INFLUENCE OF WASTEWATER TREATMENT PLANT DISCHARGE ON THE WATER QUALITY OF THE VELDWACHTERS RIVER, WESTERN CAPE.

by

SIHLE MLONYENI

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Faculty of Applied Science

Cape Peninsula University of Technology

Supervisor: Prof Beatrice Olutoyin Opeolu

Co-supervisor: Dr Omoniyi Pereao

District Six Campus

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DECLARATION

I, Sihle Mlonyeni, declare that the contents of this dissertation/thesis represent my own work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed:

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23 November 2022
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ABSTRACT

The importance of the Veldwachters River for ecosystem function in our climate-changing world cannot be overemphasized. The Veldwachters River is a non-perennial river, and it is completely dry in some parts during the summer months. It is recharged with the effluent discharge from a wastewater treatment plant (WWTP) that receives domestic and municipal wastewater. The aim of the study is to investigate the influence of a wastewater treatment plant discharge on the water quality of the Veldwachters River, as well as possible associated ecological risks of the WWTP effluent. The temporal and spatial physico-chemical characteristics of the Veldwachters River water samples and ecological risks of discharged effluents were assessed. Laboratory measurements using standard methods and bioassay experiments were carried out over four seasons - summer, autumn, winter, and spring. Effluent samples were classified using the hazard classification system for wastewaters discharges into aquatic environments. The Veldwachters River water samples, WWTP's influent and effluent samples were further analysed for Microplastics (MPs). Influent and effluent samples were characterised to determine the WWTP's removal efficiency for MPs. Values of samples pH, dissolved oxygen (DO), temperature, total dissolved solids (TDS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) ranged between 4.7 – 9.75; 1.7 – 9.5 mg/L; 14.2 – 29.5 ^{o}C; 376 – 840 ppm, 0.8 – 175.58 mg/L and 0.83 – 912.15 mg/L respectively. The ecotoxicological results showed that crustaceans *Daphnia magna* was classified as *Class III (acute toxicity)* for all sampled seasons, meanwhile, *Tetrahymena thermophila* was more sensitive to the effluent compared to *D. magna* and *Raphidocelis subcapitata*. The ecotoxicological results indicated that the use of ecotoxicity assessment methods for municipal WWTP effluent is beneficial and may contribute positively to existing water monitoring strategies. The most prominent MP forms found in the water samples were fibres, with the most common colours being black/grey. Observations during our reconnaissance survey suggest that the discharged effluent contributes to the river health downstream. There is a need for consistent monitoring of the river system and effluent quality prior to discharge into the Veldwachters River.

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DEDICATION

This thesis is dedicated to:

My parents,

Thembelani and Nomathamsanqa Mlonyeni

&

The following angels that have departed this earth:

My grandparents

Maphanga Mlonyeni (Nkala)
(1933 – 1973)
and
Nomalungisa Mlonyeni (Magcaka)
(1947 – 2008)

My great grandparents

Sikolweni Ngqubeka (Jola) and Nowati Ngqubeka (Mamzima)
&
Gedlana Mlonyeni (Nkala) and Gedliwe Mlonyeni (Mantsethe)

I wish you were still around to see the success of your children, grandchildren, and great grandchildren.

It all seems impossible until it's done, ***Nelson Mandela***

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GLOSSARY

Abbreviations and Acronyms

ABBREVIATION	MEANING
BOD	Biochemical Oxygen Demand
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
CSIR	Council for Scientific and Industrial Research
DEA	Department of Environmental Affairs
DO	Dissolved Oxygen
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
EC	Electrical Conductivity
GE	Germination Rate
NaCl	Sodium Chloride
NWA	National Water Act
O_2	Oxygen gas

pH	Power of Hydrogen
RE	Root Elongation
RHP	River Health Programme
SA	South Africa
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
UNESCO	United Nations Educational, Scientific and Cultural Organisation
WHO	World Health Organisation
WSA	Water Services Act
WWTP	Wastewater Treatment Plant
ORP	Oxidation-reduction potential

Classification of Basic Terms

TERM	DEFINITION
Ecological Risk Assessment	The process for evaluating how likely it is that the environment may be impacted due to exposure to one or more environmental stressors such as chemicals, land change, disease, invasive species, and climate change (USEPA, 2016).
Wastewater	Any liquid waste, whether containing matter in solution or suspension, and includes domestic liquid waste and industrial effluent, but excludes storm water (CoCT, 2013).
Wastewater Treatment	The removal of impurities from wastewater - water that is no longer needed or is no longer suitable for use - before they reach aquifers or natural bodies of water such as rivers, lakes, estuaries, and oceans (Ambulkar and Nathanson, 2019).
Effluent	Any liquid discharged into the coastal environment as waste, and includes any substance dissolved or suspended in the liquid; or liquid which is a different temperature from the body of water into which it is being discharged (DEA, 2014)
Influent	The untreated wastewater or raw sewage coming into a wastewater treatment plant (Baharvand & Daneshvar, 2019).
Waterborne Diseases	Pathogenic microorganisms that most commonly are transmitted in contaminated fresh water cause waterborne diseases, i.e., Cholera, Malaria (Weiss, 2015).

CHAPTER 1

INTRODUCTION

1.1 Research Problem Statement

The quality of water is just as important to water security as its availability (Zhuwakinyu, 2012). South Africa's (SA) water resources continue to deteriorate, and inadequate wastewater and sewage treatment operations and maintenance of infrastructure are regarded as major causes of the deteriorating water quality in the country (CSIR 2010; Water Research Commission, 2014). Poor wastewater and sewage treatment infrastructure directly affects both the environment and human health since wastewater treatment plants (WWTPs) discharge considerable volumes of faecal pollution indicators and harmful microorganisms into receiving waters. These WWTP effluents contribute a diverse variety of contaminants to freshwater ecosystems because not all toxins from sewage waters are removed (Aristi *et al.*, 2015). Investigating the compliance of WWTP's effluent discharged into receiving waters with the regulatory standards is important (Aniyikaiye *et al.*, 2019). Water quality monitoring and sampling becomes the best way to assess how the water is contaminated. When assessing the water quality parameters, it is also important to complement the physicochemical characterisation with ecotoxicity tests, which consider the effects of the contaminants on aquatic organisms. In this study, the physicochemical properties of the WWTP were studied in both raw and treated wastewater, as well as three points along the receiving river. A battery of tests was further used to assess the ecotoxicology of the effluent discharge on the adjoining river. Three aquatic organisms, each representing a trophic level served as models, *Daphnia magna* (a primary consumer) that feeds on algae, *Raphidocelis subcapitata* (a primary producer) and *Tetrahymena thermophila* a protozoan (a decomposer). Finally, the occurrence of microplastics was also investigated in influent and effluent samples of the WWTP and the receiving waterbody. These tests assisted in obtaining a quantitative estimate of the potential effects of the WWTP on the receiving river.

1.2 Delineation of Study

Many factors contribute to water quality degradation in the Veldwachters River; however, this study is limited to the influence of only the WWTP's effluent discharged into the Veldwachters River. The quality of the water was determined by the analysis of physicochemical parameters;

ecotoxicological tests of the effluent that only included *R. subcapitata*, *D. magna* and *T. thermophila,* and microplastic occurrence.

1.3 Background

Water plays a very important role in sustainable development and is among the major essentials that nature provides to support life. Without adequate water supplies, no life is possible, and all life activities would be unrecognisable, as most of the things that we do require the use of water. Consequently, the most valuable natural resource on Earth is water (Apeh and Ekenta, 2012). In addition, communities depend on water as a basic resource for their health, well-being, economic development, and growth (UNESCO, 2015). Water is essential for drinking, health, sanitation, and agriculture, among other things. Industry, power generation, mining operations, and tourism all rely on water (CSIR, 2010).

However, the demand for this valuable resource already exceeds supply in many regions, and the world is far from being water secure (Zhuwakinyu, 2012). Poor surface water quality has significant effects for many countries, threatening their food security and livelihoods (Wagner, 2019). Moreover, according to Ganoulis (2009), there is currently a shortage of freshwater in developing nations such as, India, and many African countries, including other developed countries which were initially considered as water rich.

South Africa (SA) is a semi dry country; its freshwater resources are scarce and extremely limited (Cohen *et al.*, 2019). With an annual rainfall of roughly 450 mm, SA is the 30th driest nation in the world. This is far less than the 860 mm per year global average (DWS, 2018). As a result, SA is categorised as a water stressed country, with an annual freshwater availability below 1700 m^3 per capita. The comparison of SA's available water per capita, with other countries, highlights the challenge that the country is currently facing (CSIR, 2010).

The available water resources are not distributed in an equitable manner and is used inefficiently (Zhuwakinyu, 2012). The future pattern of water supply and demand is unknown, but it is certain that they will change. Population growth and higher water consumption per capita in the expanding urban, home, and industrial water sectors are two factors that influence demand (Cosgrove & Loucks, 2015). Thus, water is a valuable resource that SA's Department of Water Affairs monitors and regulates (DWA, 2012).

The response to water scarcity and deteriorating water quality in SA has been to develop wastewater treatment technologies. This implied that water needed to be reused, which required effluent to be purified and returned to the aquatic environment. Although the country has benefited from the technologies used, they are now proving to be inadequate to handle the rapidly increasing load caused by population growth (WRC, 2014).

The significance of a well-functioning WWTP is rooted in the fact that they serve as the final barrier between untreated, polluted, and used water and a healthy and functioning ecosystem, and subsequently public health. Our dependence on treated water has grown to incalculable measures, and threats to that supply are comparable to the worst natural and man-made disasters, posing a threat to neighbouring countries in the river basins that SA shares (Gray, 2008; WRC, 2014). Hence, for SA to maintain the economic growth while meeting the needs of water, there is a need for steps to be taken to protect and maintain our rivers so that they can serve to supply us with good quality of water. Therefore, it is important to ascertain the WWTP's efficiency as well as the compliance level of the effluent that is discharged into the receiving water to the relevant regulatory standards.

This study, which is limited to the WWTP that discharges its effluent into the Veldwachters River, provides an assessment of the potential influence the WWTP has on the Veldwachters River. The evaluation of physicochemical properties and aquatic toxicity testing of effluent samples provide an overview on how the WWTP influences the river. Furthermore, the study also attempts to provide information on the occurrence of microplastics in the influent and effluent samples of the selected WWTP as well as points along the river. Identification of point sources of contaminants may assist in giving insights for intervention strategies that aims at reducing pollution. Information generated can serve as a baseline data that policy makers and enforcement agencies can use for an integrated pollution control strategy.

1.4 Research Question

The influence of a wastewater treatment plant on the water quality of the Veldwachters River has initiated the following research questions:

1. Will seasonal variations affect the quality of the WWTP effluent and the receiving waterbody - Veldwachters River water?
2. Is there any effect on the water quality of the Veldwachters River due to effluent discharge from the WWTP?
3. How effective is the WWTP system for the removal of contaminants such as BOD, COD and microplastics from wastewater?
4. What are the possible ecological health of the WWTP’s effluent and the Veldwachters River?

1.5 Objectives of Study

The broad objective of this study was to assess the implications of the WWTP effluent on the ecological health of the receiving river.

The specific objectives are to:

1. Evaluate seasonal variations on quality parameters of the WWTP effluent and the receiving waterbody- the Veldwachters River
2. Assess the effect of the effluent discharge has on the water quality of the Veldwachters River
3. Investigate the effectiveness of the WWTP for the removal of contaminants such as BOD, COD and microplastics from wastewater
4. Study the ecological health implications of the WWTP effluent on the Veldwachters River

1.6 Significance of Study

The occurrence of various chemicals found in aquatic environments may have negative impacts on aquatic life and availability of water resources. The current study attempts to identify these problems and determine the current state of the Veldwachters River. The study also provides scientific evidence that can assist in improving the effective management strategies of the river. Furthermore, the results provide insights that can assist the authorities to put into action efficient mitigation strategies for the discharge of effluents into the waterbody.

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of Rivers

Rivers are complex ecological systems with a significant hydrological function as well as essential ecosystem services. They provide humans with a variety of socioeconomic services, including water supply, power generation, shipping, aquiculture, and landscape entertainment, among others, for use in various anthropogenic activities (Tickner *et al.,* 2017*)*. Due to the vast importance of ecosystem benefits and social services that rivers provide, they are subject to increasing exploitation and degradation (Yan *et al.,* 2012*;* Belle *et al*., 2018). In SA, most freshwater resources are found in rivers; rivers are, therefore, an essential freshwater resource. Additionally, rivers are often seen as the mirrors of the environment, as they reflect the activities in the catchment that they drain. The entire occurrences within a catchment area are mirrored in the quality of the water, and the health of a river is an excellent indicator of the way of life within a community through which it flows (Rand Water, 2019). Moreover, a river that is not adversely affected by human activities has low levels of pollution. This is because such rivers can dilute pollutants, thus, protecting the biodiversity living within and around the river (Yan *et al.,* 2012).

2.2 Water Pollution

Water is polluted if it cannot be used for a certain purpose. Natural processes can lead to water pollution; however, anthropogenic activities are major causes of water pollution in many parts of the world (Cesh, 2010). Polluted water is mostly dangerous to irrigated plants and animals as well as individuals that get their water directly from rivers or dams. Polluted water affects public health directly or indirectly. This is because poor water quality not only restricts its utility; but it also adds to society’s economic burden because highly polluted water resources require additional treatment costs and the more polluted the water, the costlier it is for treatment CSIR (2010). It further declines the living standards and social wellbeing of human (CSIR, 2010; Apeh and Ekenta, 2012; Cullis *et al.,* 2018).

Point sources and non-point sources are the two types of pollutions sources. Point source pollution is a contamination discharged through a pipe or other discrete, identifiable location, which is easily quantified, and impacts can be directly evaluated (Cesh, 2010). Pollution from point sources such as a WWTP is common in aquatic ecosystems. Non-point source pollution occurs when water pollution arises not from one single source but from many scattered sources (Aristi *et al.,* 2015; Woodford, 2019).

Water pollution contributes to the global ‘water crisis’ by reducing the quantity of freshwater resources available to human and the ecosystem, it further displays itself in form of impairment of the quality of the water (Aniyikaiye *et al.*, 2019). The quality of water at a certain point along the river reflects important influences such as industrial or municipal wastewater caused by anthropogenic input (Apeh and Ekenta, 2012; van der Laan *et al.,* 2012). Moreover, the United Nations (2014) states that the quality of any water body is a function of either both natural influences and/or anthropogenic influence. “*Without human influences water quality would be determined by the weathering of bedrock minerals, by the atmospheric processes of evapotranspiration and the deposition of dust and salt by wind. By the natural leaching of organic matter and nutrients from soil, by hydrological factors that lead to runoff, and by biological processes within the aquatic environment that can alter the physical and chemical composition of water*” (United Nations, 2014). This means that the quality of water will be polluted either way; however, humans can try to reduce the impact that they cause.

