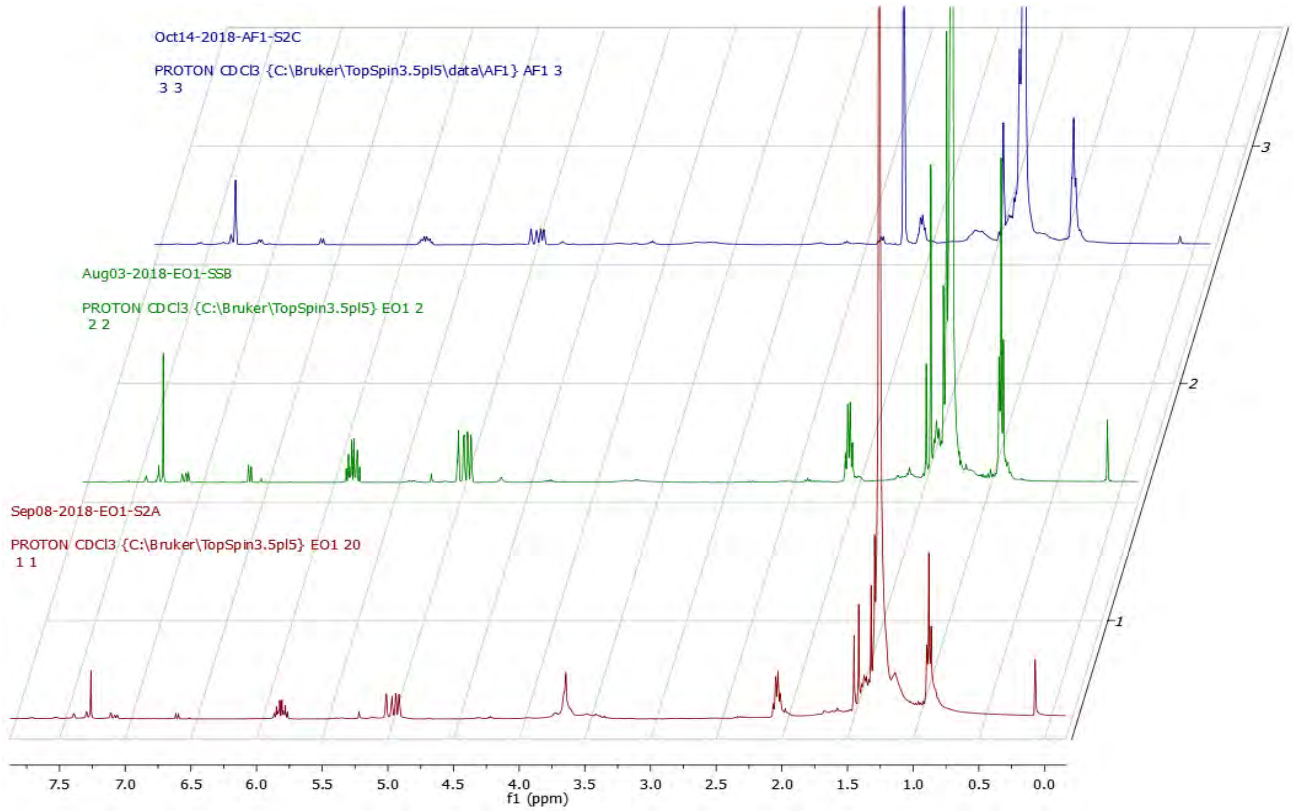
Appendix III-E: ^{1}H NMR chemical shift for Buffalo River midstream samples



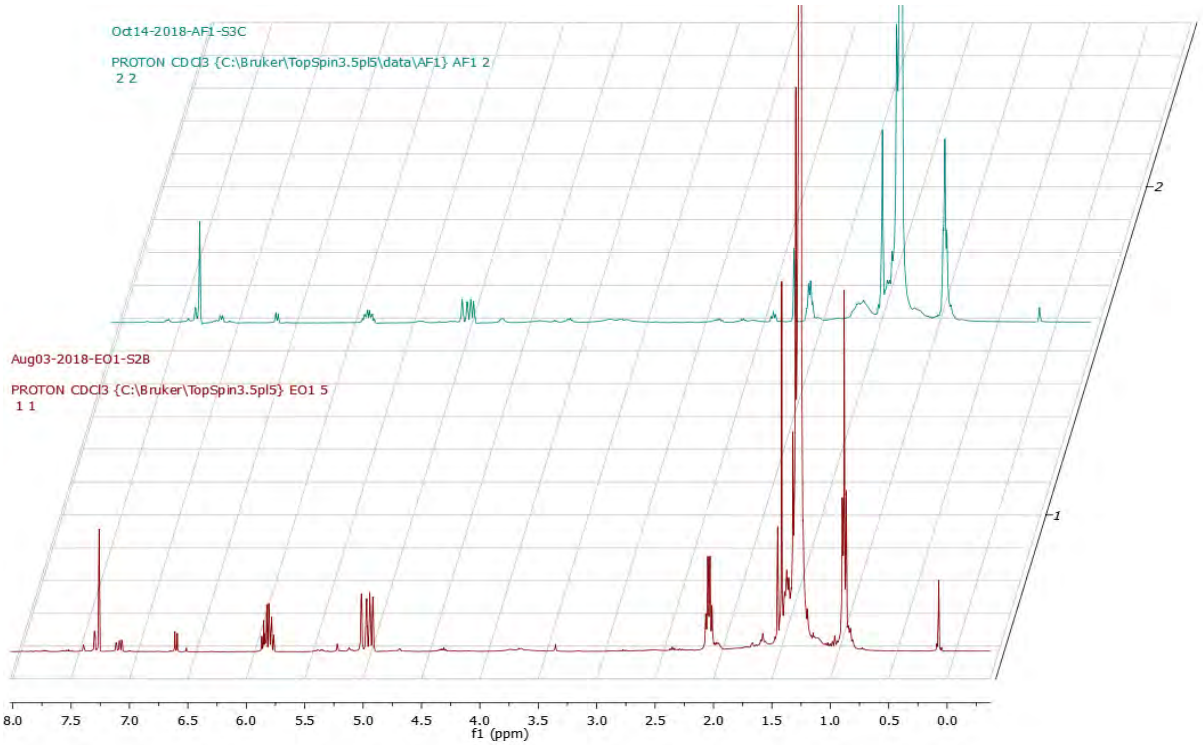
Appendix III-F: ^{1}H NMR chemical shift for Buffalo River downstream samples



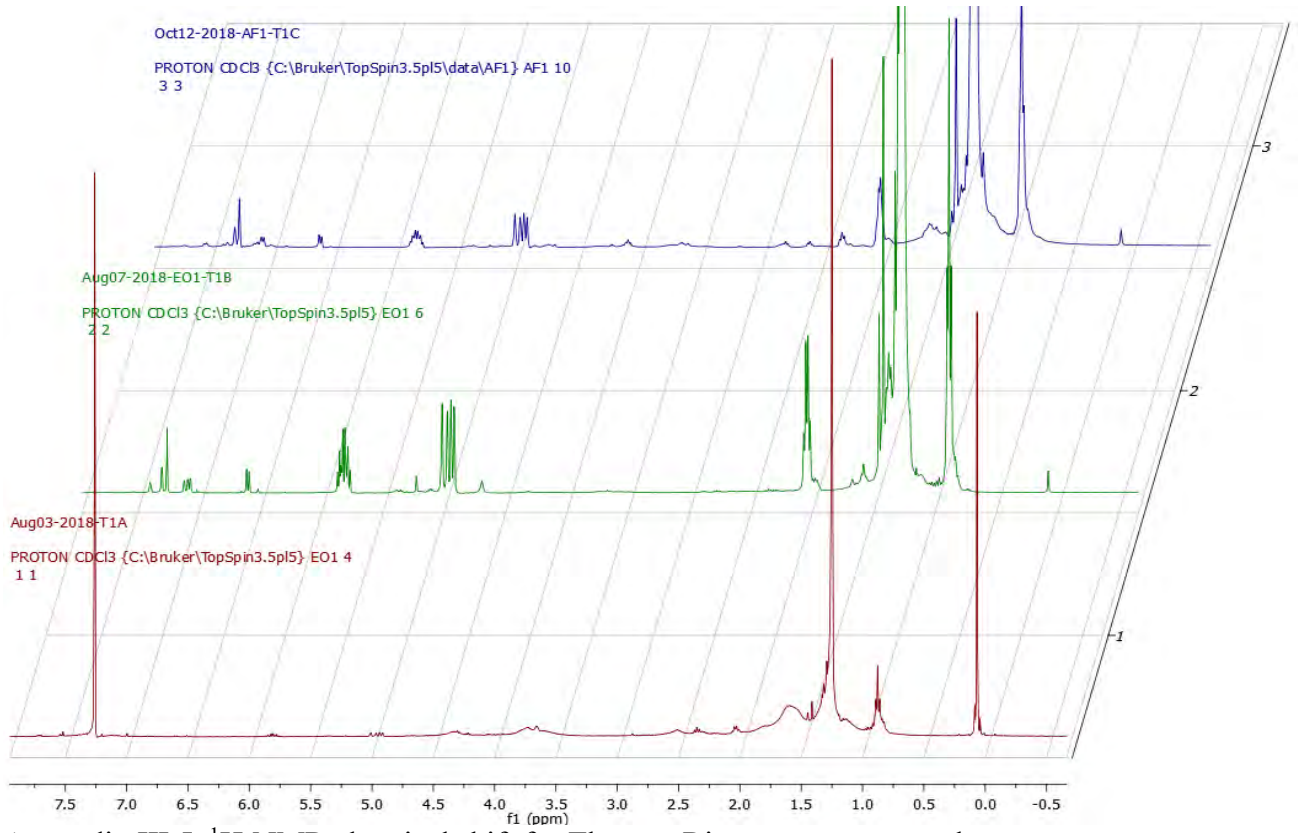
Appendix III-G: ^{1}H-NMR chemical shift for Swartkops River upstream samples



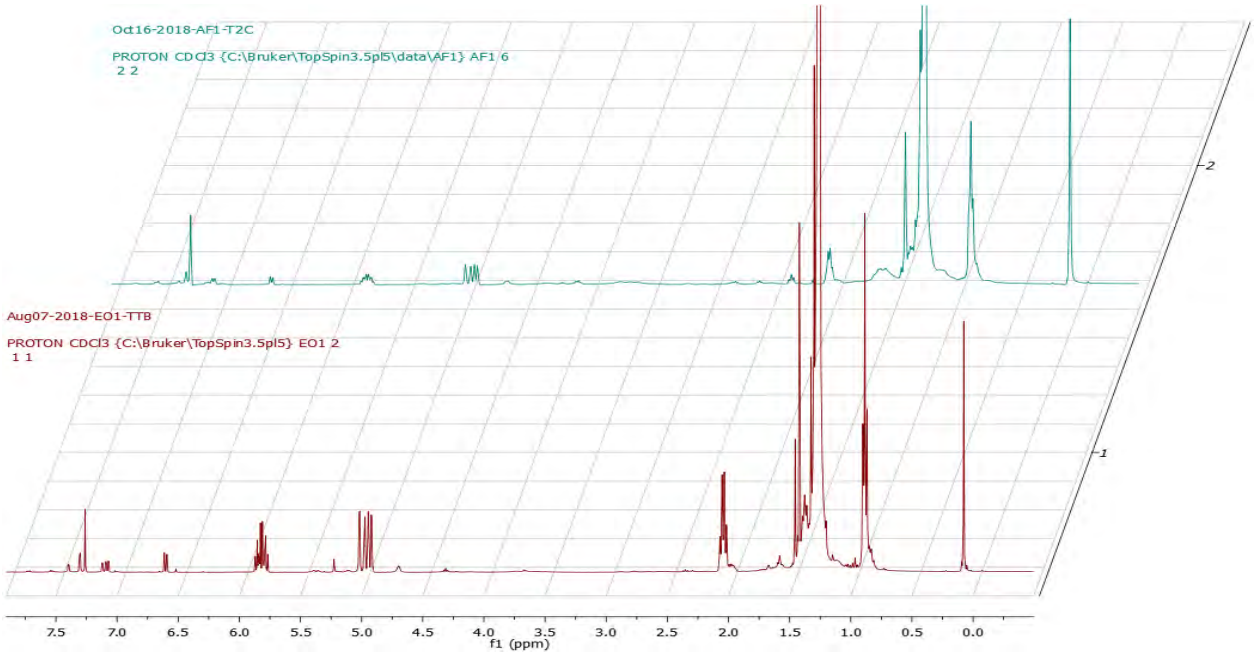
Appendix III-H: ^{1}H-NMR chemical shift for Swartkops River midstream samples