Human health is affected as it increases waterborne diseases such as diarrhoea, cholera, and bacterial infections. These waterborne diseases, particularly those leading to diarrhoea, are suspected to cause 3 – 5 million deaths yearly among children. These diseases, along with a few others, continue to be among the major causes of mortality and disability globally and continue to dominate the global burden of water-related diseases (CSIR, 2010; Yang *et al.,* 2012). Additionally, Yang *et al.,* (2012), reported that despite significant improvements in biomedical sciences and public health measures that have made it easier to control many infectious diseases over the last century, emerging and re-emerging infectious diseases have become more common and have spread over the globe.

2.3 Water Quality

The DWAF (1996) uses the term water quality to define the chemical, physical, and biological qualities of water, in terms of its suitability for an intended use. These qualities are influenced by substances that are either dissolved or suspended in the water. Revermann *et al.,* (2018) suggests that there is a need for protection of water resources for sustainable use, and water quality research is critical for providing scientific data to inform policy decisions in water resources management. Moreover, before remedial actions and other interventions can be successfully implemented, health of a river needs to be established. This is done by monitoring the quality of the water body.

According to the recent surveys by the United States Environmental Protection Agency (USEPA, 2008), many rivers and streams are too polluted and unfit for swimming, fishing, and drinking. Nutrients pollution is the most prevalent form of contamination in freshwater sources. Although these minerals are necessary for the development of plants and animals, agriculture waste and fertilizer runoff have made them a serious contaminant. The toxic load is also influenced by the discharge of municipal and industrial waste (Denchak, 2018).

Thus, extreme care must be taken to ensure that the river water quality is protected and monitored at regular intervals. The quality of a river is significant as it helps researchers in predicting and learning from natural processes in the environment, it further identifies human impacts on ecosystems, and ensuring that environmental standards are upheld (Fondriest Environmental, 2019).

2.4 Water Quality Standards

The SA government is concerned that soon, the country will be unable to meet the needs for various water uses (Belle *et al.,* 2018). The Department of Water Affairs and Forestry (DWAF), now called Department of Water and Sanitation, has developed several standards or guidelines, to describe the quality of water in a watercourse (DWAF, 1996). The guidelines are intended to support the development and execution of risk management plans that ensure the safety of drinking water sources by monitoring hazardous water constituents (WHO, 2011). These guidelines are based on health-based targets, and the criteria used to determine these standards are constantly being reviewed. As a result, each country’s drinking water standards may differ in nature and form and no single approach is universally applicable (WHO, 2011).

It is important to note that even though the guidelines describe a quality of water that is suitable for long term consumption, the formulation of these guidelines, including the values, should not be interpreted as indicating that the quality of drinking water may be degraded to the recommended level. Indeed, continual effort should be made to preserve the greatest feasible level of drinking-water quality level (WHO, 2011). The South African Water Quality Guidelines are grouped according to the intended use (e.g., irrigation, recreational and domestic use) in the 1996 Water Quality Guidelines (DWAF, 1996).

2.5 Water Quality Parameters

Testing of water is very important, prior to use for drinking, domestic, agricultural, or industrial purposes. There are various classes of water quality parameters; however, this study focuses on the physicochemical water quality parameters. Selection of parameters for testing depends on the intended use of the water. There are various floating, dissolved, suspended and microbiological as well as bacteriological impurities found in water (Patil *et al.*, 2012). The analysis of the physicochemical parameters namely pH, dissolved oxygen (DO), temperature, electrical conductivity (EC), total dissolved solids (TDS), Oxidation-reduction potential (ORP), chemical oxygen demand (COD), biological oxygen demand (BOD), were carried out.

2.5.1 Temperature

Temperature influences several physical, biological, and chemical aspects of surface water. The temperature of the water influences fish growth, reproduction, and immunity by controlling the rate of all chemical reactions. Extreme temperature changes can accelerate chemical processes and can be fatal to fish and other aquatic organisms (Cesh, 2010; Patil *et al.*, 2012; Bhateria and Jain, 2016). Increased water temperature can impair the water's ability to contain DO, and unexpected temperature "shocks" can kill many aquatic organisms. The depth of the water has an impact on the temperature of the water; surface water is generally much colder at greater depths than shallow water. Furthermore, fish respond to temperature fluctuations in the water by moving to new areas when the temperature changes by 1 to 2^{o}C (Cesh, 2010). Freshwater fishes have ideal growing temperature that ranges from 25-30 ^{o}C to which they grow quickly. About 35 ^{o}C is commonly regarded the maximum tolerance for the survival of aquatic life (Khan *et al.*, 2015). There are many factors that cause temperature changes, these include, among other things, weather, removal of shading stream bank vegetation, discharge of cooling water (Spellman, 2014).

2.5.2 pH

The ‘power of hydrogen’, pH, is a measured value on a scale, like temperature. Water pH cannot be measured physically in terms of concentration volume. It ranges from 1-14 that determines how acidic or alkaline a body of water is. A water sample with a pH value of seven is considered neutral; a pH value lower than seven is acidic and one that is higher than seven is more alkaline (Oram, 2014). This parameter is important when determining the corrosive potential of water. The lower the pH value, the higher the corrosiveness ability of the water (Bhateria and Jain, 2016). Meanwhile, alkaline water does not necessarily pose a health risk but suggests that there is a disinfection in the water. The water can have an unpleasant smell and taste and may damage water carrying equipment and pipes (Patil *et al.,* 2012; Oram, 2014; Rahmanian *et al.*, 2015). Most water found in rivers and lakes generally have a pH range of 4 to 9. Fish have a specific range of pH levels that varies by species. Furthermore, water that is outside the normal pH range for a certain species of fish can cause physical harm to the skin, gills, and eyes and in severe cases, death. Likewise, low pH can cause metals to dissolve, whereas high pH can induce ammonia toxicity in fish (Cesh, 2010).

2.5.3 Dissolved Oxygen (DO)

Dissolved oxygen (DO) consists of microscopic oxygen gas (O_2) bubbles in water and is essential for aquatic organism’s survival. It is defined as the amount of free, non-compound oxygen present in water or other liquids. The DO is a key criterion in assessing water quality, the self-purification strength of water bodies and contamination levels by organic matter due to its influence on the living organism’s aquatic ecosystems (Agoro *et al.,* 2018). It measures the amount of oxygen dissolved in water and illustrates the amount of oxygen available to living aquatic organism within the waterbody. Furthermore, DO is affected by temperature, salinity, atmospheric pressure, and oxygen demand from aquatic organisms. It is measured in parts per million (ppm), milligrams per litre (mg/l), or percent saturation (Cesh, 2010). Moreover, Agoro *et al.,* (2018), states that DO can be used as a guide for all physical and biological processes in the water.

2.5.4 Electrical Conductivity (EC)

Electrical conductivity (EC) is the ability of any medium; water in this case, to carry an electric current. It is significantly correlated to, among others, water temperature, pH alkalinity, total hardness, TDS, and COD (Bhateria and Jain, 2016). It is directly proportional to the concentration of ions in water, such as nitrate, nitrite, and phosphates, so different ions vary in their ability to conduct electricity (Khan *et al.*, 2015). This ability is associated with the concentration of ions in the water. These conductive ions are produced by inorganic substances and dissolved salts such as alkalis, chlorides, sulphides, and carbonate compounds (Rahmanian *et al.*, 2015; Fondriest Environmental, 2019). Moreover, electrolytes are the compounds that dissolve into ions. Therefore, the ionic strength of water is positively correlated to electrical conductivity of the water (Fondriest Environmental, 2019). The geology of an area through which a river flows has an impact on its EC, thus, discharge to rivers can change EC levels depending on its composition. Moreover, due to the presence of chlorides, phosphates, and nitrates, faulty sewer systems will increase EC while oil spills tend to reduce it (Bhateria and Jain, 2016).

2.5.5 Total Dissolved Solids (TDS)

Total dissolved solids (TDS) is a unit of measurement for the total concentration of dissolved solids in water. Inorganic salts and small amount of organic matter contribute to TDS. The TDS concentration is directly proportional to the electrical conductivity (EC) of water. Since EC is significantly easier to measure than TDS, it is frequently used as a TDS concentration estimate (DWAF, 1996). High values of TDS limit the suitability of the water for drinking and irrigation. It affects or increases turbidity, restricting light penetration, which will affect photosynthesis to occur. Furthermore, a high concentration of TDS does not pose a health hazard, however, it contributes to producing water hardness. According to the Penstate (2009), under the Federal Safe Drinking Water Act, the U.S. Environmental Protection Agency (USEPA) categorises TDS as a secondary maximum contaminant level (sMCL). This implies that while a maximum level of 500 milligrams per litre (mg/L) is recommended, public water systems are not required to meet this level.

2.5.6 Oxidation Reduction Potential (ORP)

Oxidation reduction potential (ORP) is a vital indicator of the characteristics of natural waters and wastewaters. It is used as a measurement of a lakes or river’s ability to purify itself or to decompose wastes, such as pollutants and dead plants and animals. ORP consists of a measure of the oxidising and the reducing potential of a water body. It is measured in volts (V) or millivolts (mV) (Goncharuk *et al.*, 2010). Moreover, natural waters interacting with the atmosphere are considered to have more positive ORP values unlike the underground waters interacting with silicates, sulphides and organic matter. Low ORP values for household and industrial wastewaters indicate the presence of reducing agents such as nitrites, ammonia and organic substances that can be oxidized. Meanwhile, the presence of oxidizing agents and a high amount of oxygen in the water are indicated by high ORP values. As a result, the ORP measurement is the only approach available to estimate the antioxidant properties of drinking water (Goncharuk, *et al.*, 2010). In addition to dissolved oxygen, ORP is also measured as it provides additional information about water quality and contamination levels.

2.5.7 Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand (BOD) is a measure of oxygen consumed by microorganisms under specific conditions in water, specified in mg/L. It is the amount of oxygen needed to remove waste organic matter from water during the breakdown process by aerobic bacteria. Living bacterial organisms, which require oxygen to function, decompose the waste organic matter to stabilize or render it harmless. BOD in water is essentially determined by the difference in the DO levels of water samples prior incubation and after the five-day incubation. The BOD is measured by determining the remaining DO at different times (Baharvand and Daneshvar, 2019). Furthermore, WWTPs use BOD as an indication of the degree of organic pollution in the water (Patil *et al.,* 2012).

2.5.8 Chemical Oxygen Demand (COD)

The determination of chemical oxygen demand (COD) in water primarily involves the reaction of the water sample with a potent oxidising agent which oxidises the organic matter present in it. COD is the well-known and short alternative test to BOD for determining the concentration of organic matter in wastewater samples. COD is an alternative measure of organic material contamination in water measured in mg/L. It is the amount of dissolved oxygen needed to cause chemical oxidation of the organic material present in water. COD and BOD are regarded as important indicators of the environmental health of a surface water supply. They are normally used in wastewater treatment but hardly in general water treatment (Patil *et al.,* 2012). This COD test is commonly used to find the severity of domestic and industrial sewage pollution (Baharvand and Daneshvar, 2019).

2.6 Wastewater Treatment

Wastewater consists of domestic, commercial, industrial as well as storm water and runoffs from lands, which require treatment prior to being discharged into the environment, to avoid any risk or harm it may have on human health and environment (Naaidoo and Olaniran, 2014; Edokpayi *et al.,* 2017). There are different types of wastewaters, the major ones are shown in Figure 2.1.

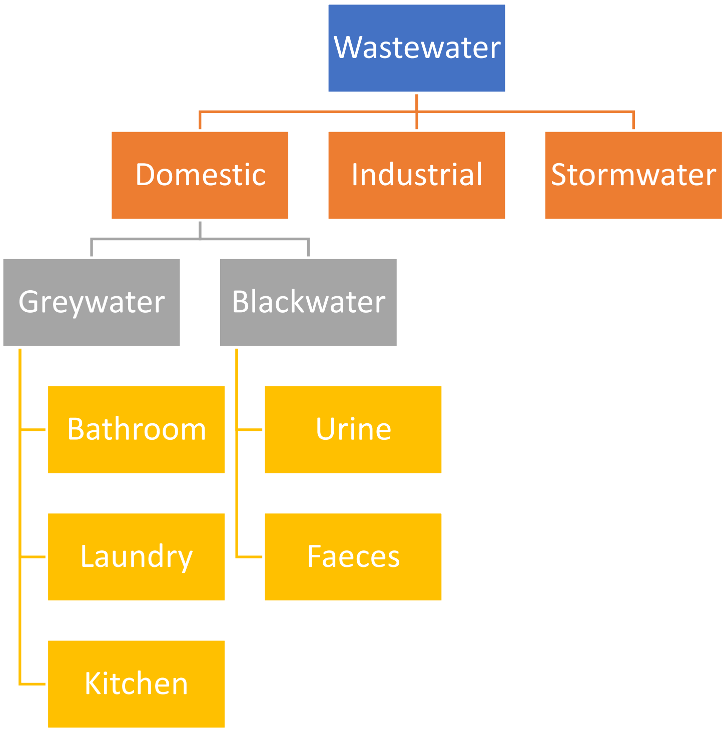

Figure 2.1: Major types of wastewaters. Source: Edokpayi *et al.*, 2017

The objective of a wastewater treatment plant is to safely dispose wastewater generated during water use, by reducing or removing contaminants and not impacting human health and the environment (Edokpayi *et al.*, 2017; Agoro *et al.*, 2018). According to Iloms *et al.*, (2020), various studies show that most SA municipal WWTPs are unable to adequately treat their wastewater to the acceptable standards, resulting in direct discharge of effluents, which then pollute receiving waters. Poor and insufficient WWTPs are considered as SA's primary cause of water pollution, which is evident through cases of non-compliance with the national water resources legislations, policies and norms and standards set to protect the SA's water resources. (Ntombela *et al.*, 2016). Population growth is one of the main contributors because the technologies used are unable to handle the increasing loads and the level of development in various municipalities plays a great role (Naidoo and Olaniran, 2014). WWTPs operate based on different processes for the removal or reductions of harmful contaminants found in wastewater. The treatment process is categorised into four stages, namely, preliminary treatment, primary treatment, secondary treatment, and tertiary treatment, each with different biological, physical and chemical processes as shown in figure 2.2 (Tempelton and Butler, 2011; Naaidoo, 2013; Naidoo and Olaniran, 2014).

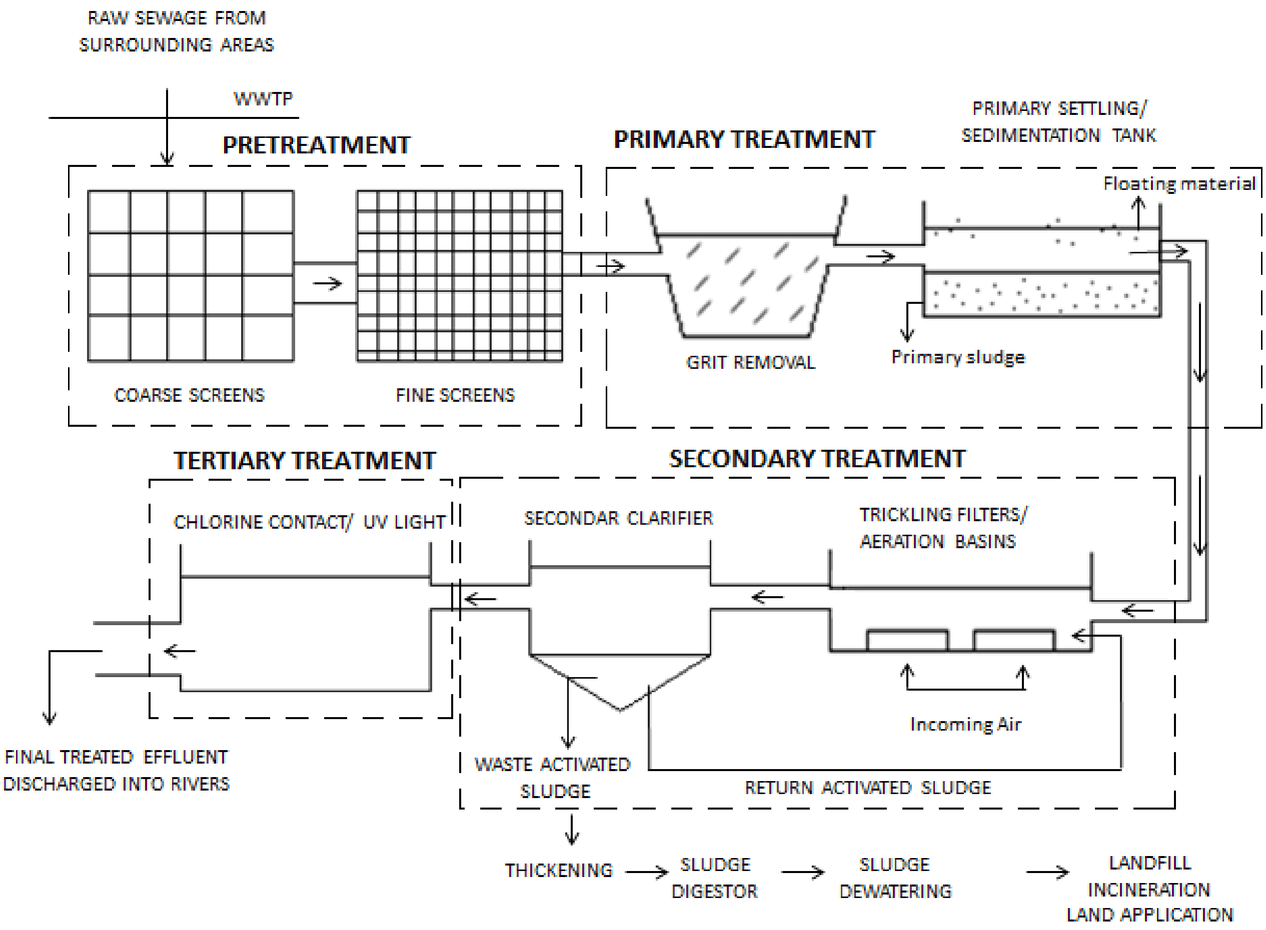

Figure 2.2: An overview of the different stages of a WWTP. Source: Naaidoo and Olaniran, 2014.

2.7 Preliminary treatment

The first stage in WWTPs is the preliminary stage (screening and grit removal) which involves the removal of wastewater constituents, likes paper, plastic or any material that could interfere with the sewage flow or damage the plant equipment (Templeton and Butler, 2011; Naaidoo, 2013; Naaidoo and Olaniran, 2014). Screening involves removal of large floating debris that are contaminated with raw faecal material that are odorous which are taken to landfill sites or incinerated (Templeton and Butler, 2011). While grit involves the removal of heavy inorganic particles such as sand, slit and gravel to avoid abrasive wear of equipment, prevent pipe clogging by deposition of grit and for the reduction of grit in settling tanks and digesters (Templeton and Butler, 2011; Naaidoo and Olaniran, 2014).

2.8 Primary treatment

Following the Preliminary treatment, the primary treatment stage is primarily the physical removal process of any suspended solids such as oils, grease, fats sand and grits that are found in the wastewater and reduced using the settling and sedimentation processes (Naidoo, 2013; Naaidoo and Olaniran, 2014). At this stage the wastewater still contains dissolved organic and inorganic compounds as well as suspended solids that are removed or reduced during the separation of solid and liquid phase. Moreover, this process allows the solids with higher specific gravity than the liquid to settle at the bottom of the settling tank, and those with lower specific gravity will move up, allowing the sludge to be treated anaerobically (Naaidoo, 2013). The wastewater in the settling tanks flows slowly to allow the wastewater to settle for hours, allowing the heavy material to get to the bottom of the tank to form primary sludge and reducing the solids in the wastewater (Naaidoo and Olaniran, 2014).

2.9 Secondary treatment

The secondary treatment is mainly based on biological processes which remove the remaining organic matter in the wastewater. This stage allows for the oxygenation of the liquid that flows from the primary settling tanks (Naidoo, 2013). There are several secondary treatment technologies such as the activated sludge process; bio-filters and different kinds of ponds and constructed wetland (Naaidoo, 2013; Naaidoo and Olaniran, 2014; Hansen 2015). The activated sludge process is the most common, at this stage, the effluent from the primary clarifier chamber is pumped into the aeration basin that is known as the anoxic which is filled with microorganisms that grow well under low-oxygen environments. The second part is the oxic zone which allows diffusers to break up the air, providing an oxygen overdose to the microorganisms (Naaidoo, 2013; Hansen, 2015). The biofilter (trickling filter, rotating biological contactors) is another commonly used system where effluent flows into a humus tank from the biofilter underdrain. The humus tanks use sedimentation to remove particles from the effluent discharged by the biofilter while the sludge from trickling filters settling tanks is transferred to sludge processing facilities or returned to the primary clarifiers to be settled with primary solids (Naaidoo, 2013). Different types of ponds and constructed wetlands are another common type of a secondary treatment that effectively polish and disinfect the effluent. Other than improving the final effluents water quality, they serve as buffers in the case of breakdowns that occur in the WWTPs.

2.10 Tertiary treatment

The tertiary treatment involves the chemical treatment and is simply an additional treatment to improve the quality of the effluent and remove contaminants or any other pollutants before it is discharged into the receiving waterbody (Naaidoo, 2013; Naaidoo and Olaniran, 2014;

Hansen, 2015). Tertiary treatments are quite expensive but can remove almost all impurities found in the wastewater. Phosphorus and nitrogen removal or chemical disinfection are the commonly used methods in the tertiary stage. Different chemicals are used to effectively remove both phosphorus and nitrogen in the effluent. While disinfection, as the name suggests has the objective to remove or inactivate pathogenic microorganisms using chemicals such as chlorine. Following the final treatment, the effluent is discharged into a waterbody and the quality if often based on the intended use of the effluent (i.e., Agricultural, or industrial use).

2.11 Effects of wastewater on surface water quality in other Countries

Wastewater quality differs based on the types of influents that WWTPs receive, including and not limited to urban runoff, atmospheric deposition, and agricultural runoff (Edokpayi *et al.*, 2017). In a study of impacts of industrial effluents on water quality in Uganda, Walakira and Okot-Okumu (2011), reported that most factories that discharge their effluents in the receiving rivers along the Lake Victoria basin have no WWTPs and those that have them, are poorly designed and constructed. The discharged effluent is untreated and poses a threat to the integrity of the river, which has led to their study of assessing the quality of the streams receiving effluents from the different industries. Water quality parameters such as pH; EC; turbidity; colour; BOD; COD; total nitrogen (TN); total phosphorus (TP); sodium (Na); chloride (Cl); calcium (Ca); lead (Pb); copper (Cu) and cadmium (Cd) were assessed following the Standard Methods for Examination of Water and Wastewater by the American Public Health Association (APHA, 1999). The results showed that most of the industries discharged effluents that were not within the Ugandan national regulations. Moreover, these results revealed a typical example of what is happening in most developing nations, that there is inadequate enforcement of environmental regulations.

In Iran, a study of the impact of treating wastewater on the physiochemical variables of environment by Baharvand and Daneshvar (2019) proved that having WWTPs that are efficient can improve the quality of the effluent it discharges to receiving waterbodies. Previous studies have been done in this area and the physicochemical parameters: total suspended solids (TSS); temperature; pH; NO_3, phosphorus (P); BOD; COD and DO used were determined based on the literature. Except for DO and COD values, the results showed that the values obtained for the different parameters were well within the Environmental Protection Organisation of Iran, for effluents to be discharged in rivers. The removal efficiency is estimated to be 80-92% to the total suspended solids; nitrate; BOD and COD when the influent and effluent values were compared. The DO values are said to have increased from influent to effluent due to the aeration processes during the treatment stages. While COD, only decreased to some extent, with a removal efficiency of 80-89%. Although not all the parameters were within the standards, this study shows an efficiency purification of 80% and more plans to further enhance the treatment processes are recommended.

Benit and Roslin (2015), conducted a study on physicochemical properties of wastewater collected from various sewage sources in India. The study highlights the three major categories of pollutants that cause pollution in water, which are namely: disease causing agents; oxygen demanding wastes and water-soluble inorganic pollutants. They further indicate that anthropogenic activities are the main drivers of water pollution and state that studies on wastewater quality helps improve knowledge on the kind of water that is being discharged into receiving environments. Physicochemical parameters such as colour, odour, pH; EC; TDS; BOD; COD; DO; $NO_{3;}$ sulfate, sodium, and potassium of the wastewater were tested. The results obtain varied between the sampling points and showed that almost all the parameters tested were above the acceptable limited required by USEPA and WHO.

To address the problem of water scarcity and pollution in urban rivers, the Kunming City government in China started recharging urban rivers with WWTP effluent in 2009. The government further invested billions to support the different types of pollution control projects to reduce pollution in the Dianchi lake. Jin *et al.*, (2017), studied how the WWTPs effluent impacts an urban river of Dianchi, China. The study used a paired-sample t-test, factor analysis and canonical correspondence analysis, to analyse the changes on the water quality and the plankton community and their relationship. The results showed a decrease in the effluent's TSS; COD; BOD and TP, while the concentration of NO_3; TN; and nitrate nitrogen (NO_3-N) increased. After the effluent was discharged into the river, the phytoplankton changed from Chlorophyta to Bacillariophyta, which was caused by the high NO_3-N and high temperature. The zooplankton was less sensitive to changes and their structure was influenced by temperature. The overall results of this study showed that the WWTP's effluent was beneficial for reducing river water pollution particularly when the nitrification process of the WWTP was working properly. To further reduce high levels of TN and TP in the river after the recharge, different methods can be applied.

2.12 Effects of wastewater on surface water quality in South Africa

In SA, most WWTPs discharge effluents directly to nearby rivers, consequently, the increased discharge of these effluents, deteriorates the water quality in SA's river systems (Sibanda *et al.*, 2015). Iloms *et al.*, (2020), reported in their study of WWTPs in Free States Province of South Africa that WWTPs are not effectively maintaining wastewater to acceptable standards, which was further confirmed by Odjadjare and Okoh (2009); Mema, (2010) and Edokpayi *et al.*, (2017). Their findings show that poor investments in wastewater treatment infrastructure, a scarcity of competent manpower and bad planning or corruption results in poor performances in WWTPs. According to different case studies in a report by Mema (2010), low-income communities are majorly affected, and an example is the Eastern Cape with over 80% of treated effluent that is not in line with the required standards. However, even SA's most developed regions like Gauteng and the Western Cape are also affected.

According to Edokpayi *et al.*, (2017), WWTP effluents contribute to oxygen demand level of receiving waters, caused by the organic substances found in these effluents which decreases the amount of DO required by aquatic organisms. Many studies show that levels of DO in the effluents of different WWTPs are typically lower than the required standard of 7.5 mg/L for discharged effluent into water bodies. For instance, Olabode *et al.*, (2020), reported their monthly variation in DO in two WWTPs in the Western Cape province, the values ranged between 1.30 and 5.50 mg/L. DO levels below 5 mg/L cause adverse effects to aquatic ecosystem in the receiving surface water (Agoro *et al.*, 2018; Iloms *et al.*, 2020). Whereas aquatic organisms require DO levels of 4-5 mg/L and the results in their study for both WWTPs show that 67% of the recorded values are in that range. Igbinosa and Okoh (2009), reported DO values of 4.15 – 5.38 mg/L in their research on impact of discharge effluents of a receiving watershed in the Eastern Cape Province. The DO content in the effluent depleted faster than that of the receiving waterbody, which was attributed to the effluent's organic matter content. Iloms *et al.*, (2020), reported low DO levels that ranged between 1.0 – 2.7 mg/L in a study in the Free States. DO is a key parameter used as a guide of the physical and biological processes in water and control the water quality (Igbinosa and Okoh, 2009; Agoro *et al.*, 2018).

Edokpayi *et al.*, (2017), states that although environmental impacts are important to note, they take a long time before they establish whereas health impacts cause negative impacts on people using the contaminated surface water because the pathogens found in the water. Moreover, there have been various disease outbreaks such as cholera and diarrhoea in South Africa with

WWTP effluents being the major contributor. Table 2.1 gives a general summary of South African provinces' diverse water quality issues and their numerous causes.

Table 2. 1: A review of the state of rivers' overall water quality in different South African provinces. Source: Sibanda *et al.*, 2015.