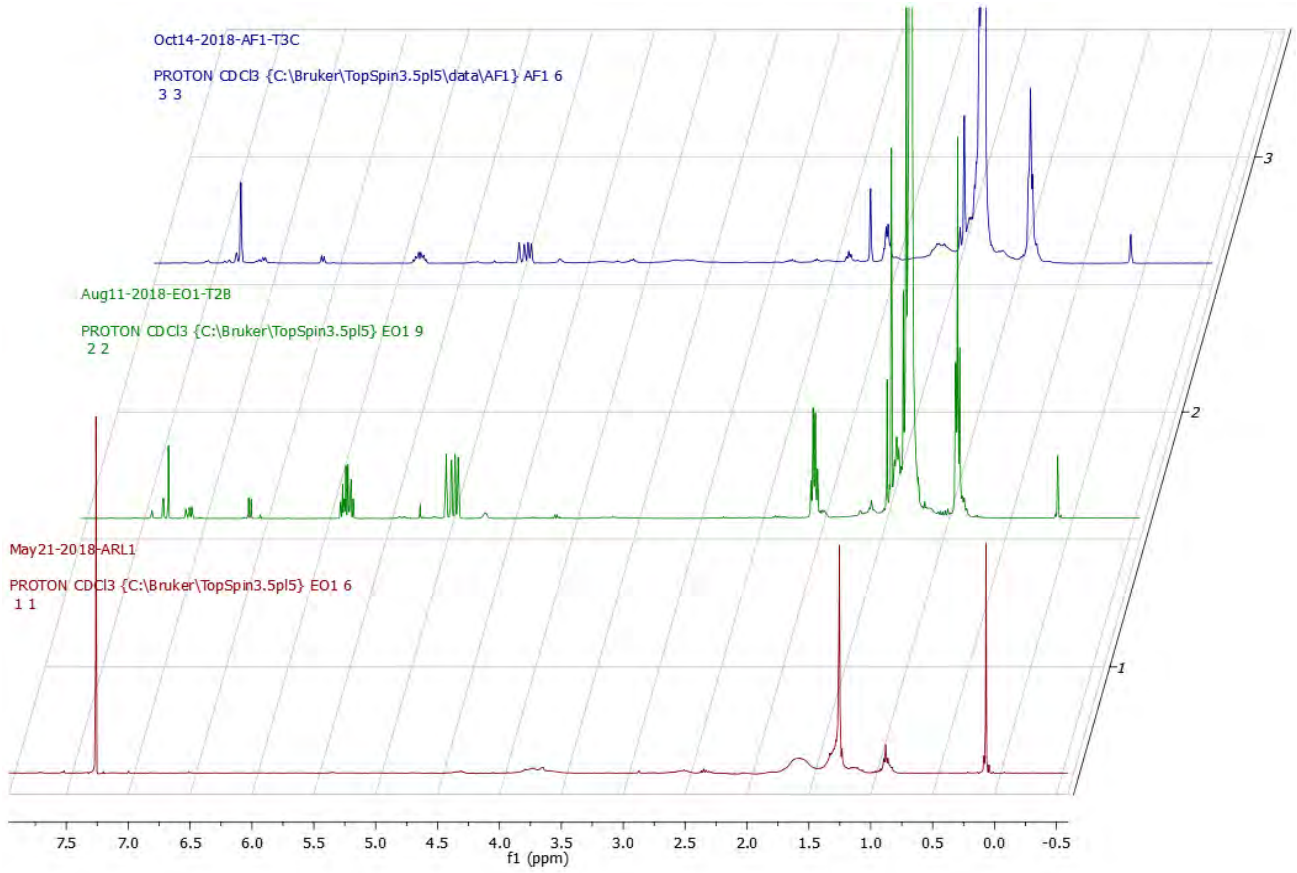
Appendix III-I: ^{1}H-NMR chemical shift for Swartkops River downstream samples



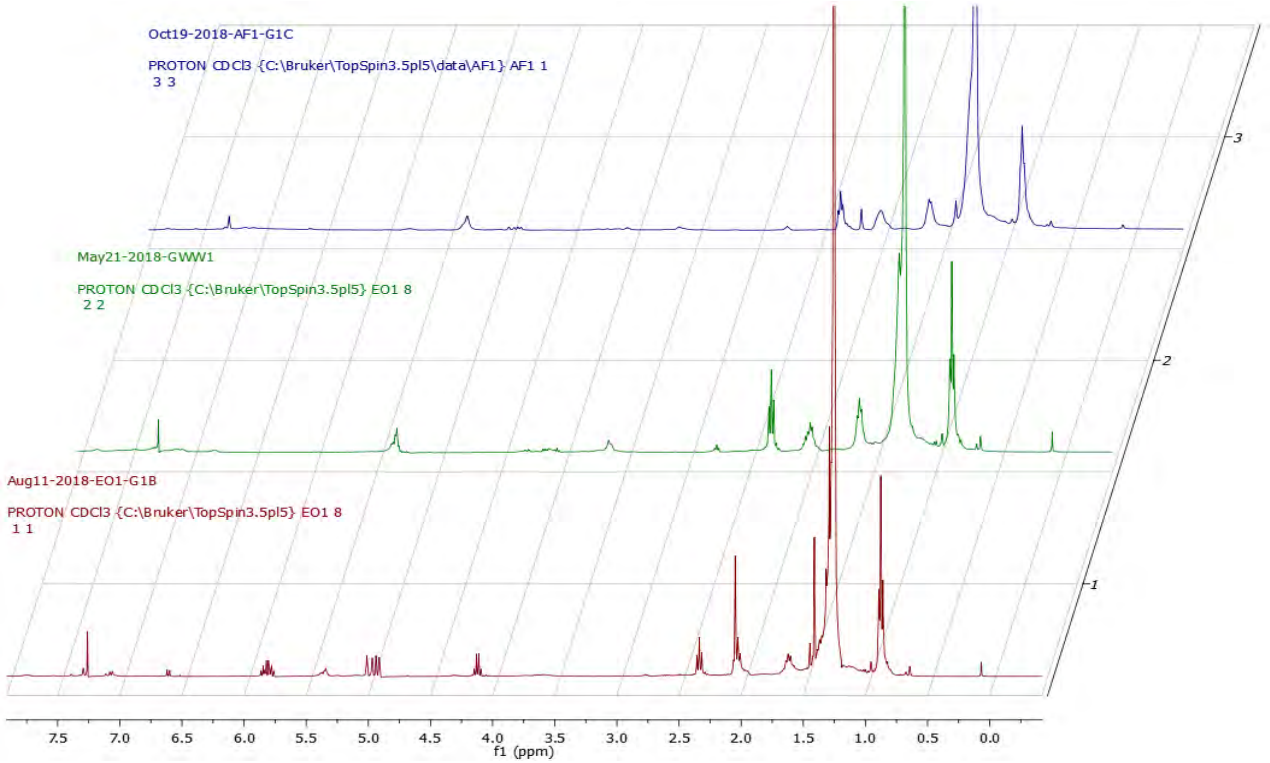
Appendix III-J: ^{1}H-NMR chemical shift for Thyume River upstream samples



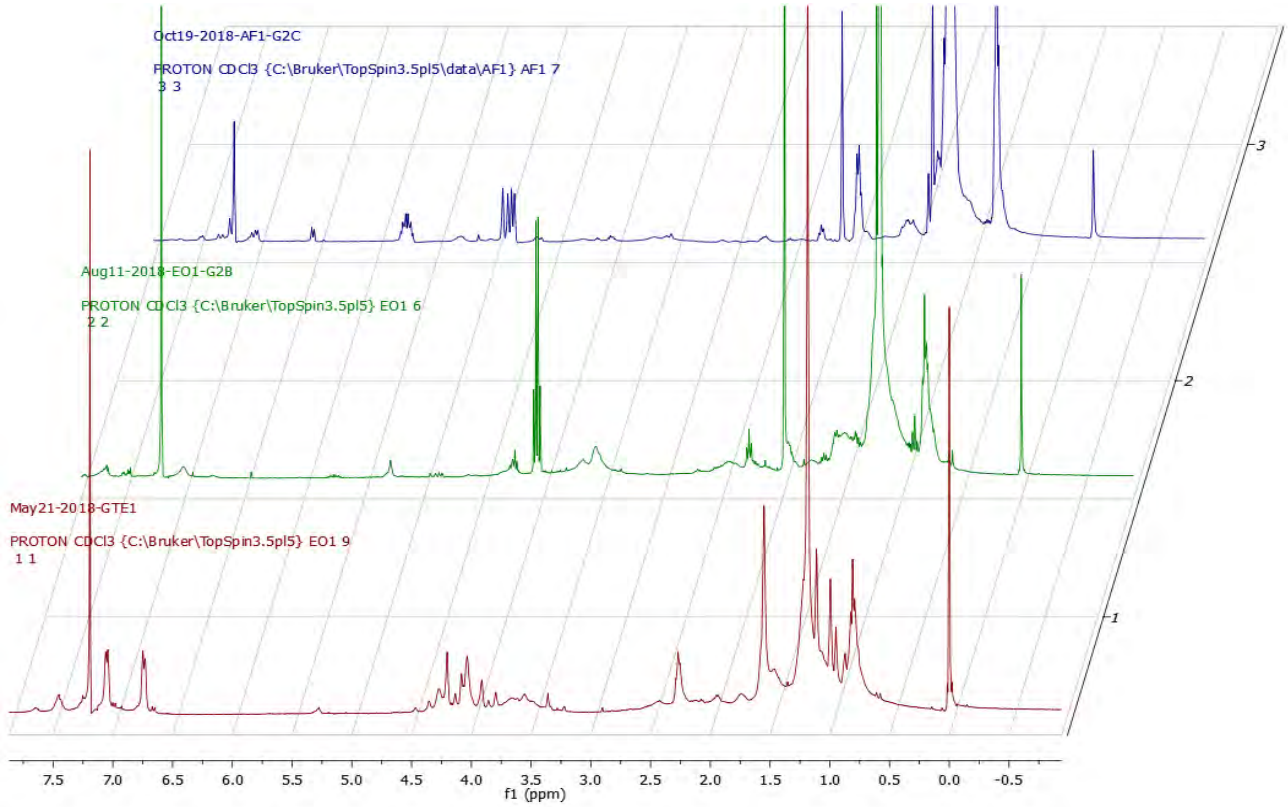
Appendix III-K: ^{1}H-NMR chemical shift for Thyume River midstream samples



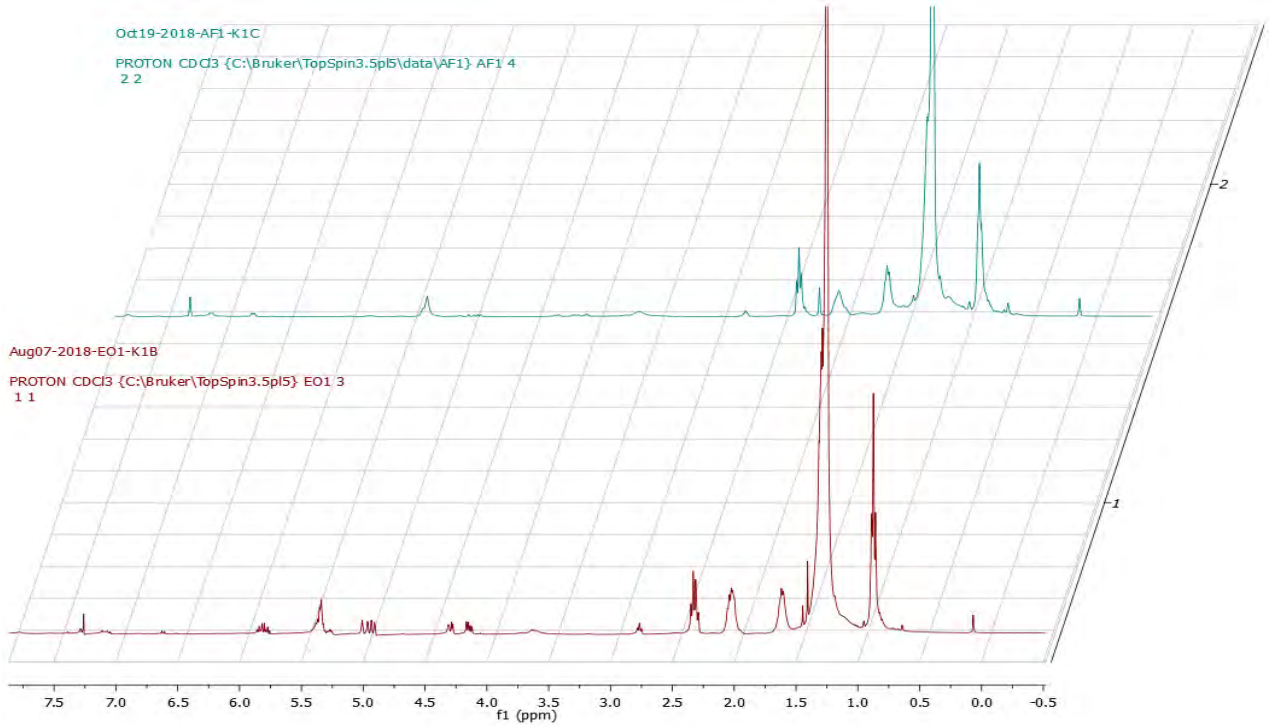
Appendix III-L: ^{1}H-NMR chemical shift for Thyume River downstream samples



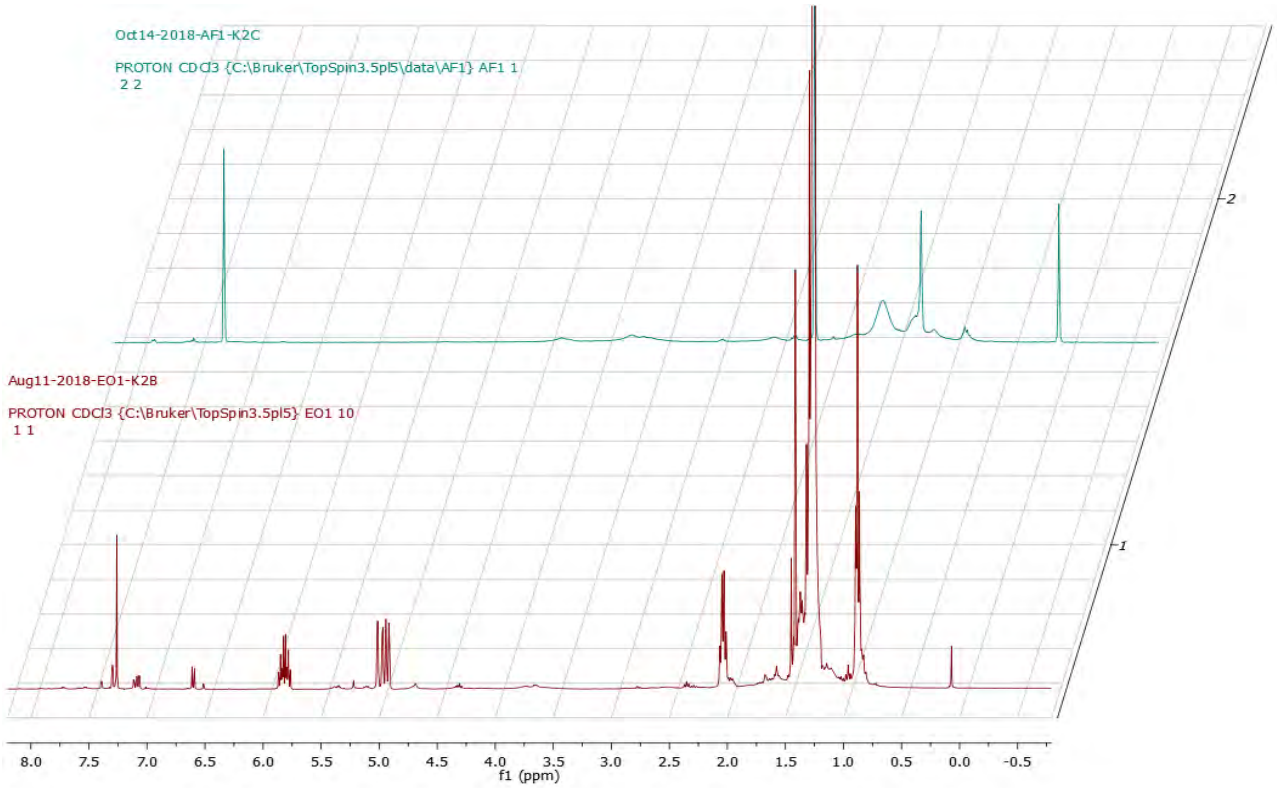
Appendix III-M: ^{1}H-NMR chemical shift for Grahamstown wastewater



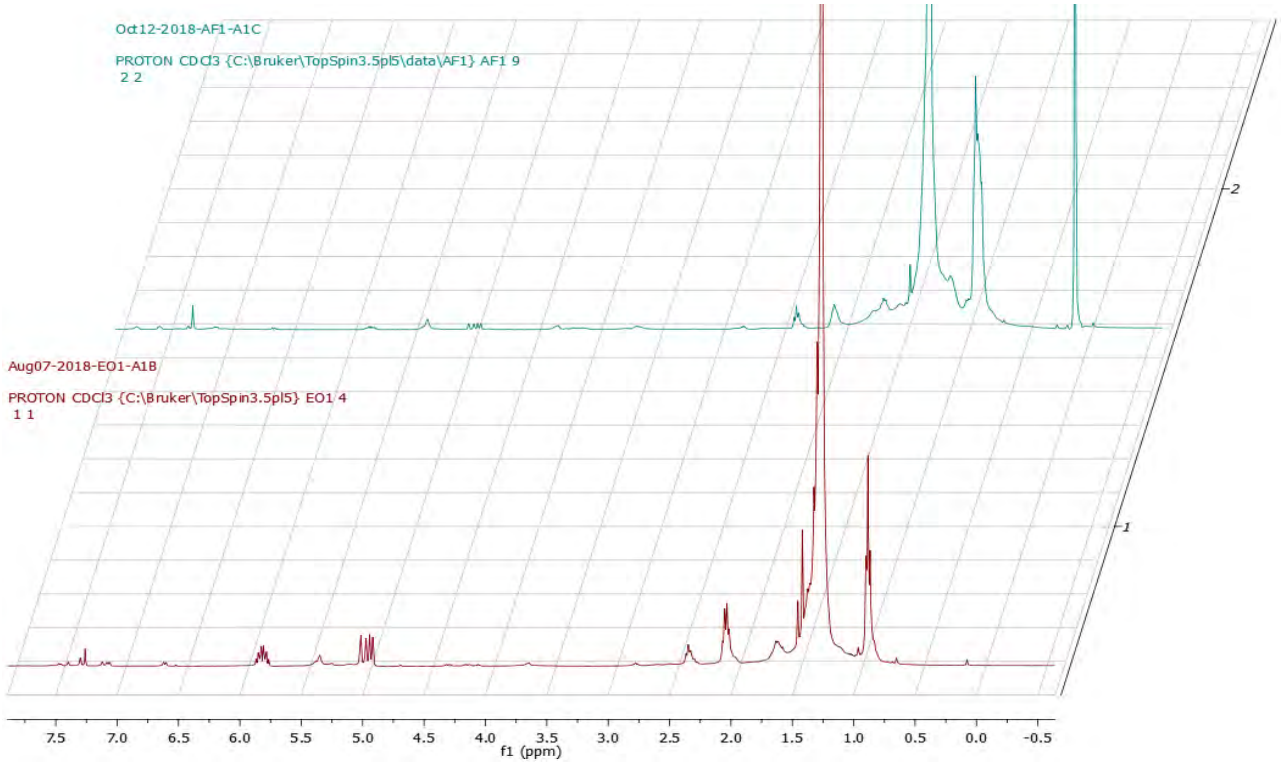
Appendix III-N: ^{1}H-NMR chemical shift for Grahamstown treated effluents



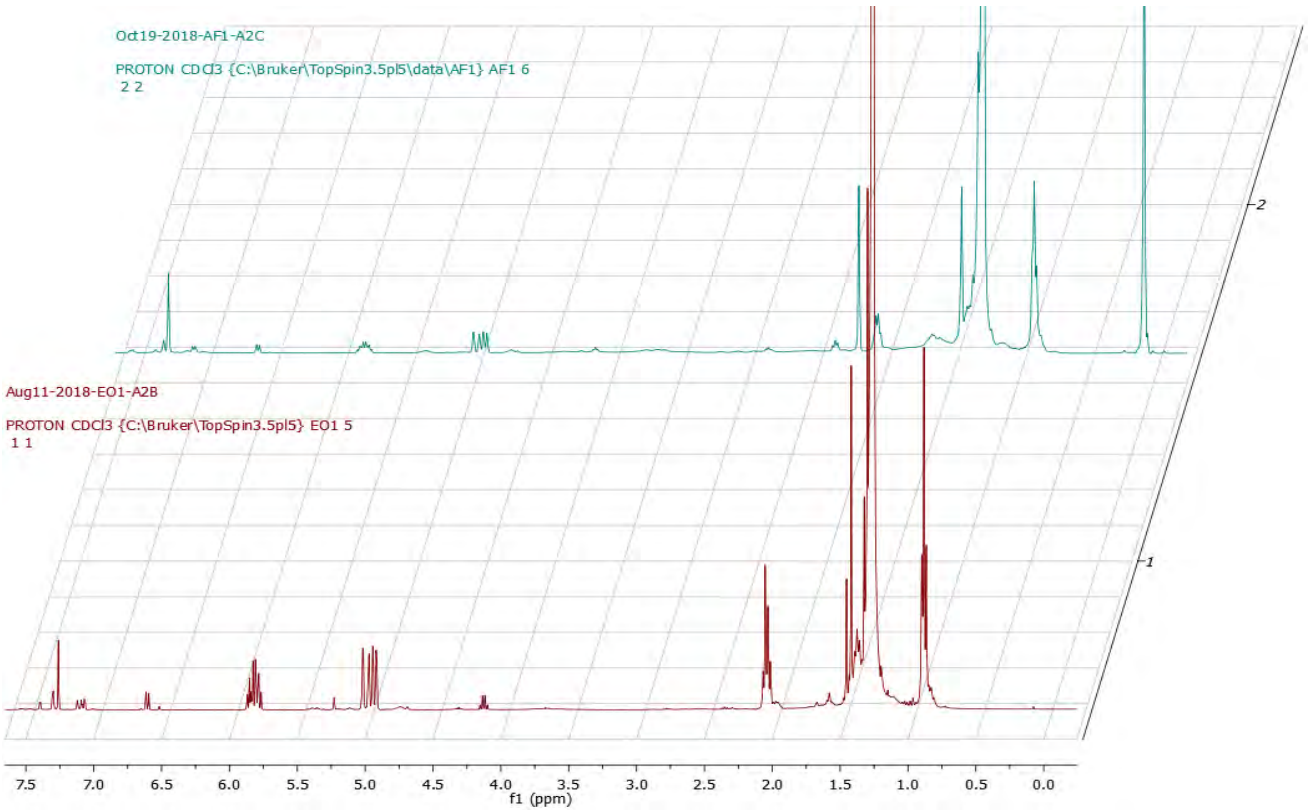
Appendix III-O: ^{1}H-NMR chemical shift for Kings Williams Town wastewater



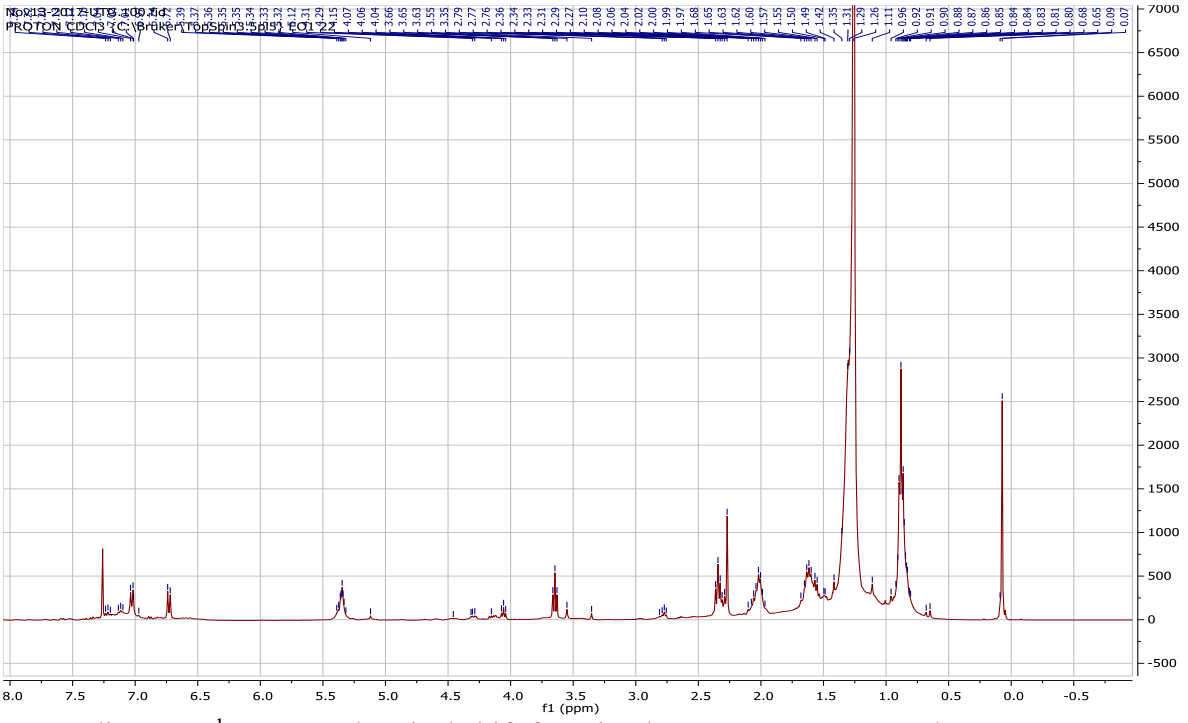
Appendix III-P: ^{1}H-NMR chemical shift for Kings Williams Town treated effluents