Province	**River Systems**	**Impacts detected/describer**	**Source of pollution**
Eastern Cape	Mthatha area	-Rivers contain large numbers of pathogenic organisms and high concentrations of nutrients, salts and endocrine-disrupting compounds (EDCs)	-Treated, partially treated and untreated urban and industrial effluent
	Buffalo River system	-Elevated concentrations of dissolved salts and metal ions in the lower reaches of the river. -Large numbers of pathogenic organisms; high concentrations of nutrients, salts and EDCs -Frequent toxic blooms of cyanobacteria *Microcystis aeruginosa* in the major downstream reservoirs	-Saline effluents discharged from tanneries -Discharges of treated, partially treated and untreated urban and industrial effluent
KwaZulu - Natal (KZN)	Umgeni River system	-Elevated concentrations of pesticides and nutrients -Large numbers of pathogenic organisms and high concentrations of nutrients, salts and EDCs	-Return flows and seepage from agricultural lands -Contaminated runoff from urban centers and informal settlements, combined with discharges of treated, partially treated and untreated urban and industrial effluent

	Thukela River system	-Large numbers of pathogenic organisms and high concentrations of nutrients, salts and EDCs -Elevated concentrations of pesticides and nutrients reaching the river -Lowered pH values and elevated concentrations of total dissolved salts, especially sulphate.	-Discharges of treated, partially treated and untreated urban and industrial effluent, contaminated runoff from urban centers and informal settlements -Return flows and seepage from agricultural lands (principally livestock ranching, dairy farming, cultivation of crops, sugar cane) and forestry -Operating and defunct coal mines contribute large volumes of acid mine drainage (AMD) to the river system.
Free State	Caledon and Modder river systems	-Large numbers of pathogenic organisms, high concentrations of nutrients and salts and moderately high concentration of EDCs -Periodic blooms of toxic cyanobacteria Microcystis aeruginosa have been recorded from the Krugerdrift Dam	-Discharges of treated, partially treated and untreated urban effluent, as well as contaminated runoff from urban centres and informal settlements -Return flows and seepage from agricultural lands result in elevated concentrations of pesticides and nutrients reaching the rivers

Gauteng/ Northwest / Free State	Vaal River system	-Lowered pH values and elevated concentrations of metal ions and total dissolved salts, dominated by sulphate, as well as relatively high levels of radioactivity in certain tributary rivers -Large numbers of pathogenic organisms and high concentrations of nutrients and salts, as well as low to moderately high concentrations of EDCs -Blooms of toxic cyanobacteria (Microcystis aeruginosa)	-Numerous active and defunct gold and uranium mines in the Witwatersrand complex contribute large volumes of AMD -Discharges of urban and industrial effluents, as well as contaminated runoff from larger cities, smaller urban centres and informal settlements
Mpumalanga / Limpopo	Eastern River systems; Upper Olifants River system	-Lowered pH values (sometimes to <3.0) and elevated concentrations of metal ions (especially aluminium, iron, cadmium, zinc and cobalt) and total dissolved salts, dominated by sulphate v -Large quantities of inorganic and organic compounds in the Olifants River -Large numbers of pathogenic organisms and high concentrations of nutrients, salts and low to moderate concentrations of EDCs	-Operating and defunct coal mines contribute large volumes of AMD -Heavy industries in the Witbank and Middelburg area (mainly iron and steel works) -Discharges of urban and industrial effluents, as well as contaminated runoff from larger towns, smaller urban centres and informal settlements (many lacking proper and/or functioning sanitation systems)

Northwest	Crocodile (West) River system	-Large numbers of pathogenic organisms and high concentrations of nutrients, salts and low to moderately high concentrations of EDCs (all these substances pose health risks to humans and livestock that may consume the water)	-Discharges of large volumes of treated, partially treated and untreated urban effluent, especially from the northern areas of the Witwatersrand, as well as contaminated runoff from urban centres and informal settlements
Western Cape	Cape Town urban rivers	-Receiving urban rivers contain large numbers of pathogenic organisms and high concentrations of metal ions, nutrients, salts and EDCs	-Contaminated runoff from urban areas and informal settlements: discharges of treated, partially treated and untreated domestic and industrial effluent

2.13 Legislation Overview

The development of international environmental laws has led to the emergence of various principles such as the precautionary principle, the polluters pay principle and the preventative principle. In addition, the Environmental Impact Assessment (EIA) is also used as a monitoring tool that helps with the implementation of the aforementioned principles (UNEP, 2015). Only a few countries and continents have regulations that focus on wastewater management, and other countries have both sectorial legislations and a framework environmental legislation, while others have one, the other or none. New Zealand is one of the countries that have an environmental act that regulates wastewater, under the Environmental Protection Act 1970 known as the Code of Practice for Management of Domestic Wastewater. While many countries (Bolivia, Democratic Republic of Congo, Ecuador, Kenya, the Maldives, Nicaragua, Uruguay and South Africa) have recognised the right to water in their constitutions, only a few of them recognise the right to sanitation. Additionally, in terms of drinking water, many countries have laws that protect drinking water sources, an example of a national legislation is the Safe Drinking Water Act (SDWA) in the United States, which is known as the main federal law to ensure good quality drinking water.

In South Africa, the policy on wastewater treatment management is based on a human rights approach, acknowledged by the South African Government in the hierarchical suit of environmental legislations, that also ensure that WWTPs are compliant and reduce their environmental and human health risks. These legislations can be broadly summarised as: The Constitution of the Republic of South Africa (South Africa, 1996), which is the supreme law of the country; framework environmental legislation such as the National Environmental Management Act No. 107 of 1998 (NEMA); and sectorial environmental legislation such as The National Water Act No 36 of 1998 (NWA); The Water Services Act, No. 108 of 1997 (WSA); The National Water Resources Strategy and The South African Target Water Quality Guidelines (TWQGR). Additionally, legislations indirectly relating to wastewater management such as the National Health Act (Act 63 of 1977) (NHA); Local Government: Municipal Structures Act (Act 117 of 1998) and Local Government: Municipal Systems Act (Act 32 of 2000).

- The Constitution of the Republic of South African (South Africa, 1996) in section 27 declares that ‘everyone has the right to have access to sufficient water’. Section 24 of the Constitution further declares that ‘everyone has a right to an environment that is not harmful to their health or wellbeing and have the environment protected for the benefit of present and future generations through reasonable legislative and other measures that prevent ecological degradation through pollution’. Accordingly, water restrictions are enforced in areas where there are water shortages, and this is based on water levels in dams and population size. These restrictions are made to encourage communities to conserve water and hence, ensure consistent availability (DWA, 2012).

- National Environmental Management Act No. 107 of 1998 (NEMA):) is the constitutional framework that enforces Section 24 of the Constitution. This act aims to provide for co-operative, environmental governance by establishing principles for decision-making on matters affecting the environment, institutions that will promote co-operative governance and procedures for co-ordinating environmental functions exercised by organs of state; and to provide for matters connected therewith. Moreover, the Polluter Pays principle, Precautionary principle and principles of Sustainable Development and Environmental Justice are all applicable to the management and operation of WWTPs. Section 28(1) NEMA further stipulates the responsibilities related with the duty of care and remediation of environmental harm where responsible individuals shall take reasonable measures to prevent pollution from occurring, continuing or recurring.

The Department of Water and Sanitation (DWS) leads and regulates the water and sanitation sectors in SA, develops policy and strategy, and provides support to the sector. Two Acts govern DWS, which are currently being incorporated (DWS, 2018).

The two radical legislative frameworks have been formed to address water access disparities:

- The Water Services Act, No. 108 of 1997 (WSA 108 of 1997): aims to provide for the right of access to water supply and basic sanitation. The Act makes provision to secure sufficient water and an environment not harmful to human health or wellbeing. Section 3 stipulates the right of access to basic water supply and basic sanitation

- The National Water Act, No. 36 of 1998 (NWA 36 of 1998): ensures that SA's water resources are protected, used, developed, managed, and controlled, in a way, which inter alia, considers the reduction, prevention and degradation of water resources. Section 19(1) establishes a general duty of care, stating that an owner of land, a person in control of land or a person who occupies or used the land on which any activity or process is or was carried out, which causes, has caused or is likely to cause pollution of a water resource, must take all reasonable measures to prevent any such pollution from occurring, continuing, or recurring.

Both acts advocate for the use of water in promoting socio-economic development and gives national authorities a constitutional obligation to provide suitable infrastructure for water resources management. With these Acts, governance and regulatory frameworks, and national strategic objectives, DWS creates a favourable environment for efficient management and use of water resources.

- The National Water Resources Strategy (NWRS): aims for the facilitation of the management of the country's water resources. It determines the framework for the use, improvement, protection, control and managing of water resources. The NWRS gives information regarding the features of water resource management.

- The South African Target Water Quality Guidelines (TWQGR): serves as the primary source of information for determining the water quality requirements of different water uses and for the protection and maintenance of the health of aquatic ecosystems.

- National Health Act (Act 63 of 1977) (NHA): aims in providing measures for the promotion of the health of the inhabitants of the Republic of South Africa. Section 20(1) states that every local authority shall take all lawful, necessary, and reasonably practicable measures to avoid any nuisance, unhygienic condition, or any offensive condition. The local authority must furthermore prevent the pollution of any water intended for the use by its inhabitants, irrespective of whether such water is obtained from sources within or outside its district or must purify such polluted water.

- Local Government: Municipal Structures Act (Act 117 of 1998): Section 84(1) of this act sets out the division of functions and powers between district and local municipalities. In terms of sections 84(1) a district municipality's functions and powers include management of domestic wastewater and sewage disposal systems.

- Local Government: Municipal Systems Act (Act 32 of 2000): Section 55(1) stipulates that the municipal manager as head of administration is, subject to the policy directions of the municipal council, responsible and accountable for provision of services to the local community in a sustainable and equitable manner. Furthermore, the core services to be provided by municipalities include: the provision of clean drinking water, sanitation, clean drinking water, waste removal amongst other things.

South Africa has excelled in the development of laws and supporting legal tools, including the Acts on water quality; and relating its legislations and policies to sanitation and wastewater management based on health and safety, environmental protection and human rights to water and the environment. When compared to other countries, SA is considered a leader in terms of wastewater treatment research and development (UNEP, 2015).

Considering the different challenges faced with implementing all the aforementioned enforcement protocols, in 2008 the DWS introduced the Green Drop Programme. This is an incentive-based programme which aims to facilitate compliance through motivation and rewards, rather than direct regulation. DWS has realised that rewarding positive behaviour is more efficient and effective, as opposed to negative behaviour. The Green drop programme seeks to sustainably enhance wastewater quality in SA, by recognising and developing the important competencies needed to achieve the programmes aims. The DWS, through this programme, ensures that WWTPs meet the minimum requirements to protect human health and the environment. As per the 2013 Green Drop report, a cumulative risk rating for each municipal WWTP and a weighted Green Drop score for each municipal WWTP was established. WWTPs with a score of 90% or higher obtained a Green Drop certificate, while those with a score of less than 30% were considered critical and received a Purple Drop.

2.14 Microplastics in Aquatic Ecosystems

Plastic occurrence in the environment is currently a global concern (Andrady, 2011; Conley, *et al.,* 2019). The themes for United Nations World Earth Day and World Environment Day for 2018 are both focused on plastic pollution. This is because recent empirical data obtained from different parts of the world have indicated occurrence, distribution, effects, and possible other risks associated with plastics. Plastics occur as macro, meso, micro and nano fragments in aquatic ecosystems. These fragments are referred to as macroplastics (>25 mm), mesoplastics (5-25 mm) and microplastics (>5mm) and nanoplastics (<0.1 µM) depending on their particle sizes. MPs enter waterbodies through waste discharges, wastewater treatment plants, and household products, among others. Classes of plastics that have been found in aquatic environment include low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polystyrene (PS), foamed polystyrene, nylon, thermoplastic polyester, and polyvinyl chloride (PVC) (Andrady, 2011; Mattsson *et al.,* 2015; Comanita *et al.,* 2016). These contaminants originate from packaging materials, netting, plastic bags, cigarette filter, etc. They may also occur as microplastics when degraded by microorganisms, sunlight, temperature, or hydrolysis (Andrady, 2011; Mattsson *et al.*, 2015). Aquatic species may consume these pollutants, which could have a negative impact on ecosystem function. Plastics can harm aquatic species' gastrointestinal tracts, respiratory systems, and locomotive appendages when consumed (Mattsson *et al.,* 2015; Comanita *et al.,* 2016; Bayo *et al.,* 2019).

Plastics may potentially cause additional toxins, such as persistent organic pollutants, which could affect the environment (Comanita *et al.,* 2016). They can accumulate in organisms, especially aquatic predators and transported in the food chain. Plastics can cause eye and respiratory tract irritation, acute skin rashes, birth abnormalities, dyspepsia, and liver dysfunction in humans, among other things. They contain a host of hazardous endocrine disrupting chemicals. Furthermore, they are potential carcinogens that can alter insulin resistance, reproductive system, and brain function of organisms (Mattsson *et al.,* 2015; Comanita *et al.,* 2016; Bayo *et al.,* 2019).

A study investigating the abundance, concentration, and variability of microplastics based on different water parameters and environmental factors, their possible sources and removal efficiency, was conducted at an urban WWTP in Spain (Bayo *et al.*, 2019). The MPs detected, consisted of a 46.6% of total microliter, with a statistically significant removal of 90.3% in the final effluent of the WWTP. Five different shapes were isolated; the most prominent MPs forms in the final effluent were fragments and fibres, with the most common size class being 400 - 600 µm. Furthermore, seventeen different polymer families were identified, with low-density polyethylene being the most common one (52.4%) in a film form (27.7%), mostly from agriculture greenhouses near the sewage plant and single plastic bags. Influent wastewater with high concentrations of suspended solids proved to have a low MPs burden with a larger MPs size, possibly due to a hetero aggregation with particulate matter (Bayo *et al.,* 2019).

2.15 Ecotoxicological Monitoring of Wastewater

Ecotoxicology is a discipline that examines pollutants in the biosphere and their effects on constituents of the biosphere, including humans. The term is defined as "*the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animals (including human), vegetable and microbial in an integral context*" (Kahru and Dubourguier, 2010). Aquatic plants and animals are an important component in the aquatic ecosystem and the assessment of ecotoxicological properties of chemicals aims to prevent the probable hazards caused by polluted water to the ecosystem. The evaluation of environmental impact of chemicals and water quality of a river; using only physicochemical characterisation is insufficient for adequate classification of sample toxicities (Arias-Barreiro *et al.*, 2010). To monitor wastewater quality, toxicity evaluation is crucial as it shows how test organisms react to all compounds in the waste, and it determines the toxicity of a substance in an aqueous solution (Weyman *et al.,* 2012; Phungula, 2016). Furthermore, in WWTPs these tests have advantages of protecting receiving watercourses from toxic effluents and assist in monitoring the effectiveness of WWTPs (Mendonca *et al.,* 2013).

Toxicity tests can be carried out using various experimental models and laboratory techniques to assess the risk of exposure (Arias-Barreiro *et al.,* 2010). Many model species have been used in toxicity testing and the ones commonly used for aquatic systems include algae, luminescent bacteria, protozoa, daphnia, and fishes (Aydin *et al.,* 2015; Rotini *et al.,* 2017). The tests cover a variety of organisms, from microbes to larger organisms and plants and are now widely accepted as important for hazard evaluation of wastes, wastewaters, and industrial chemicals (Aydin *et al.,* 2015). Moreover, in several countries, for wastewater management ecotoxicity tests are used as part of site-specific risk assessments or hazard-based standards by promoting best available technology for certain industry sectors (Mendonca *et al.,* 2013). A study by Aydin *et al., (*2015), determined toxicity levels of pharmaceuticals wastewaters by comparing luminescent bacteria *Vibrio fischeri* with microalgae *Scenedesmus obliquus.* While de Melo *et al.,* (2012), conducted a toxicity evaluation of cosmetics industry wastewater using three aquatic toxicity bioassays *Daphnia similis*, *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata* algal growth. Another study by Mendonca *et al.,* (2013), conducted a battery of five microbiotests which represented different trophic levels; primary producers (microalgae and higher plants); primary consumers (crustaceans) and decomposers (bacteria). According to Mendonca *et al.,* (2013), when assessing toxicity effects in wastewater, due to variations in the relative sensitivities of the organisms, it is essential to study effects at various trophic levels. In this study, to characterise the WWTP and the Veldwachters River organisms bearing different functions at the ecosystem level were used. The aquatic toxicity tests were performed using the alga *Raphidocelis subcapitata,* crustacean *Daphnia magna*, and protozoan *Tetrahymena thermophila* which are further discussed below:

2.15.1 Algae (*Raphidocelis subcapitata)*

Raphidocelis subcapitata, formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata* is a microalga that plays an important role in sustainable wastewater treatment. These photosynthetic microorganisms provide the oxygen required by bacteria to mineralize organic matter, enhance nutrients removal, and provide the highest pathogen removal efficiencies among biological wastewater treatments (Ruiz-Marin *et al.,* 2009; de Godos *et al.,* 2010). Moreover, microalgae-based treatments are powered by sunlight, which reduces the energy input to the process. This lower energy consumption, and the natural uptake of carbon dioxide (CO_2) during microalgal growth, mitigates a significant part of the greenhouse gas emissions related to wastewater reclamation (de Godos *et al.,* 2010).

A study done in Brazil on the wastewater generated from the cosmetic industry revealed that hair care products consist of high COD, which arises from the poorly biodegradable and toxic compounds such as surfactants, natural oils, dyes, and fragrances. The use of aquatic toxicity tests for this research was beneficial as the results allowed for the identification of the toxins and the development of strategies to eliminate them from wastewaters (de Melo *et al.,* 2012). *Pseudokirchneriella subcapitata* algal growth inhibition (Method 8112) as described in the Standard Methods Baird *et al.,* (2017), was one of the bioassays used in the study. Organism sensitivity was monitored by periodically measuring toxicity to the reference substance copper sulphate for *P. subcapitata*. The results were expressed as the 72 h IC25 (%), while the effluent concentration that inhibited algal growth by 25%, was estimated using a linear interpolation method available from the USEPA. To facilitate data interpretation, toxicity results were converted to toxic units (TU) using the following equations: TU = 100/IC25 for *P. subcapitata* (de Melo *et al.,* 2012).

2.15.2 Crustacean (*Daphnia magna)*

Daphnia magna (water flea) is a freshwater crustacean. It is a filter feeder, and can survive in culture by eating algae, bacteria, or yeast. *D. magna* been the subject in numerous literatures, demonstrating its long history in scientific research. Due to its significance in freshwater ecosystems, *D. magna* is probably one of the most extensively researched topics in ecology and is regarded a keystone species in aquatic toxicology (Heinlaan *et al.,* 2010; Stollewerk, 2010). *D. magna* exhibits a remarkable ability to contend with environmental challenges and it is commonly used due to its short doubling time, high sensitivity, and simplicity (Stollewerk, 2010).

A similar study was done in Bangladesh using *D. magna* to evaluate the risk of exposure of the industrial effluent. The study area, in Dhaka, the capital city of Bangladesh is densely populated with the tanning industrial zone, which pollutes waterways. Untreated chemical wastes are discharged into low-lying areas and many water bodies that are major sources of water supply for the city. Three water samples were taken at three different sites for the testing. Acute toxicity to *D. magna* was examined using the DAPHTOXKIT F^{TM} according to the manufacturer's manual based on the ISO 6341 and OECD test guideline 202 OECD (Arias-Barreiro *et al.,* 2010). The results of the study showed that for samples one and three there was no significant toxicity for *D. magna*. Sample 2, however, exhibited strong toxicity with EC_{50} of 31.5% for *D. magna* with the 48 h acute toxicity test. These findings suggest that the Buriganga basin was disturbed by the discharge of the effluents from the Hazaribagh tannery, which poses detrimental effects on the broad spectrum of organisms in the ecosystem.

2.15.3 Protozoan (*Tetrahymena thermophila)*

Tetrahymena thermophila is a ciliated protozoa that has been used for many years as a model organism for cellular and molecular biology as well as for environmental research studies (Mortimer *et al.,* 2009). Furthermore, in contrast to other commonly used unicellular model organisms, it contains numerous genes that are conserved across different eukaryotes, it serves as a useful eukaryotic model system for mechanistic studies.

A study ‘Detection and Quantification of Genotoxicity in Wastewater - Treated *Tetrahymena thermophila* Using the Comet Assay’ was conducted. The study focused on the eukaryotic microorganism *Tetrahymena thermophila* that was used as a test organism in the comet assay. These ciliated protozoa are widely used in toxicity tests for determining impairment growth concentrations (IGC 50%), and their physiology and genetics have been well studied. *T. thermophila* was obtained from Micro biotest (Deinze, Belgium) as part of the Protox F™ kit. The findings were statistically significant and generally reliable. The test using *T. thermophila* has the potential to becoming a useful tool for quickly screening complex environmental water samples and for assessing ecotoxicity hazard assessment (Lah *et al.*, 2004).

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

3.1 Introduction

This chapter presents the research design and methodology used in the study to answer the research questions. The study followed a quantitative research method. Sample preparations and the equipment used, both onsite (pH; DO; TDS; Redox Potential and Temperature) and in the laboratory (BOD; COD; *R. subcapitata*; *D. magna* and *T. thermophila*), will be discussed including the different sampling points, and how they were chosen.

3.2 Selection Criteria of Sampling Points

A site inspection was conducted, to critically observe the study area and select sites that would meet the objectives of the study. The selected five sampling points were points in the plant, outside the plant and in the vicinity of the plant. The selection of sampling points upstream and downstream of the river were considered based on accessibility and water availability for most part of the sampling period. Influent and effluent samples were also collected from the WWTP and point of discharge into the river. A Global Positioning System (GPS) was used to obtain geographic coordinates of the sample sites.

Table 3. 1: A detailed description of the sample points and their GPS coordinates

Sample point name	Sample description	GPS coordinates
Influent (INF)	Point of entry of wastewater into the WWTP.	-33.944150, 18.823977
Effluent (EFF)	Treated effluent of the WWTP prior to discharge into the river.	-33.944658, 18.825017
Point of Discharge (POD)	Point of discharge into the river.	-33.946234, 18.822940
Upstream (UPS)	A point before WWTP effluent discharge.	-33.945239, 18.822227
Downstream (DOWNS)	A point after WWTP effluent discharge.	-33.951003, 18.822910

3.3 Map locality of sample site

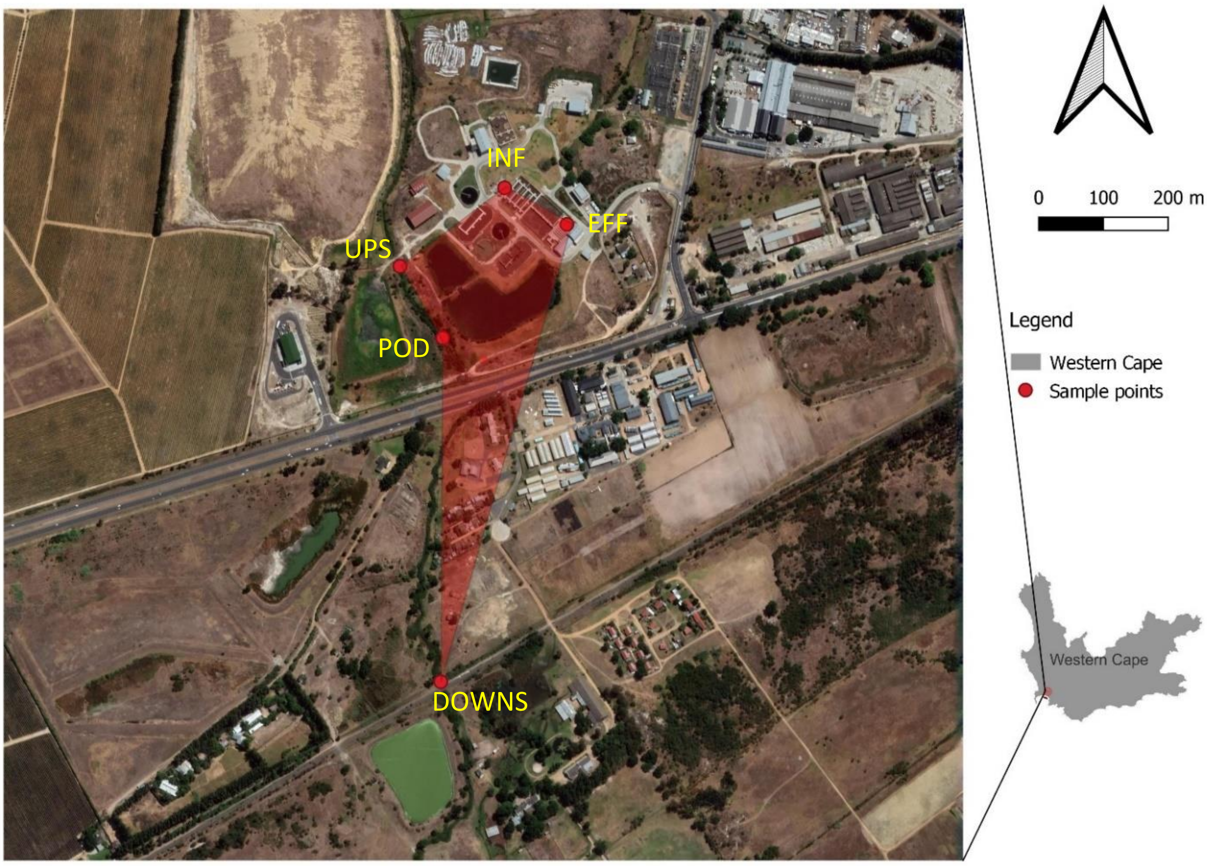

Figure 3.1: Location of the Wastewater Treatment Plant. Source: QGIS, 2022. INF – Influent; EFF – Effluent; POD – Point of discharge; UPS – Upstream; DOWNS – Downstream.

The WWTP is fully functional and receives wastewater from both municipal and industrial sources. The WWTP treats 74-85% domestic and 15-26% industrial sewage from its local area via a combination of gravitational sewers and 9 pump stations. Moreover, the WWTP has a treatment capacity of 35 megalitres per day, average dry weather flow (ADWF). The plant consists of the membrane bioreactor (MBR) with a treatment capacity of 27 ml/d ADWF and the modified old, activated sludge process (ASP) with a treatment capacity of 8 ml/d ADWF. The final effluent of this WWTP is being discharged into the Veldwachters River. The river flows through agricultural areas and is the tributary of the Eerste River, that supplies farmers in the area with water for irrigational purposes.

3.4 Sampling procedure and determination of physicochemical parameters

Field sampling commenced in September 2019 and ended in June 2021. The plan was to sample for 12 consecutive months to cover all four seasons and provide information of possible seasonal variation within one year. However, due to Covid 19 and lockdown restrictions the sampling regime extended to 13 inconsecutive months. The samples were collected using glass bottles with a storage capacity of 2.5 litres. A portable multi parameter reader (SensoDirect 150 – Lovibond, Germany) was used to obtain the physical parameters (pH, DO, TDS, ORP and temperature) values. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were evaluated in the laboratory using methods of the American Public Health Association, (1999). Prior to sampling, the portable parameter reader was cleaned thoroughly and before taking any physical measurements, the device was calibrated in accordance with the manufacturer's instructions. The sampling bottles were cleaned by soaking in detergent for 24 h, followed by rinsing with clean water until free of detergent and then thoroughly rinsed with distilled water. The clean bottles were used to collect the samples in the river. Each bottle was labelled according to the sampling site name and the date of sampling. The bottles were first rinsed with the river water three times, then plunged into the river and filled with the water. In cases where it was impossible to access the river surface and plunge the bottle inside the water, a scoop was used instead, to collect the water and fill the bottle. The bottles were tightly closed and placed in a cooler box filled with ice and closed to avoid photodegradation. All the onsite measurement data was recorded on prepared sheets. After collection, the cooler boxes containing the samples were transported to the laboratory and kept in the fridge at about 4^{o}C prior to BOD, COD and ecotoxicity bioassay experiments with a maximum holding time of 48 h. A total of 26 influent and effluent samples; and 37 river water samples were collected and analysed.

3.5 Biochemical Oxygen Demand Determination

Analysis of the BOD based on a pressure measurement in a closed system was determined using the BD 600 BOD system (Lovibond, Germany). The system can measure BOD in the range of 0 – 4000 mg/l, for this study, the 0-400 mg/l range was measured. Nitrification inhibitor B (Allyl Thiourea, or ATH) was added to inhibit nitrification, the right amount of nitrification inhibitor B is related to the measurement range as per instructions on manual. 3-4 drops of 45% potassium hydroxide (KOH) solution were added to absorb carbon dioxide in the sample. Readings were recorded after 5 days of the experiment.

3.6 Chemical Oxygen Demand Determination

The COD was determined using the waster tester photometer-system MD 100 (Lovibond, Germany). The system has a COD quantification range of 0 – 15000 mg/l depending on the sample size. Samples were added to the vials, contents were mixed thoroughly and pre-heated in the thermoreactor in accordance with the manufacturer's instructions. After cooling, the vials were placed in the chamber for readings.