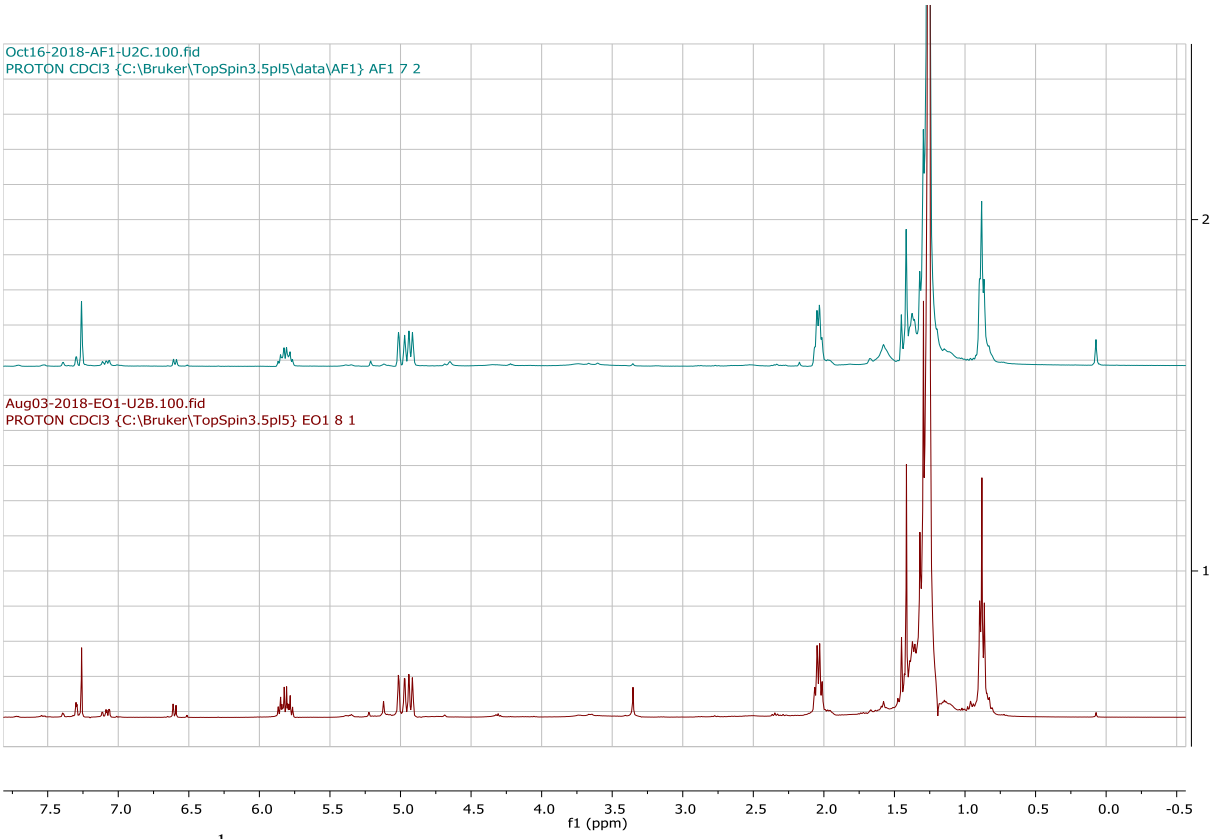
Appendix III-Q: ^{1}H-NMR chemical shift for Alice wastewater



Appendix III-R: ^{1}H-NMR chemical shift for Alice treated effluents.

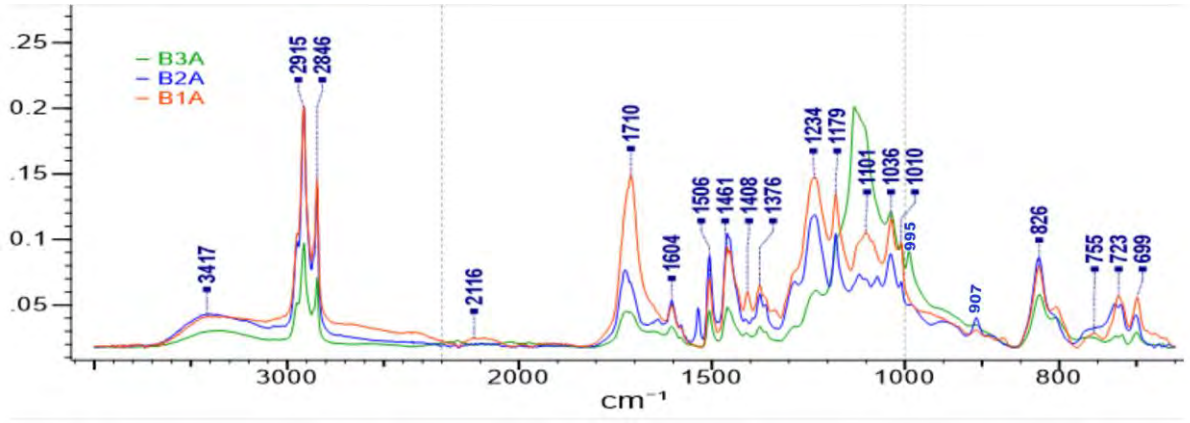


Appendix III-S: ^{1}H-NMR chemical shift for Uitenhage wastewater sample

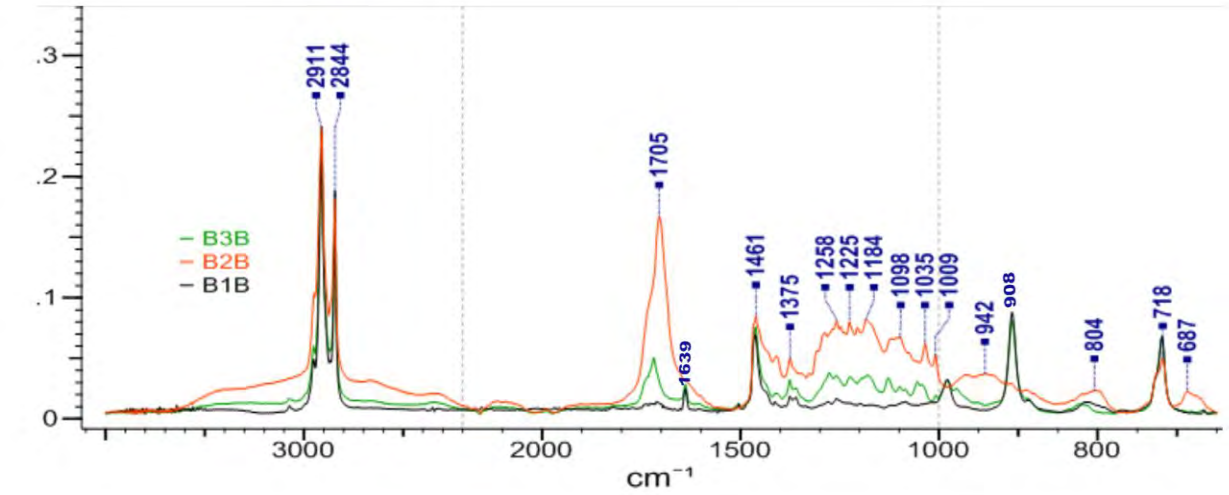


Appendix III-T: ^{1}H-NMR chemical shift for Uitenhage treated effluents

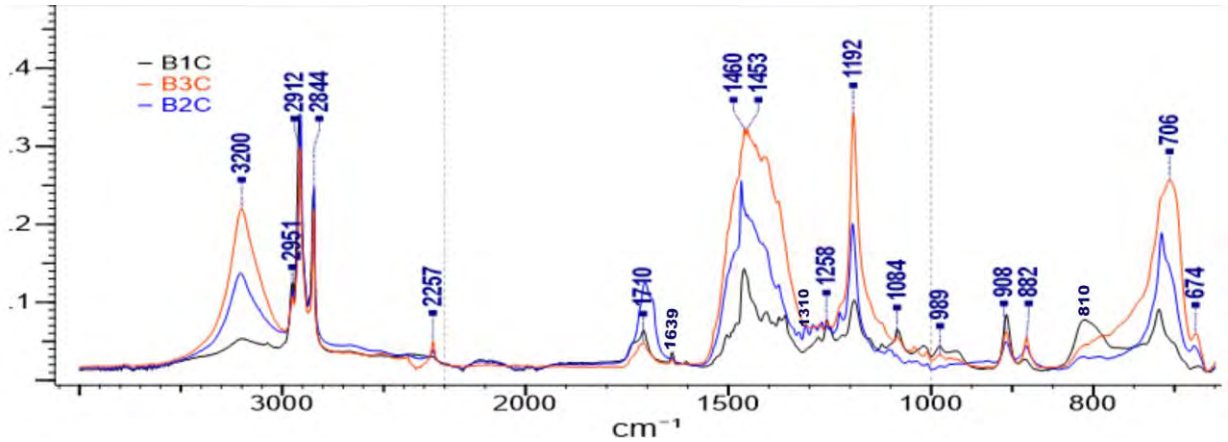
APPENDIX IV



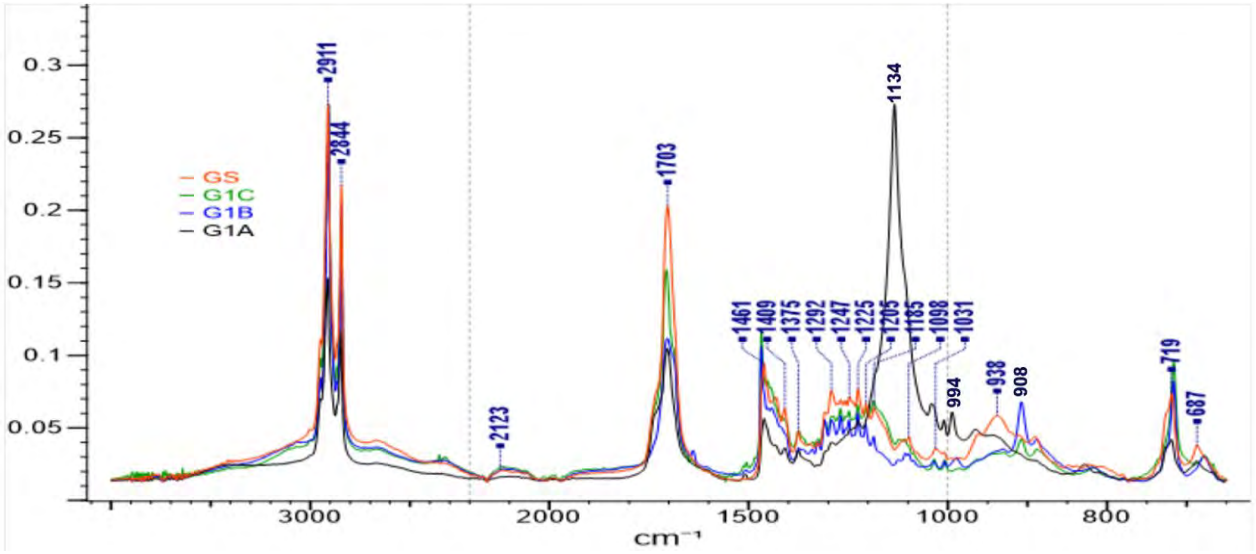
Appendix IV-A: Absorption peaks of the Bloukrans River samples for autumn: upstream (B1A), midstream (B2A) and downstream (B3A).