3.7 Microplastic Extraction from environmental matrices and analyses

3.7.1 Water sampling

Two procedures were used to collect samples for microplastics analyses. A 20 L sample of surface water, per site was collected into a bucket and taken to the laboratory for further analysis and was processed within 24 h. The other procedure was to collect five replicates of 20 L samples on each site and were all processed onsite. Each of the 20 L samples were filtered onsite through a 250-µm sieve; the remaining particles on the mesh was transferred into correctly labelled falcon tubes, stored on ice, and taken to the laboratory for further analysis. The samples were frozen or refrigerated in the laboratory if they were to be used soon after collection.

3.7.2 Sludge sampling

The WWTP sludge was collected in terms of the MPs sediment procedure using a metal spoon. Five replicates of sludge were collected at 5 m apart from each other; the focus was to scoop 5 cm deep of the sludge sample. The samples were stored in a zip plastic bag, into a cooler box with ice and transported from the field to the laboratory for further analysis.

3.7.3 Microplastics extraction from water samples

The 20 L bucket water was filtered through a vacuum pipe system with 20-µm mesh, and five (four L) replicates were filtered through a vacuum pipe system on a 20-µm mesh. Microplastic was analysed using a microscope. A stock solution of 10% potassium hydroxide (KOH) was prepared. To prepare 1 L solution, 100 g of KOH was added to 900 ml filtered RO water. The solution was stored in a dark bottle and labelled 10% KOH until use. The KOH was added to the water sample, with a ratio of 1:2 water sample. The solution was placed in the oven for 24 hours at 50 ^{o}C. Thereafter, if the digestates had too many sediments in it, a hypersaline solution (NaCl 360 $g \cdot \ell^{-1}$) was prepared.

Hypersaline was prepared by adding 360g/L of salt to the filtered reverse osmosis (RO) water. The RO water was placed into a glass beaker onto the hotplate stirrer which was set to 60 ^{o}C and a magnetic stirrer was added. The pre-weighed salt was added slowly into the water, to prevent stirrer to not function and the glass beaker was covered with foil while the solution was dissolving. Once dissolved, the hypersaline solution was filtered through a 10 µm mesh and was stored until use.

The hypersaline solution was added to the digested sample with a volume three times the actual sample. The solution was stirred vigorously for 2 minutes and was left to settle for 15 minutes. The process allowed biological material, heavily dense particles to sink to the bottom and microplastics to float on the surface. The supernatant was filtered through a pre-cut 20 µm mesh using a vacuum pump, keeping the filtered to repeat the process three times for each replicate. Each 20 µm mesh was place in a petri dish correctly labelled and allowed to dry before the microscope analysis.

3.7.4 Microplastic extraction from sludge samples:

The frozen sludge samples were defrosted, weighed, and transferred to aluminium containers and were covered with foil. The sludge samples were dried for 48 h or until dry. A stock solution of 10% KOH was prepared. To make 1 L solution, 100 g of KOH was added to 900 ml filtered RO water. The solution was store in a dark bottle and labelled 10% KOH until use. The KOH was added to the water sample, with a ratio of 1:2 water sample. The solution was placed in the oven for 24 hours at 50 °C. Thereafter, if the digestates had too many sediments in it, a hypersaline solution was prepared. A hypersaline solution was prepared and was added to the digested sample with a volume three times the actual sample. The solution was stirred vigorously for 2 minutes and was left to settle for 15 minutes. The process allowed biological material, heavily dense particles to sink to the bottom and microplastics to float on the surface. The supernatant was filtered through a pre-cut 20 µm mesh using a vacuum pump, keeping the filtered to repeat the process three times for each replicate. Each 20 µm mesh was place in a petri dish correctly labelled and allowed to dry before the microscope analysis

3.7.5 Microplastics Quality Assurance

The use of negative controls is essential to evaluate the likelihood of sample contamination from the processing or extraction process. While several precautions were taken, to avoid contamination, namely, minimal exposure of samples to air; use of gloves and laboratory coats, covering filter membranes, blank experiment analysis etc., there were a few microfibres detected in the negative controls. This amount was subtracted from the corresponding water sample, to avoid possible manmade and airborne plastic pollution found in the laboratory, equipment used or through sampling. Although precautions to prevent contamination, were taken, it is impossible to completely avoid contamination. For instance, in a study of MPs found in WWTP, Yang *et al.,* (2021), found fibres in blank experiments and the authors subtracted the fibres from corresponding samples. A similar observation was made by Yang *et al.,* (2019), on MPs study found in WWTPs. Thus, greater attention is required to identify and reduce methodological errors increasing aerial microfibre contamination Wesch *et al.,* (2017).

3.8 Ecotoxicology Bioassays

Three different aquatic toxicity tests were used in this study -each represented a different trophic level. *Daphnia magna* (a primary consumer), *Raphidocelis subcapitata* (a primary producer) and *Tetrahymena thermophila* (a decomposer). All the tests consisted of negative controls, which were cells that were not exposed to the test samples (WWTP effluent).

Table 3.2 shows detailed information of the aquatic toxicity used. The Microbiotests Toxkits were supplied by MicroBioTests Inc., Belgium as Algaltoxkit FTM, Daphtoxkit F^{TM}, and Protoxkit FTM. The experimental data for all tests was analysed using ToxRAT Professional 3.2® for the determination of mortality and growth inhibition, statistical significance, and critical concentrations.

Table 3. 2: Specifications of microbiotests applied for toxicity assessment of the WWTP effluent (Microbiotests, 1996; Microbiotests, 2001; Microbiotests, 2004).

	Algae	Crustacean	Protozoa
Organism	*Raphidocelis subcapitata*	*Daphnia magna*	*Tetrahymena thermophila*
Analysed sampled	WWTP effluent	WWTP effluent	WWTP effluent
Exposure time (h)	72	48	24
Trophic level	Producer	Primary consumer	Primary decomposer
Source of organism	Ready to use kit	Ready to use kit	Ready to use kit
Dilution water	Algal culturing medium	Standard freshwater	Protox dilution medium
Incubation temperature (°C)	23 ± 2	20-22 (hatching); 20 (test)	30
Photoperiod	72 h light	72 h light (hatching); darkness (test)	darkness
Illumination (Lux)	1000 (sideways) OR 3000-4000 (bottom)	6000 (hatching)	
Test vessel size	200 mL flask	100 mL flask	15 ml test tube
Test volume (mL)		100	
Effect criteria (LC/EC_{50})	Growth rate inhibition	Mortality/immobilization effect	Growth rate inhibition
Test principles	Measurement of the optical density increase relative to the control	Living or dead crustaceans are visually counted	Measurement of the optical density increase relative to the control
References	Algaltoxkit F, 2004	Daphtoxkit F, 1996	Protoxkit F, 2001

3.8.1 *Raphidocelis subcapitata* Yield and Growth Inhibition Test

The OECD 201 algae growth inhibition test method was used to determine the toxicity of samples on freshwater algae, *R. subcapitata.* There were five treatments and a control; measurements were taken at 24 h intervals for 72 h. The algal beads were de-immobilized according to the manufacturer's instructions. An algal density of 1×10^6 cell/mL was prepared from the concentrated algal inoculum by measurement of the optical density of the inoculum on a spectrophotometer (Jenway 6300) at a wavelength of 670 nm. Dilution series of the samples were prepared, and each flask inoculated with 1×10^4 cells/mL as the test start concentration. There were six treatments including a control and each treatment was made in triplicates. The long cells were placed in the holding tray and were incubated at 23 ℃ with a sideway illumination of 10000 Lux for 72 h. Algal growth inhibition relative to the control were determined every 24 h. Measurement of the OD at 670 nm of the algal suspensions in the long cells was performed at 24 h intervals for 3 days during the 3 days. The data was used to determine yield and growth inhibition of *Raphidocelis subcapitata* after exposure to the water samples. The results were recorded in the results sheet and were statistically analysed using ToxRat Professional 3.2 Software.

3.8.2 *Daphnia magna* Acute Mortality Test

The crustaceans, *D. magna* were exposed to the different water samples collected for toxicity testing and hatching of the ephippia was based on supplier's Daphtoxkit F^{TM} instructions. The young daphnids were pre-fed 2 h prior to the commencement of experiments to prevent "starvation to death". Dilution series of the samples were prepared according to standard procedures and there were five treatments and a control, and each treatment had four replicates. The neonates were placed in a multi well (5 in each well) and were incubated in darkness at 20 ^{o}C for 24 – 48 h. Neonates were counted and those that were unable to swim after gentle agitation of the liquid for 15 seconds were considered immobilized. The scores were recorded in the score sheet and the results were analysed statistically using ToxRat Professional 3.2 Software.

3.8.3 *Tetrahymena thermophila* Growth Inhibition Test

The Protoxkit F™ (2001), standard operating procedure handbook was followed for the tests. The test measured growth inhibition of the ciliate protozoan *T. thermophila* after 24 h. In this period, normal growing cultures completed at least 5-generation cycles. The test was performed in disposable 1 cm polystyrol spectrophotometric cuvettes. To calculate the dilution factor that was needed to arrive at a 'theoretical' OD value of 0.04 the following formulas were used:

$$F = \text{OD value} / 0.040$$

$$V = 0.5 \times (F-1)$$

All the cells were placed into a holding tray and the tray was put in an incubator (in darkness) at 30 ^{o}C for 24 h. After the 24 h incubation, the measuring equipment was recalibrated with a test cell containing 2 ml distilled water and all cells were gently shaken to determine the OD at 440 nm (= time T24 scoring).

3.9 Effluent Toxicity Classification

The results were expressed as effective concentration (EC). Toxicities and EC values are inversely related and so, toxicity units (TUs) are used to describe concentration-based toxicity measurements. Acute toxicity unit (Tua) was used to express acute toxicity of concentration-based toxicities. . The EC_{50} values were determined at 24 h, 48 h and 72 h by ToxRat Professional 3.2 software. Thereafter, toxicity values EC_{50} were transformed into TU according to the equation $TU = 100 / EC_{50}$ by Kocbus and Oral (2015) and was classified according to Persoone *et al.,* (2003), hazard classification system for wastewaters discharges into aquatic environments as shown in Table 3.2.

Table 3.3: Hazard classification system for wastes discharged into the aquatic environment (Persoone et al, 2003).

TU		Toxicity	Symbol
< 0.4	Class I	No acute toxicity	☺
0.4 <TU< 1	Class II	Slight acute toxicity	☹
1 <TU< 10	Class III	Acute toxicity	☠
10 <TU< 100	Class IV	High acute toxicity	☠☠
TU > 100	Class V	Very high acute toxicity	☠☠☠

A weight score was calculated for each hazard class to show the quantitative importance (weight) of the toxicity in that class according to Persoone *et al.,* (2003) as shown in Table 3.3. Class weight scores were evaluated by the allocation of a test score for the effect results of each test of the battery and were calculated using equation 1.

Table 3. 4: Calculation of the class weight scores for wastewater

The test score is allocated for the effect results of each biotest in the battery where:	
No ‘significant’ toxic effect	Score 0

Significant toxic effect, but < L(E) C_{50} (= < 1 TU)	Score 1
1 - 10 TU	Score 2
10 – 100 TU	Score 3
>100 TU	Score 4

To calculate weight class score:

Class weight score = $\sum all\ test\ scores\ /\ n$ ………………………...equation 1

Where n = number of tests performed

To calculate the class score in percentage:

% Class weight score = Class score ⁄ maximum class weight score × 100…………equation 2.

3.10 Statistical analysis

The results obtained from the wastewater samples were analysed using the IBM SPSS Statistics version 28 package for statistical analysis. Pearson’s correlation analysis at 5% significance level was used to show the relationship between the concentrations of the analysed physicochemical parameters for the 2019 – 2021 sampled period. Data obtained from bioassay experiments were analysed using ToxRat Professional 3.2 Software.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results based on the data found during fieldwork and lab analysis. The results presented are the key findings of the study and are presented based on physicochemical properties, toxicity tests and microplastic occurrence in the river water, the influent, and the effluent. These results indicate the state of the study area and answer the research questions. The results are discussed based on the different seasons sampled in the year. Summer (December, January, and February); Autumn (March, April and May); Winter (June, July and August); Spring (September, October and November). However, due to logistic reasons, the BOD and COD results are presented for a period of 7 months (August 2020 – June 2021) showing all seasons while microplastics occurrence are presented for two seasons (spring and autumn). Moreover, in February 2020 and March 2021, there was no water found upstream due to high weather conditions, hence the missing results in those two seasons (summer and autumn).

4.2 Physicochemical Properties

The onsite physicochemical parameters recorded for all sampling points are presented below. The sampling period commenced from 2019 – 2021, due to Covid 19 and lockdown restrictions the sampling regime extended to 13 inconsecutive months. Moreover, the results obtained, using the methods that were illustrated in chapter 3 were compared with the accepted limits of the Department of Water Affairs and Forestry (DWAF), South Africa and the World Health Organisation (WHO), as shown in table 4.1.

Table 4.1: Wastewater limit values applicable to discharge of wastewater into a water (Government Gazette, 1999; DWAF, 1996; DEA, 2014; Akan *et al.*, 2008; WHO, 2011; Olabode *et al.*, 2020)

Parameters	Unit	South Africa (DWAF)	WHO	USEPA
pH		5.5 - 9.5	6 - 9	6.5 – 8.5
DO	mg/L	7.5	N/A	6.0 – 9.5
Temperature	^oC	≤ 35	40	≤ 35
EC	µS/cm	≤750	1000	2500
ORP	mV	N/A	700	N/A
COD	mg/L	≤ 75	≤100	≤ 1000
BOD	mg/L	3.0 – 6.0	50	≤ 500
TDS	mg/L	0 - 450	0 - 650	500

N/A: not available

4.2.1 Temperature

Temperature is one of the important guides to water quality as it affects chemical reactions, aquatic life, and suitability of water. Figure 4.1 illustrates the monthly variation in temperature for the WWTP and the Veldwachters River. The overall temperature of the effluent for the sampling period ranged between 19.4 – 28.4 ^oC. The values recorded were slightly similar to the values (16.9 – 25.3 ^oC) reported by Olabode *et al.*, (2020), for two wastewater effluents. The lowest temperature (14.2 ^oC) was obtained upstream in August 2019 and June 2021, while the highest temperature (29.5 ^oC) was obtained at the point of discharge in March 2021.

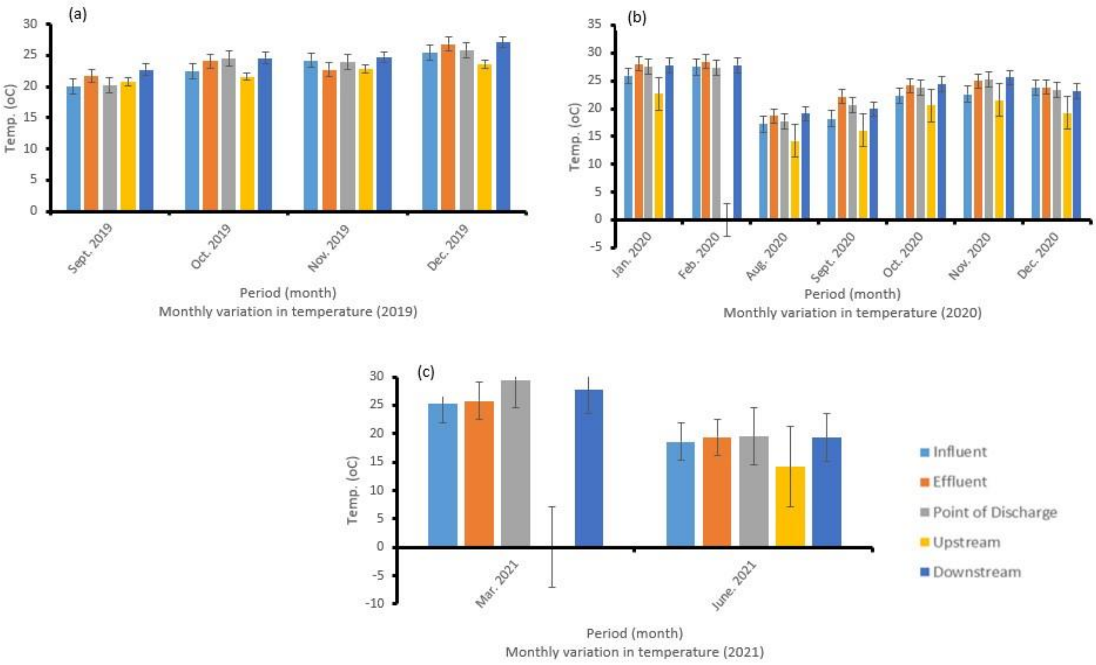

Figure 4.1: Monthly variations in temperature (a) 2019; (b) 2020 and (c) 2021

In 2019 the temperatures ranged between (20 – 27.1 °C) and continued increasing in January 2020 and February 2020 and ranged between 22.7 – 28.4 °C in all sampled sites which is due to no rainfall and intensity of sunlight. Lower temperatures were observed in August 2020 which was during the peak of winter (14.2 – 19.1 °C, while in September 2020 – November 2020 (spring), there was a slight increase in temperature. A similar trend was observed with the temperatures in March 2021 and June 2021. The lowest temperature values were observed during winter and early spring, while the highest were during summer, mid – late spring and late autumn. This suggests that seasonal variations have significant influence on the temperature. No values were recorded upstream in February 2020 and March 2021, as there was no water in the river at that point, due to prevailing atmospheric conditions observed on those sampling days. The Veldwachters River is a non-perennial river, and this was evident because there was always less water upstream, than downstream especially during summer seasons, as observed during the sampling period. There was no significant change in temperature between the effluent and downstream the river. The effluent's temperature complied with the set limits for discharged effluent into a waterbody by DWAF (≤ 35 °C) and WHO (40 °C). Based on these guidelines, the temperature of the effluent appears to pose no threat to the receiving water body.

4.2.2 pH

The results of the monthly variation in pH for the WWTP and the Veldwachters River for the sample period is presented in figure 4.2. The pH level in water outlines its utility for different purposes and is known to have an impact on the presence of micronutrients and trace and heavy metals in the water (Agoro *et al.*, 2018). The pH values for September 2019 – December 2019 (spring and summer seasons) were around neutral (pH 7), and not significantly different from one another. The pH slightly increased in all months of 2019 at the point of discharge, upstream and downstream which suggested that there were other unidentified factors, presumably coming from upstream and other nonpoint sources along the river.

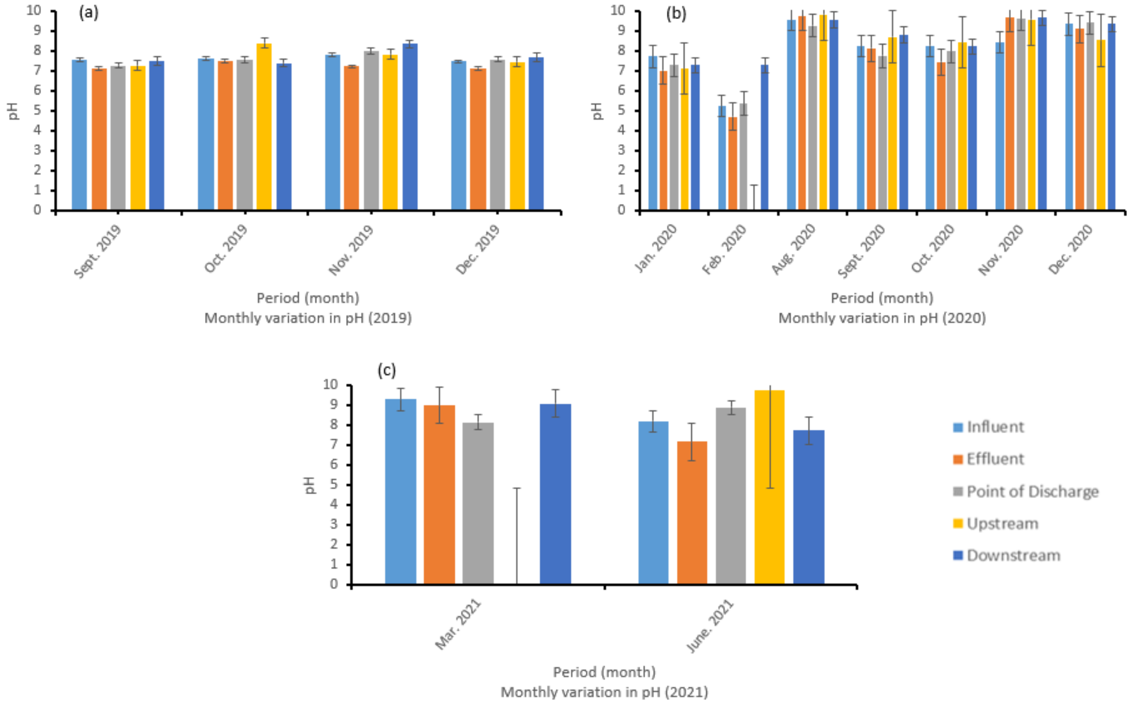

Figure 4.2: Monthly variations in pH (a) 2019; (b) 2020 and (c) 2021.

In January 2020 and February 2020 which was the peak of summer, the pH values ranged between (4.7 – 9.4) where most months were around neutral (pH 7) and then tended towards acidity which was attributed to seasonal variations. In August 2020 – December 2020, the pH values continued tending towards alkalinity, around (pH 9) for most sampling points. While the year 2021 showed no difference in the pH range to that of the previous year's results and no water was found upstream in March 2021. The water was still slightly alkaline and there was no significant change from the effluent values and downstream. This is an indication of disinfection in the water and could be due to bicarbonate, carbonate, and hydroxide caused by CO_2 and microbial decomposition of organic matter (Baharvand and Daneshvar, 2019). A similar observation was reported at the Deoli Bhorus Dam, where it was stated that changes in pH are all dependent on the photosynthetic activity of the water which influences the changes of CO_2, carbonate, and bicarbonate of the water (Kalwale and Savale, 2012). Similarly, Olabode *et al.,* 2020 reported trends of pH tending towards alkalinity could have been due to high levels of carbonate use in the treatment method and similarly to their sampling observations, this study also observed the water to produce foam in the samples collected at the point of discharge. The pH values observed for all sampling seasons for the effluent ranged between 4.7 – 9.7 which were not all within the limits for discharged effluents into a receiving waterbody both from DWAF; USEPA and WHO as shown in Table 4.1. However, when compared to other points along the river, the effluent may not pose a negative effect on the water quality of the receiving water body. This is because, there is no significant change in the values in all the points, which suggests that the effluent has no detrimental effect on the watercourse.

4.2.2 Dissolved Oxygen

As previously mentioned in chapter 2, DO is an important parameter in assessing water quality, and is a guide to the physical and chemical processes in water. Figure 4.3 presents monthly variations of DO recorded for both the WWTP and the Veldwachters River for all sampled months. In the year 2019, the values for DO ranged between (1.7 – 6.1 mg/L) for all months. The lowest DO concentration (1.7 mg/L) recorded in December 2019 (summer) at the point of discharge was significantly different to the DO concentrations found in other months, except for October 2019 at the influent (1.8 mg/L). The low DO could be due to inorganic substance found in the waterbody (Agoro *et al.,* 2018; Iloms *et al.,* 2020). This could be due to poor treatment of the wastewater effluent and due to unidentified non-point sources entering the waterbody upstream.

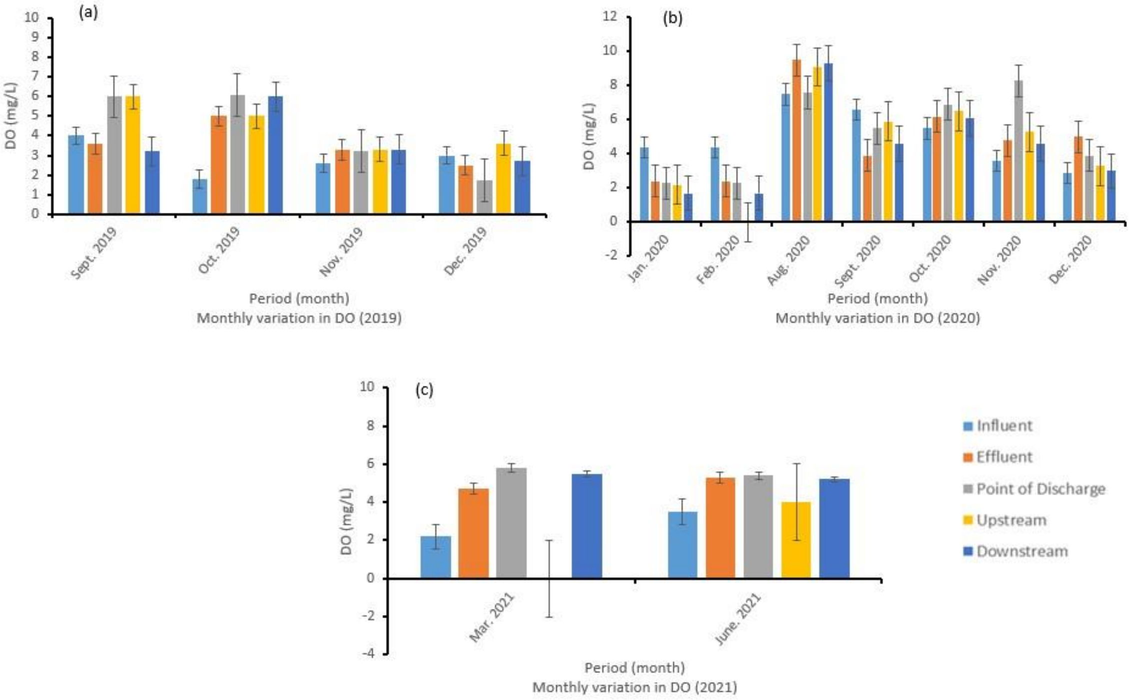

Figure 4.3: Monthly variations in DO (a) 2019; (b) 2020 and (c) 2021

Similarly, to what was reported elsewhere, that inorganic compounds such as ammonia nitrites, hydrogen sulphates, and Ferro ions are likely to decrease oxygen in a waterbody (Agoro *et al.*, 2018). The variation of DO values in January 2020 and February 2020, which was the peak of summer also had a low concentration of 1.7 mg/L downstream. This could be attributed with seasonal variations because the DO values increased in the August 2020 (winter) and started decreasing in September 2020 – November 2020 but no significant change was observed, except at the point of discharge in November 2020 (8.3 mg/L). In December 2020, the values were still low which proved that seasonal variations have an impact on the DO values. DO concentrations in unpolluted water normally range between 8 – 10 mg/L, concentrations below 5 mg/L affect aquatic life, while concentrations below 2 mg/L cause fish kills (DFID, 1999). Agoro *et al.*, (2018) reported that the acceptable standard of DO for aquatic organisms is 4 – 5 mg/L, while the result of the effluent shows that about 46% the values for DO were within that range and are good for aquatic life. However, 54% of the effluent shows low DO values, which may pose a negative effect on aquatic organisms in the receiving water body (Edokpayi *et al.*, 2017). The overall DO values (1.7 – 9.5 mg/L) ranged below the recommended limits for DWAF (7.5 mg/L) for most of the sampled sites and no limits were found from WHO.

4.2.3 Electrical Conductivity

The EC is primarily attributed to dissolved ions obtained from decomposed plant matter and is also an important indicator of salinity found in surface water (Agoro *et al.*, 2018). The results of the monthly variation in EC of the WWTP and the Veldwachters River (2019 -2021) is presented in figure 4.4 above. The EC values throughout the sampling period appeared to be similar and ranged between 559 – 929 µS/cm, except for the influent values (973 – 1241 µS/cm) which were quite higher.