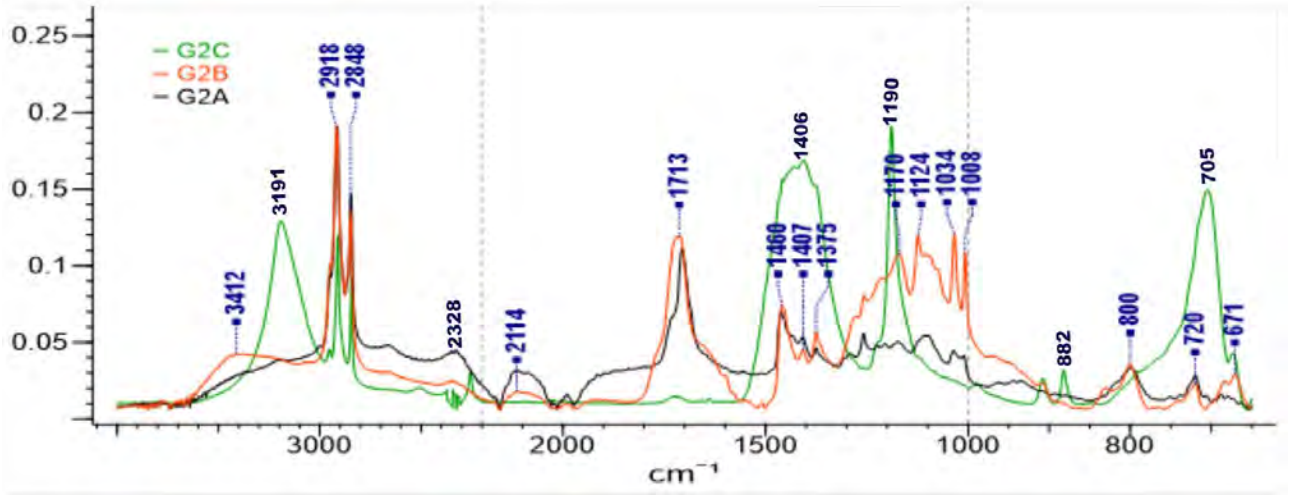
Appendix IV-B: Absorption peaks of the Bloukrans River samples for winter: upstream (B1B), midstream (B2B) and downstream (B3B).



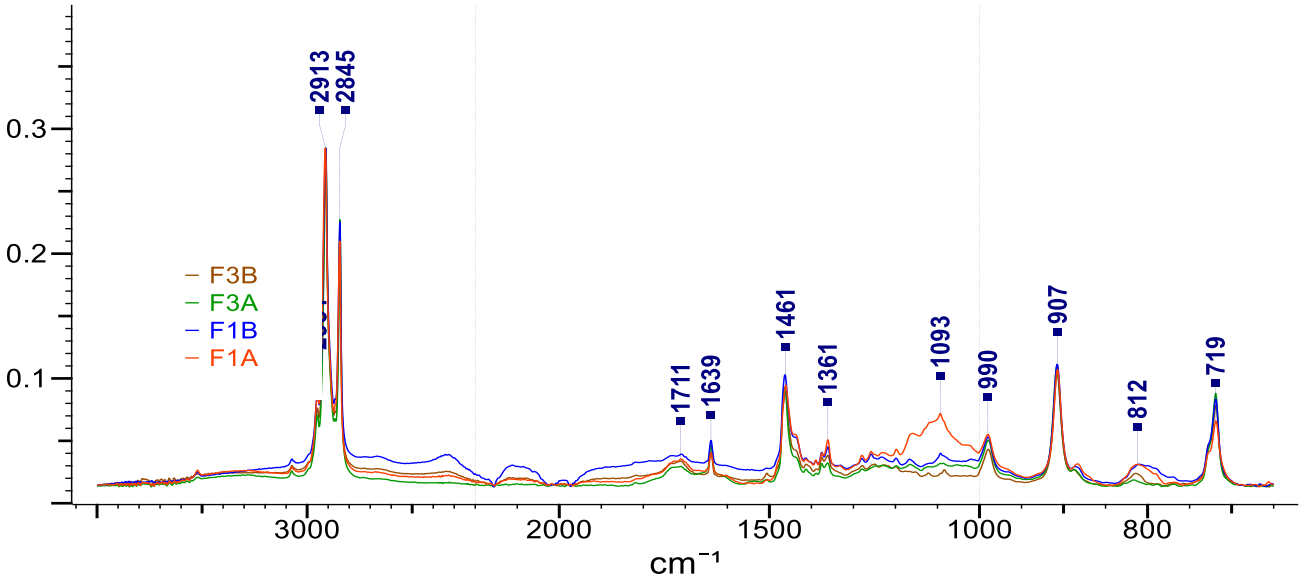
Appendix IV-C: Absorption peaks of the Bloukrans River for spring: upstream (B1C), midstream (B2C) and downstream (B3C).



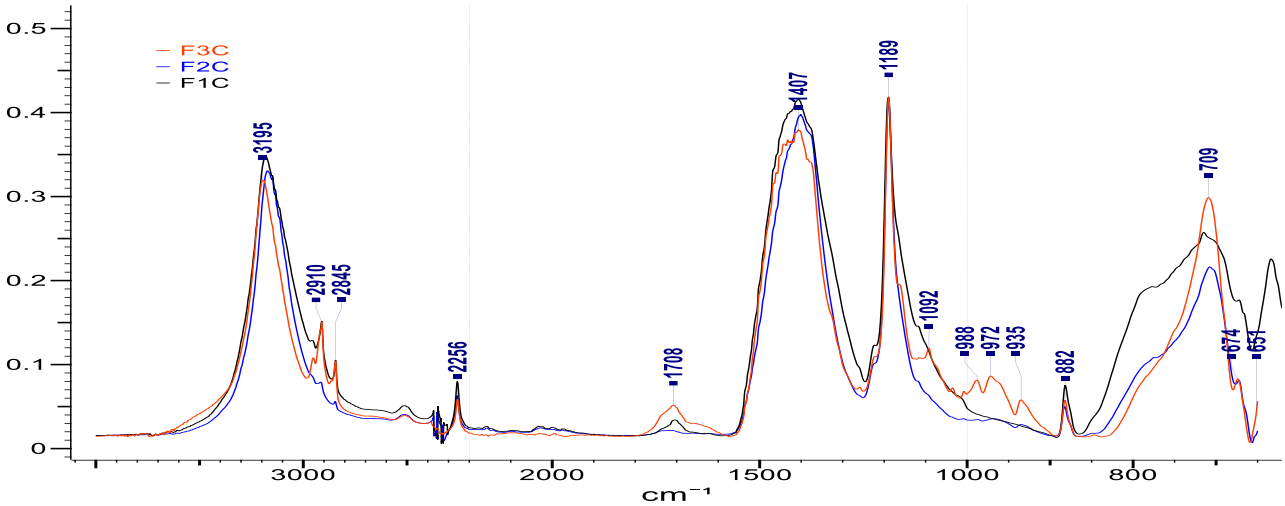
Appendix IV-D: Absorption peaks of Grahamstown wastewater influents samples: autumn (G1A), winter (G1B), spring (G1C) and sludge (GS).



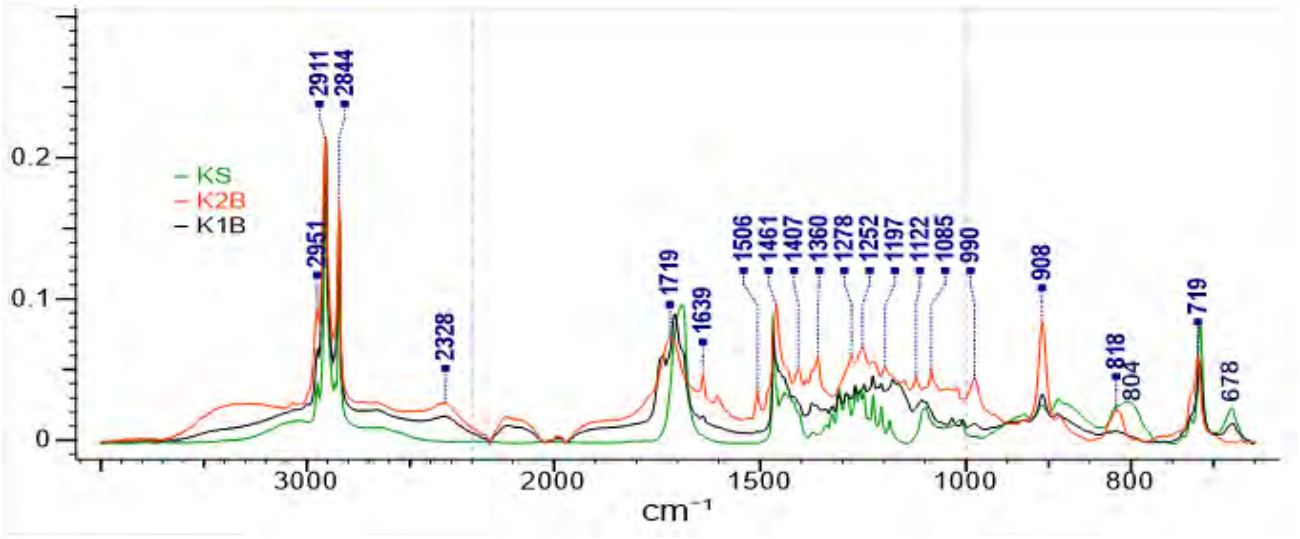
Appendix IV-E: Absorption peaks of Grahamstown wastewater effluents samples for autumn (G2A), winter (G2B) and spring (G2C).



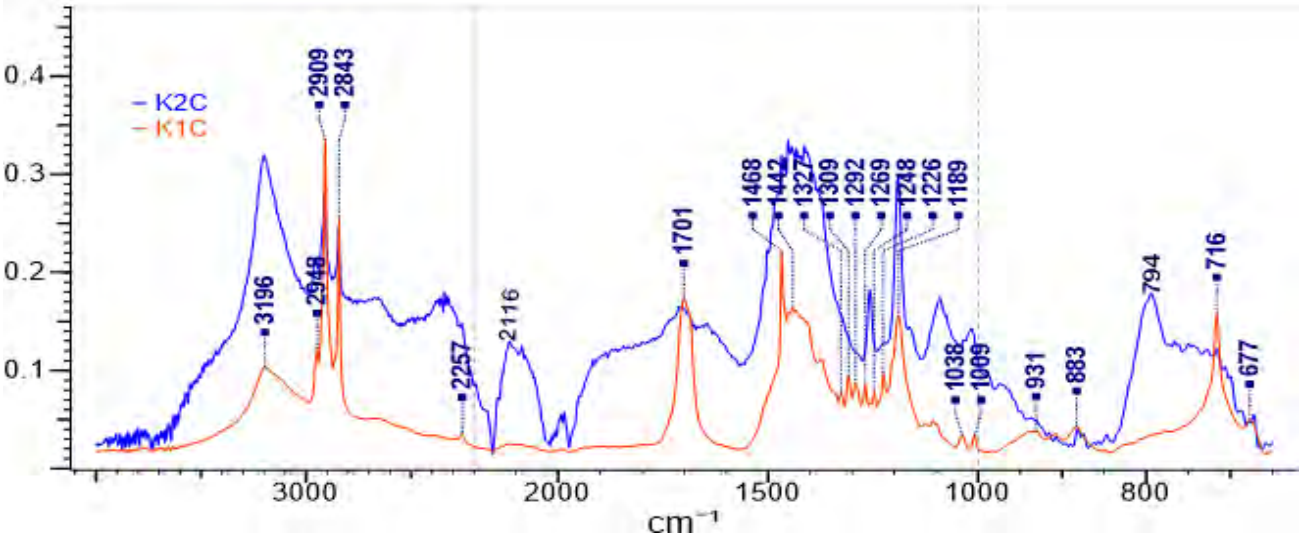
Appendix IV-F: Absorption peaks of Buffalo River samples: autumn upstream (F1A), downstream (F3A); winter upstream (F1B) and downstream (F3B).



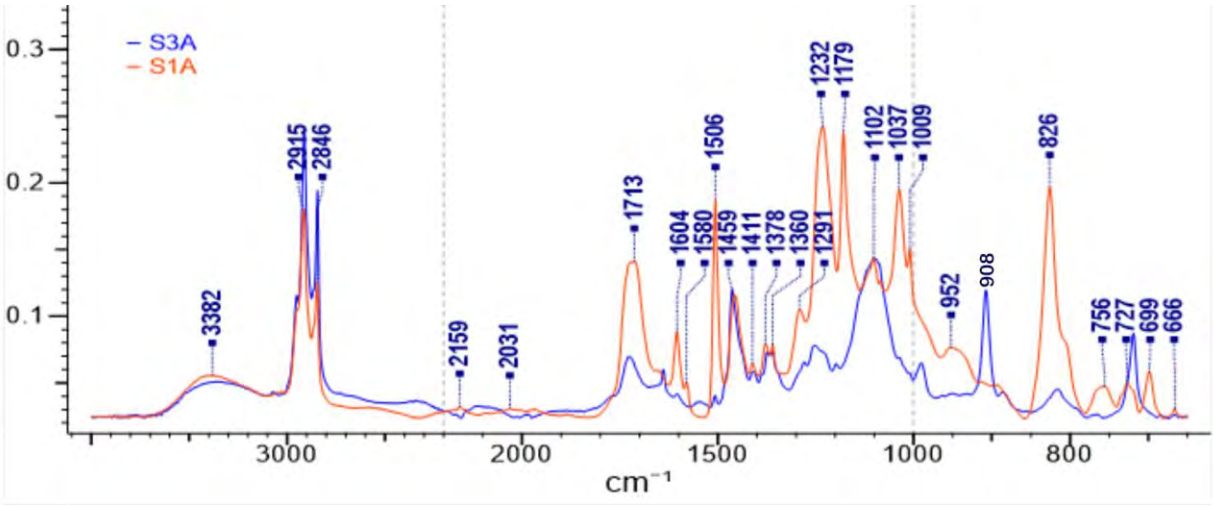
Appendix IV-G: Absorption peaks of Buffalo River samples for spring: upstream (F1C), midstream (F2C) and downstream (F3C).



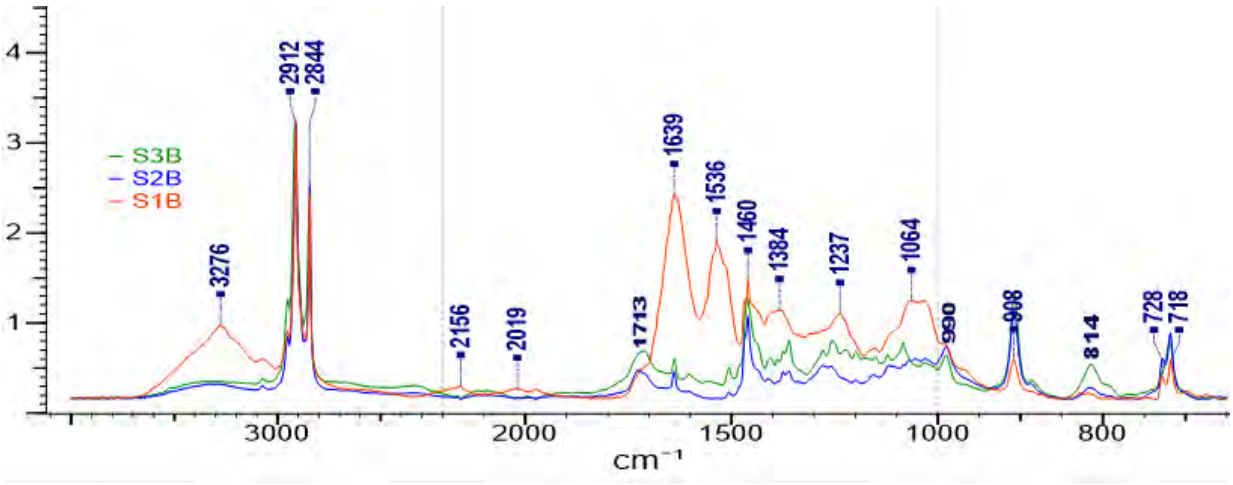
Appendix IV-H: Absorption peaks for King Williams Town wastewater samples for winter: influent (K1B), effluents (K2B) and sludge (KS).



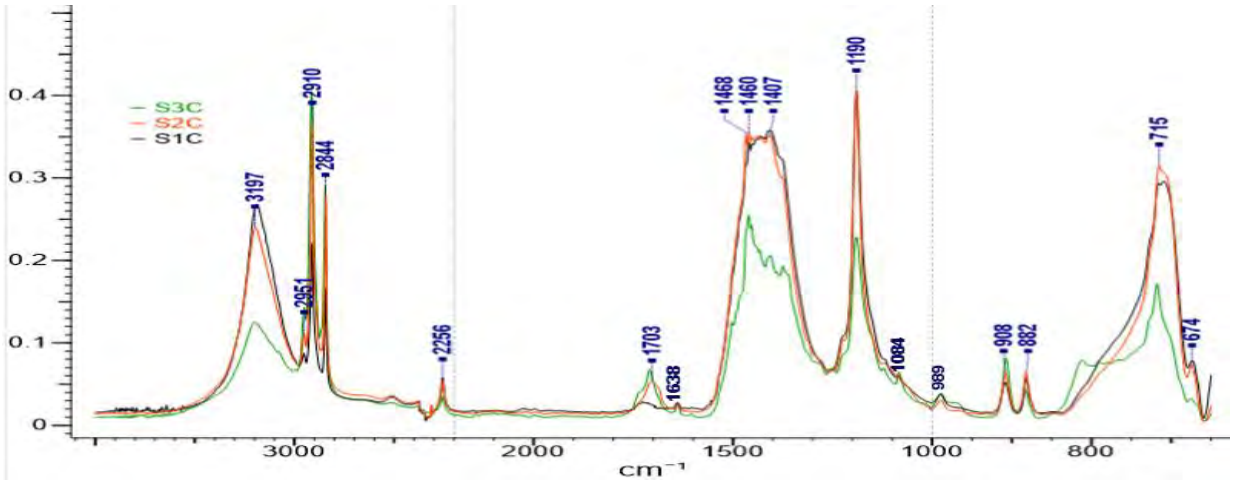
Appendix IV-I: Absorption peaks for King Williams Town wastewater samples for spring: influent (K1C) and effluents (K2C).



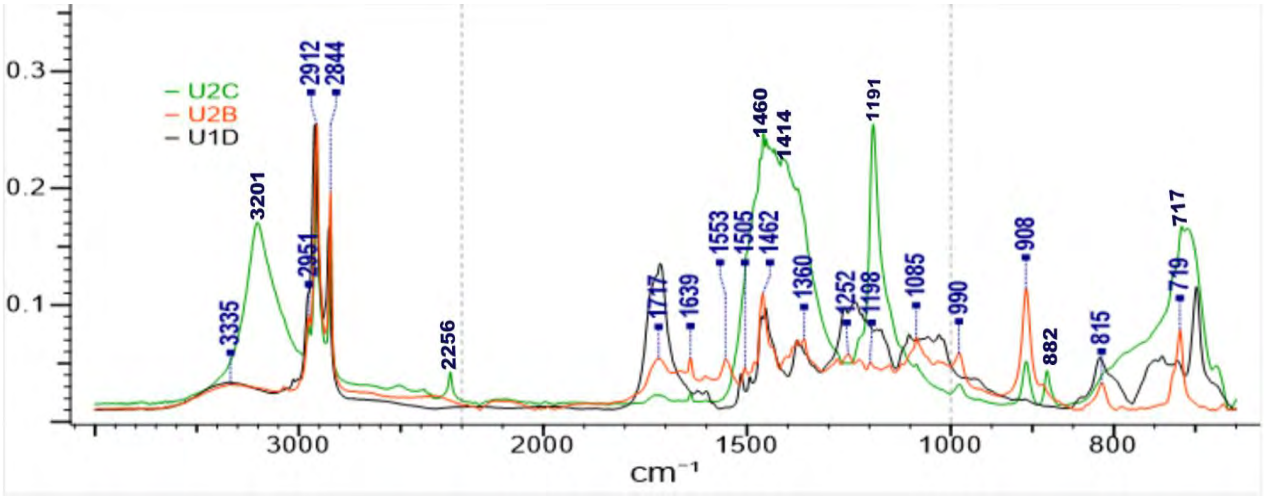
Appendix IV-J: Absorption peaks of Swartkops River samples for autumn: upstream (S1A) and downstream (S3A).



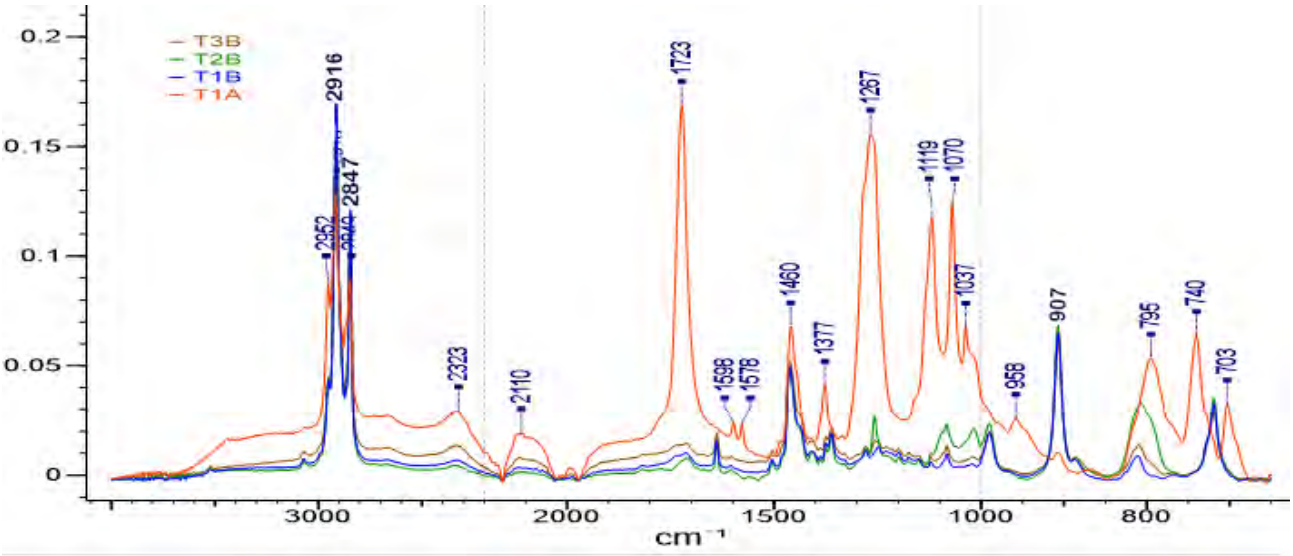
Appendix IV-K: Absorption peaks of Swartkops River samples for winter: upstream (S1B), middle (S2B) and downstream (S3B).



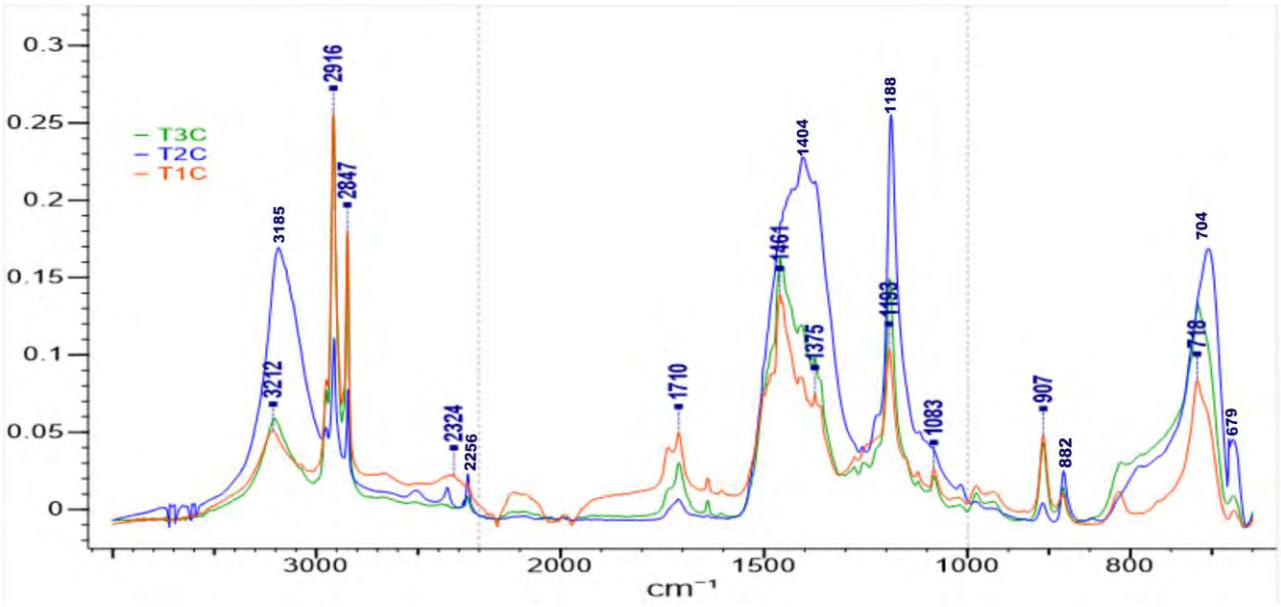
Appendix IV-L: Absorption peaks of Swartkops River samples for spring: upstream (S1C), midstream (S2C) and downstream S3C).



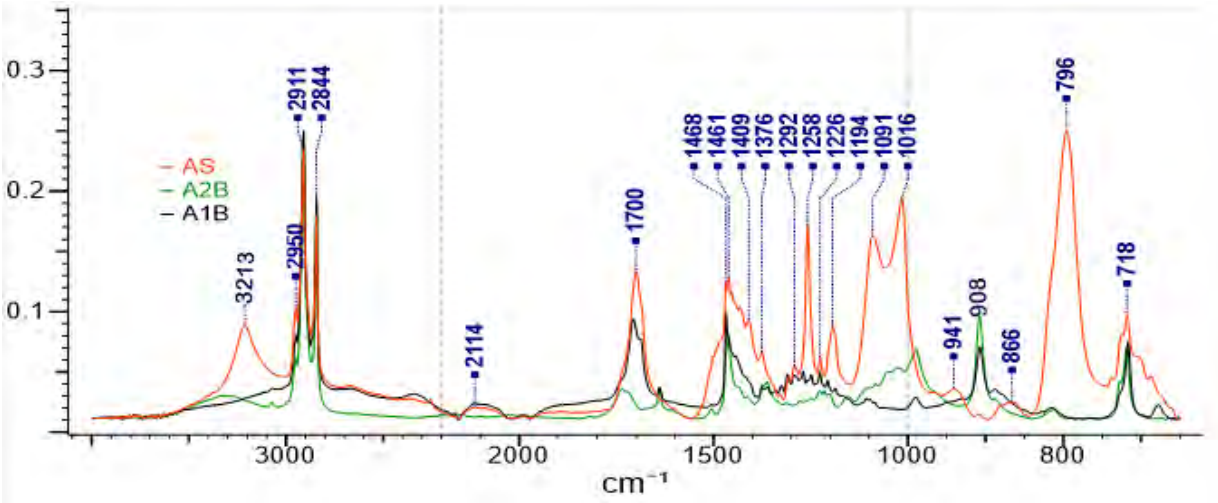
Appendix IV-M: Absorption peaks of Uitenhage wastewater inffluent (U1D); effluents samples for winter (U2B) and spring (U2C).



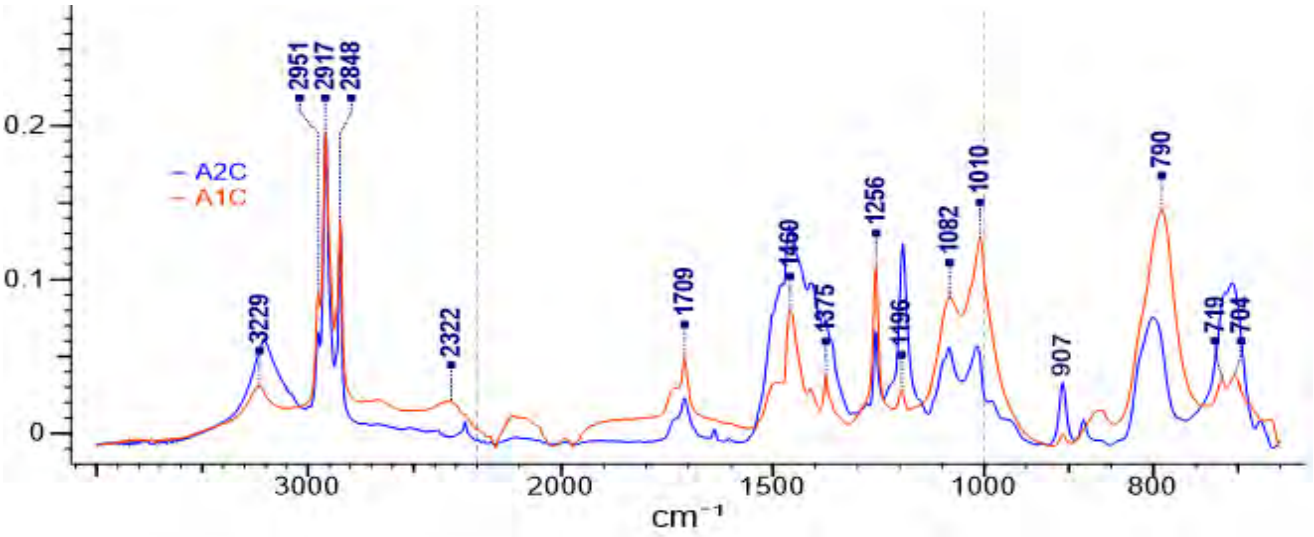
Appendix IV-N: Absorption peaks of Tyhume River samples for autumn: upstream (T1A); winter: upstream (T1B), midstream (T2B) and downstream (T3B).



Appendix IV-O: Absorption peaks of Tyhume River samples for spring upstream (T1C), midstream (T2C) and downstream (T3C).



Appendix IV-P: Absorption peaks of Alice wastewater (A1B), treated effluents (A2B) and sludge (AS) samples for winter.

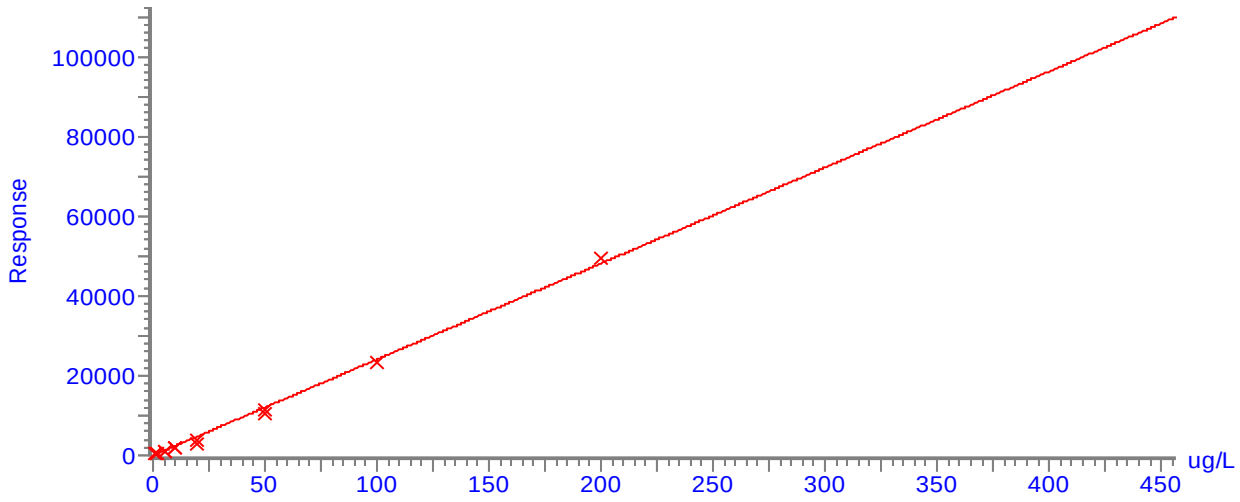


Appendix IV-Q: Absorption peaks of Alice wastewater (A1C), treated effluents (A2C) samples for spring.

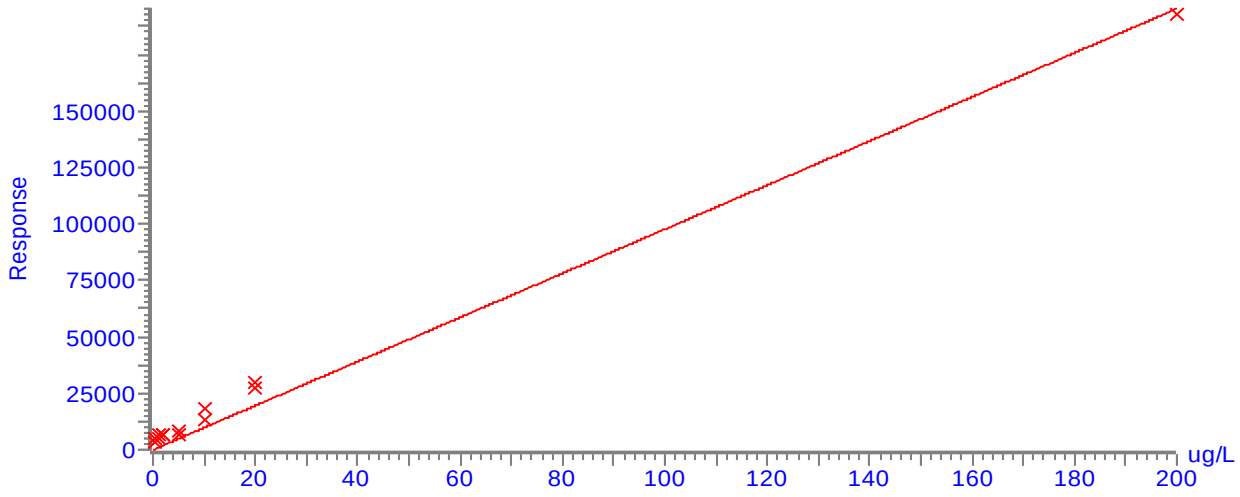
Appendix V

Calibration Curves for EDCs

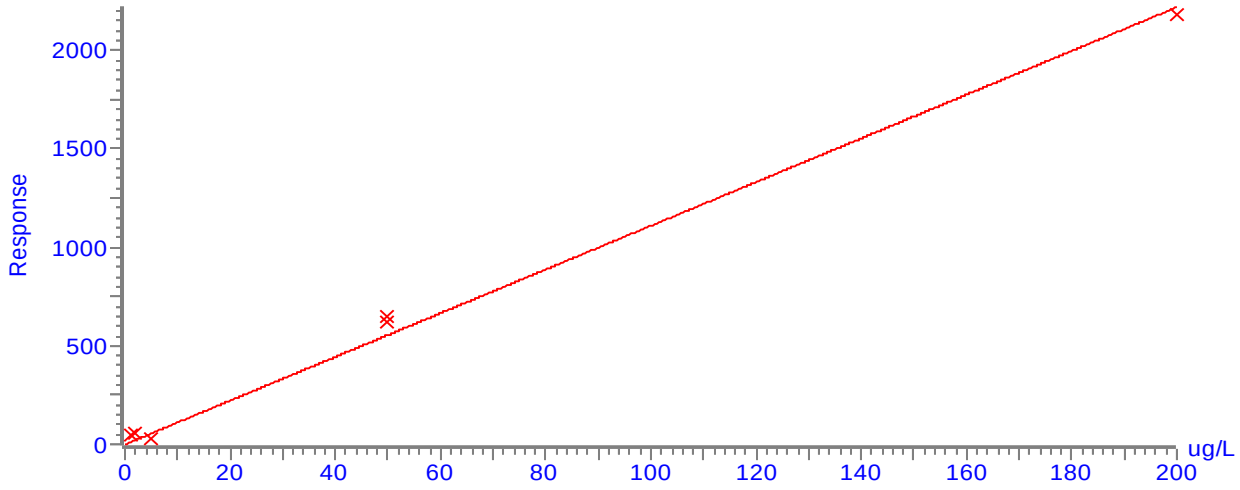
Compound name: Estradiol
Coefficient of Determination: R^2 = 0.995151
Calibration curve: 240.681 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None



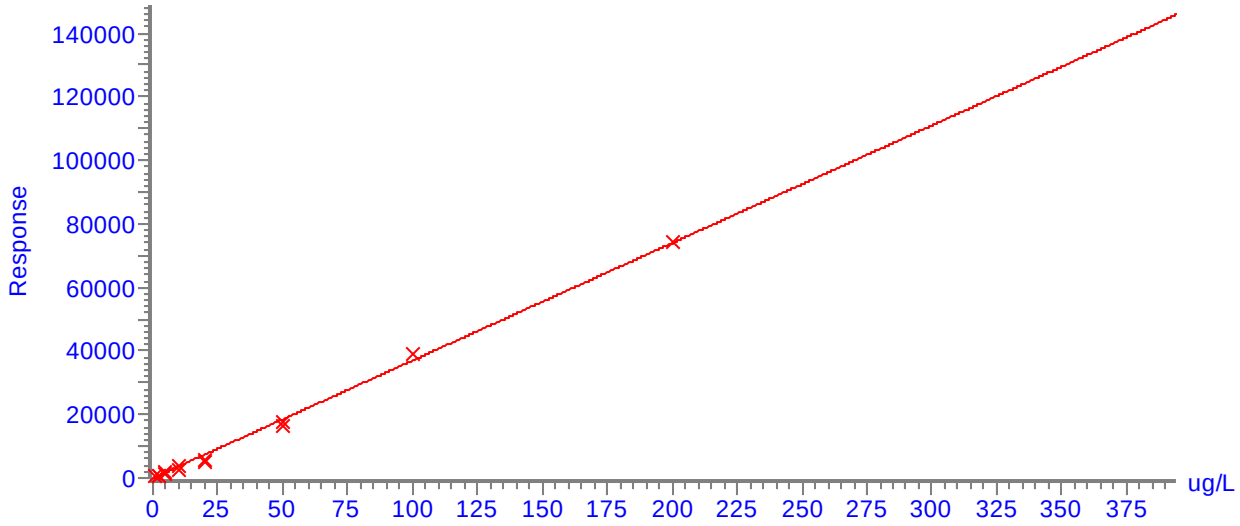
Compound name: 4-Nonylphenol
Coefficient of Determination: R^2 = 0.987601
Calibration curve: 976.732 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None



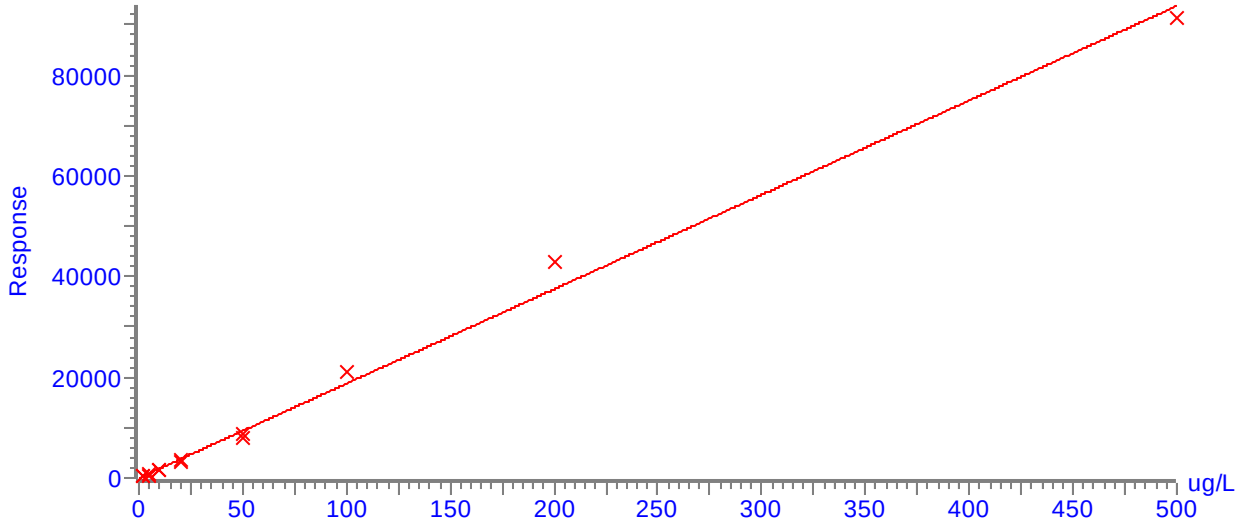
Compound name: 2_4-Dichlorophenol
Coefficient of Determination: R^2 = 0.994524
Calibration curve: 11.0972 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None



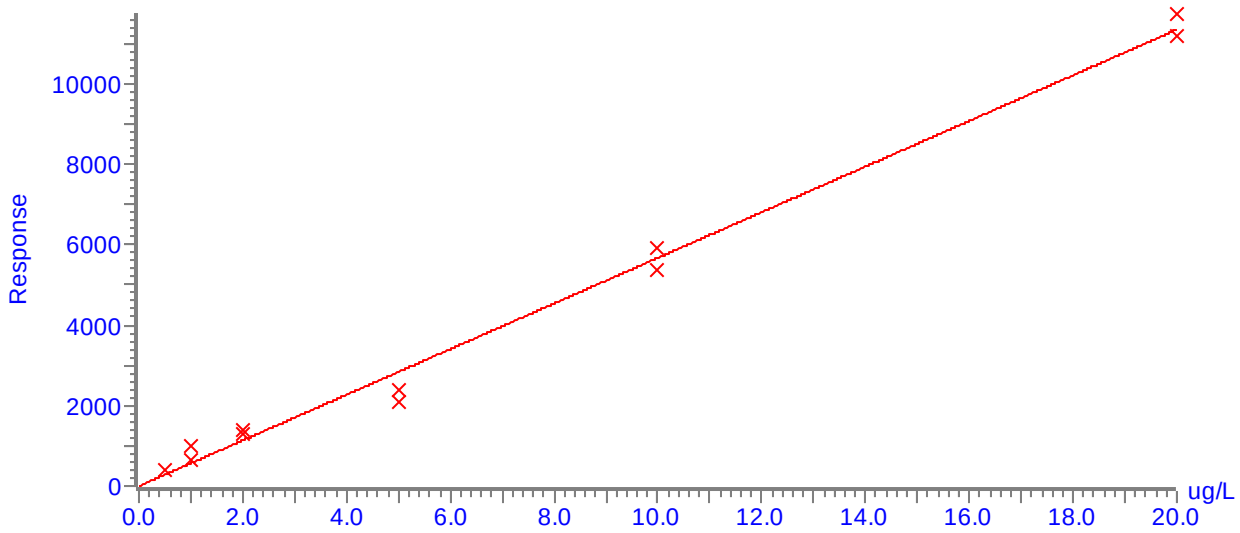
Compound name: Estrone
Coefficient of Determination: R^2 = 0.996385
Calibration curve: 369.933 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None



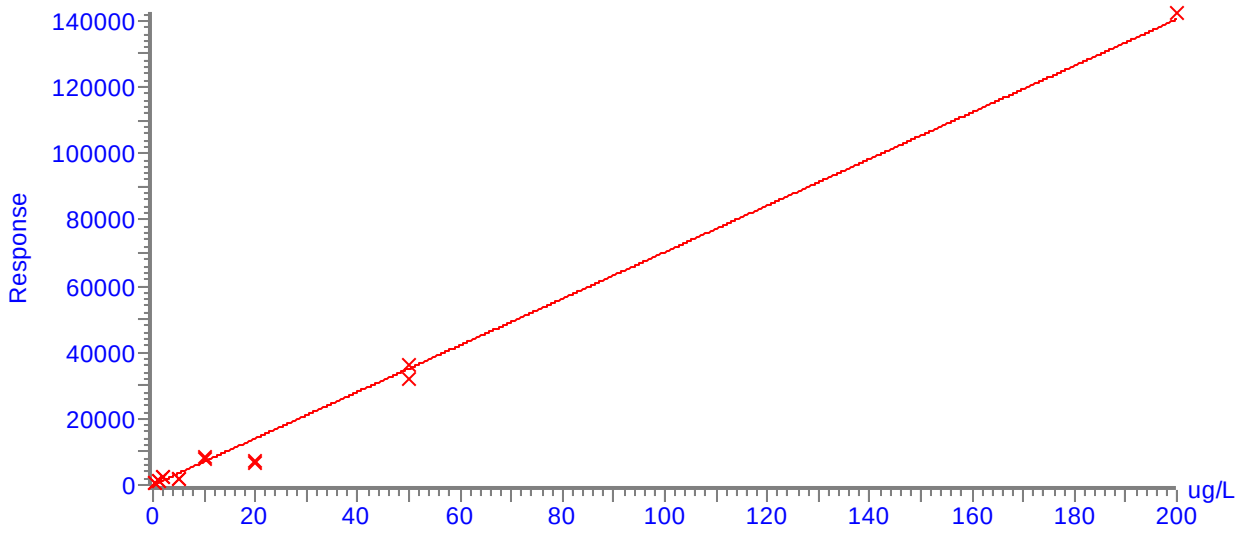
Compound name: Bisphenol A
Coefficient of Determination: R^2 = 0.994829
Calibration curve: 187.479 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None



Compound name: 4-Octylphenol
Coefficient of Determination: R^2 = 0.991987
Calibration curve: 567.095 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None



Compound name: Triclosan
Coefficient of Determination: R^2 = 0.993340
Calibration curve: 702.403 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None



Appendix VI-A: Heavy metals concentrations (µg/L) in the freshwater samples with their limits of detection.

Sample	Cr	Mn	Ni	Cu	Zn	As	Cd	Pb	Hg
LOD	**0.26**	**0.23**	**0.07**	**0.16**	**0.11**	**0.03**	**0.01**	**0.02**	**0.01**
B1A	0.9	0.7	13.1	5.7	40.7	2.0	1.2	1.1	0.14
B2A	1118.7	966.7	849.6	578.9	126.8	532.4	756.4	436.1	47.31
B3A	190.5	136.2	220.4	212.8	73.4	159.6	96.3	169.5	5.37
B1B	2.3	4.2	8.9	10.0	33.4	2.8	2.5	2.4	3.20
B2B	155.4	116.3	202.6	180.5	118.6	334.2	82.9	146.0	9.12
B3B	1.1	11.0	17.6	16.6	46.6	26.7	0.7	1.6	0.84
B1C	5.5	8.8	51.2	28.6	99.9	5.4	4.3	5.2	4.25
B2C	1.3	204.3	16.7	17.9	129.3	37.9	0.3	2.7	0.35
B3C	0.7	3.8	7.4	11.9	47.9	1.6	0.1	1.3	0.25
F1B	0.7	1.2	4.0	4.2	37.2	0.4	0.04	0.4	0.18
F2B	1368.4	839.3	1370.5	1529.4	109.9	1025.8	659.3	1195.0	821.81
F3B	256.6	237.6	323.2	287.1	52.9	231.9	148.3	232.4	24.46
F1C	3.5	2.6	9.2	7.1	41.3	6.8	1.4	1.9	0.25
F2C	1.8	2.6	26.7	14.4	51.0	11.0	1.4	1.1	0.21
F3C	5.4	10.1	792.5	21.6	68.9	10.7	4.2	6.9	0.52
S1A	0.28	2.3	17.5	9.0	46.1	0.8	0.5	0.5	0.32
S3A	7.1	4.5	53.4	24.4	92.6	17.5	162.3	3.9	0.73
S1B	0.8	2.7	10.5	8.1	34.5	1.0	1.0	0.1	0.78
S2B	12.1	18.4	37.2	32.1	83.7	16.2	11.0	10.4	0.47
S3B	1.4	1.5	18.4	10.9	77.9	1.6	0.2	0.5	0.21
S1C	99.7	72.4	114.4	112.9	101.4	66.9	45.6	74.8	22.14
S2C	0.7	2.0	6.2	11.8	60.9	1.1	0.1	0.7	0.15
S3C	9529.9	2467.0	1331.5	638.7	122.7	2205.8	4279.3	540.9	214.10
T1A	1702.9	1042.1	1839.0	2092.3	114.3	1347.9	847.3	1769.5	1187.01
T3A	0.8	1.5	3.8	8.0	39.9	0.8	0.2	0.5	0.26
T1B	64.1	71.2	97.6	78.1	53.8	66.7	39.5	49.4	8.89
T2B	0.3	1.6	3.7	4.9	29.2	0.3	<LOD	<LOD	0.16
T3B	1.8	19.5	9.7	11.4	50.3	7.4	1.0	2.4	0.06
T1C	0.4	0.8	3.6	5.5	31.5	0.2	<LOD	<LOD	0.06
T2C	19.0	10.7	42.9	36.2	68.8	21.0	14.9	10.0	2.22
T3C	15.7	40.8	30.3	30.9	56.9	16.6	11.0	16.1	0.23

Appendix VI-B: Heavy metals concentrations (µg/L) in the wastewater and treated effluent samples with their limits of detection.

Sample	Cr	Mn	Ni	Cu	Zn	As	Cd	Pb	Hg
LOD	**0.26**	**0.23**	**0.07**	**0.16**	**0.11**	**0.03**	**0.01**	**0.02**	**0.01**
G1A	0.5	68.2	6.9	17.4	60.3	1.4	0.1	0.6	0.21
G2A	69.1	64.3	120.3	107.6	128.1	187.1	41.5	60.2	5.44
G1B	67.7	61.9	99.5	103.9	81.5	71.0	38.1	63.6	10.58
G2B	2102.7	1632.6	1947.2	2244.9	197.0	1536.2	1026.8	2098.2	1064.95
G1C	1.5	108.5	10.3	13.6	53.5	0.5	0.3	1.3	0.47
G2C	0.4	10.6	14.8	10.7	76.4	8.1	0.4	0.4	0.28
K1B	1.3	2.1	8.8	23.0	82.7	0.9	0.1	1.4	0.60
K2B	17.7	6.2	45.1	36.4	69.4	19.5	13.2	15.9	1.14
K1C	1584.1	1014.8	3564.5	4352.7	299.0	2905.8	731.9	3909.2	623.75
K2C	14.5	21.8	30.1	27.9	73.9	17.6	9.5	12.2	1.48
A1B	28.1	14.8	428.0	57.0	84.8	52.8	15.6	26.2	6.41
A2B	212.1	266.3	284.8	248.0	72.0	197.3	119.3	81.4	12.78
A1C	0.9	7.8	16.4	31.7	81.7	8.7	0.6	1.5	0.14
A2C	1564.2	4187.3	551.1	154.6	108.0	297.5	734.3	109.3	32.31
U2B	106.4	135.1	236.2	142.9	78.1	153.9	73.6	74.2	9.11
U2C	1.3	47.0	49.3	24.6	111.3	5.2	2.0	0.8	0.68