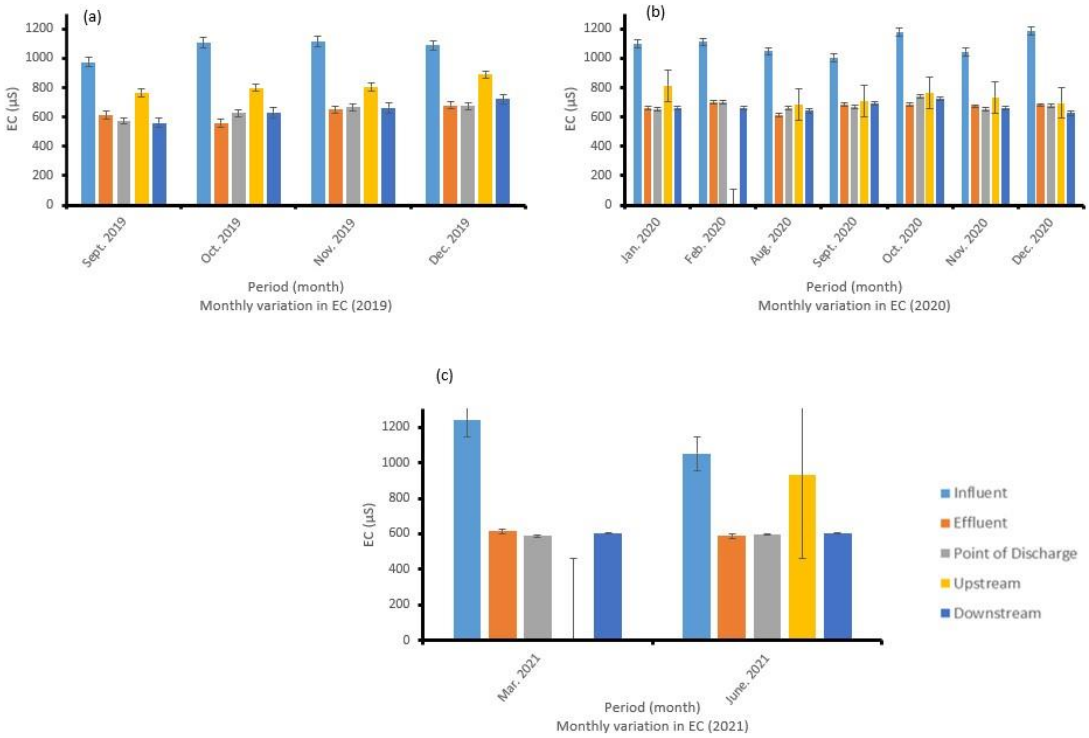

Figure 4.4: Monthly variations in EC (a) 2019; (b) 2020 and (c) 2021

High EC values in wastewater suggests that there is high total dissolved solids concentration. It is observed that the values recorded upstream are always higher, while downstream is similar or slightly lower than those recorded at the effluent for all months. Thus, suggesting that other unidentified contaminants gain access to the watershed, which conforms with a similar trend observed in two different studies on WWTPs and rivers (Osode and Okoh 2009; Agoro *et al.*, 2018). The highest values for the all the sampling points were observed in the spring and summer seasons while the lowest values were observed in the autumn and winter season, which is in line with what was reported elsewhere, and that seasonal and temperature variation affects EC, (Olabode *et al.*, 2020). Moreover, the values of the influent were too high and when compared with the values of the effluent, it shows that the WWTP is efficient in the reduction of EC. Although some effluent values were higher than the DWAF (≤750 µS/cm) standards, they were still within the WHO (1000 µS/cm) and USEPA (2000 µS/cm) standards.

4.2.4 Total Dissolved Solids

Figure 4.5 presents the monthly variation in TDS for both the WWTP and the Veldwachters River (2019 – 2021). The salt concentration present in water is determined by TDS, which is a measure of water salinity. Calcium, magnesium, sodium, potassium, iron, and manganese carbonates, bicarbonates, chlorides, sulfates, phosphates, and nitrates are among the most prevalent (Olabode *et al.,* 2020). TDS is directly proportional to EC; hence it is observed that the trends of these results corroborate with the values recorded for EC.

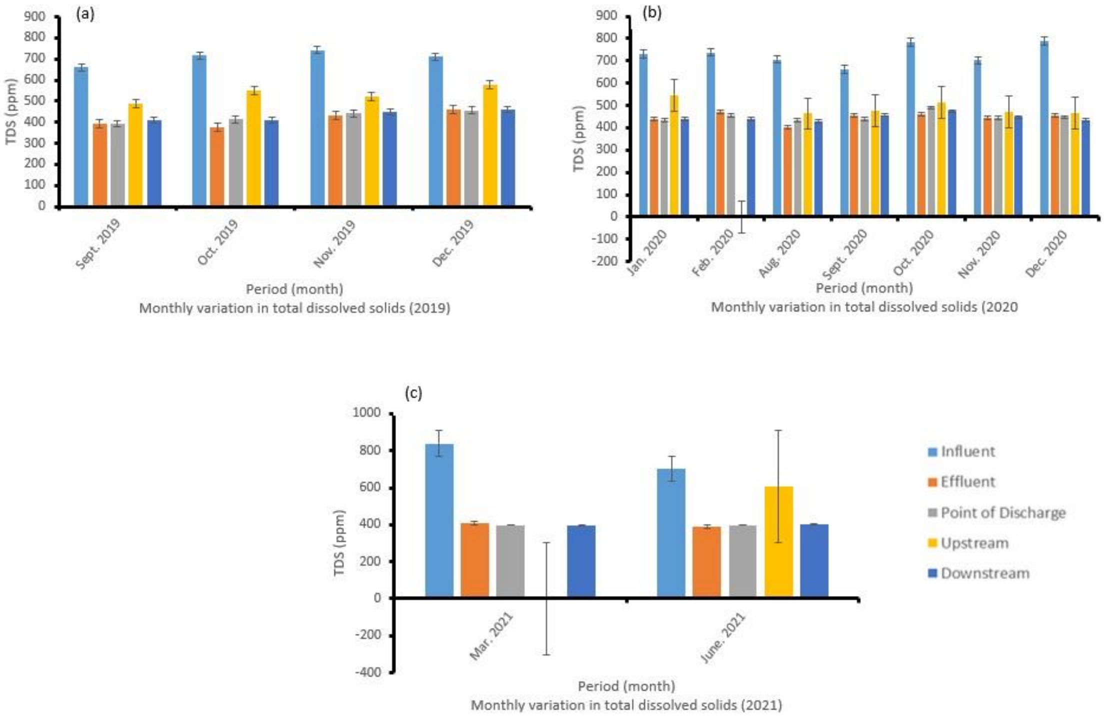

Figure 4.5: Monthly variations in TDS (a) 2019; (b) 2020 and (c) 2021

The values for the entire sampling period ranged between (376 – 607 mg/L) for all other points and were higher for the influent (661 – 840 mg/L). The difference in values for both influent and effluent suggested that the WWTP is efficient in TDS reduction and most of the recorded values for the effluent were within the DWAF (0 – 450 mg/L) while in other months, TDS values were slightly high, although all were within the WHO (650 mg/L) limits for effluent discharged into a waterbody. Moreover, the difference with the values recorded upstream and downstream suggests that there could be external pollution sources, within the vicinity of the river. A similar observation by Agoro and co-workers, in a study on physicochemical properties of wastewater further states that high concentrations of TDS are toxic to aquatic organisms and thus lead to dehydration and thermal shock, which affects the organisms osmoregulatory strength. Furthermore, this difference showed that the effluent quality normalised with that of the receiving waterbody, hence the decreased results downstream.

4.2.5 Oxidation Reduction Potential

Figure 4.6 shows the monthly variation in ORP for both the WWTP and the Veldwachters River (2019 – 2021). The ORP readings in water are considered as an important indicator of pollution levels. The values were mostly positive, except for the values recorded at the influent for all months of 2019, January 2020; December 2020 and June 2021 (-350 – (-39 mV)). The positive values indicated an oxidation water environment, while the negative values suggested that many pollutants may have contributed to the reducing status of the influent and the effluent of those months.

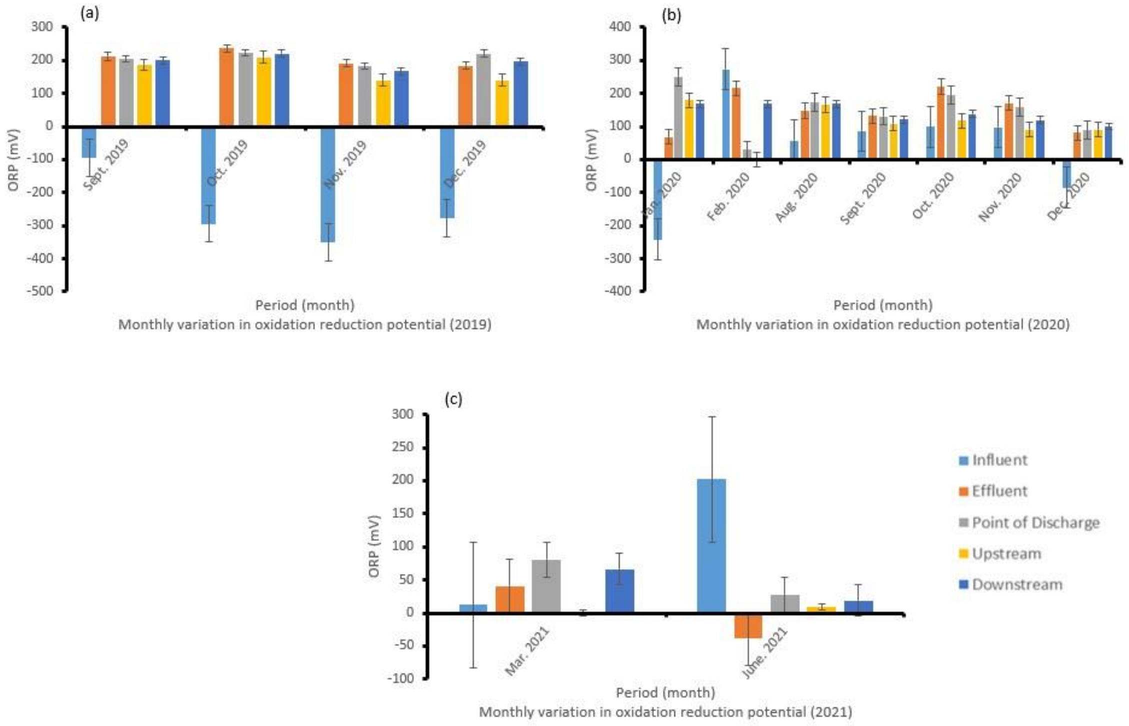

Figure 4.6: Monthly variations in ORP (a) 2019; (b) 2020 and (c) 2021

The values downstream were higher than values recorded upstream, at all points except for January 2020. The observed trends could be due to the very high flow the river receives from the WWTP, which contributes positively to the river recharge and health downstream. Currently, there are no regulatory standards for ORP in SA, therefore, ORP in this study was compared with the WHO standards. The general ORP values ranged between -39 – 273 mV and were well within the WHO standards of 700mV. This suggested that the effluent poses little to no negative effect on the river.

4.2.6 Biological Oxygen Demand

The display of biological oxygen demand content in both the WWTP and Veldwachters River was determined by calculating the amount of oxygen needed for its stabilisation as BOD as depicted in Figure 4.7. The BOD values for the effluent in sampled months ranged between 0.9 – 79.54 mg/L, while higher values were found at the influent (76.4 – 175.58).

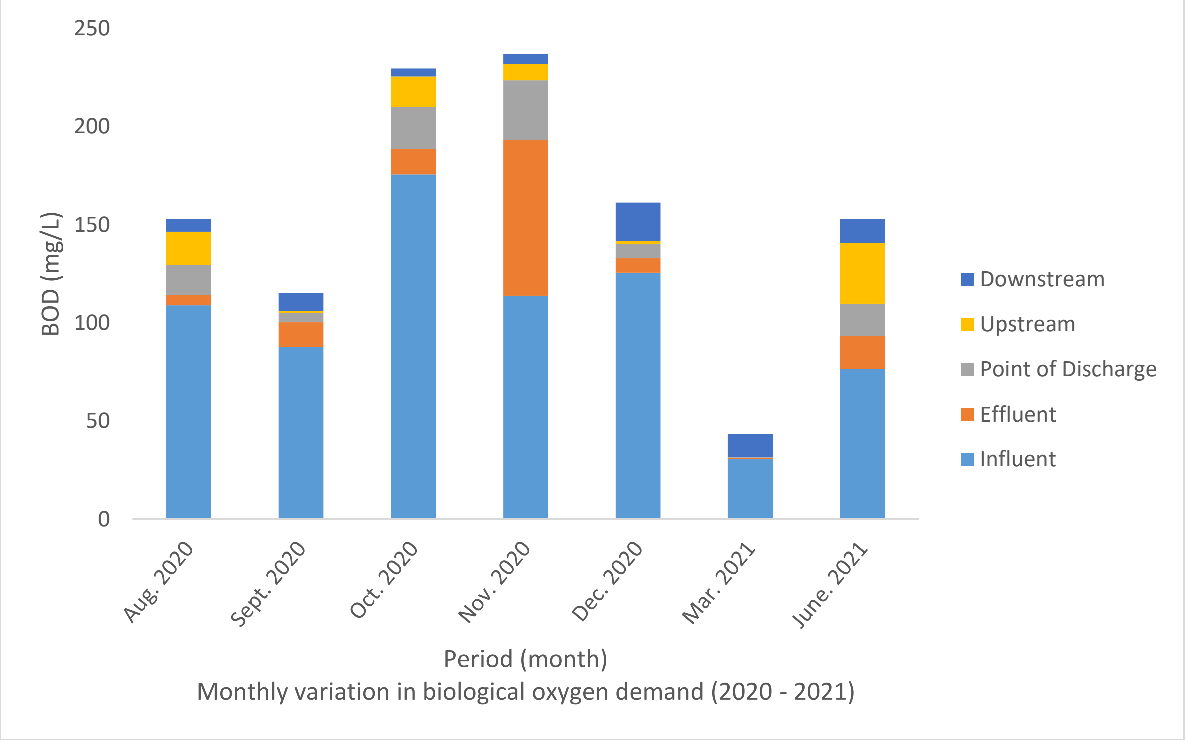

Figure 4. 7: Monthly variations in BOD 2020 - 2021

The concentration range for the effluent was lower compared to the 9.17 – 252.44 mg/L reported for physicochemical properties of wastewater effluent from two selected wastewater treatment plants in Cape Town (Olabode *et al.*, (2020). The concentrations were, however, higher than the values 3.7 – 14 mg/L reported by Agoro *et al*, 2018 in a study on physicochemical properties of wastewater in three typical South African sewage works. The high values of BOD in September – November 2020 which was in the spring season; and June 2021 (winter) could be attributed to heavy discharge of industrial and domestics effluents, crops, and animal waste. Similarly, the slightly high values found in the in the receiving river (upstream and point of discharge) suggested that other unidentified sources (agricultural runoff;

landfill site runoff and leachate) also contribute to high BOD values. These high values indicate a high level of organic matter content in the river or effluent water, which signifies a potential danger to the aquatic organisms as the quantity of dissolved oxygen in the water is reduced. The low values found at the effluent as compared to the influent indicated a high efficiency of the WWTP processes in eliminating BOD during the treatment process and further suggest that the water is good although it could be improved. The DWAF guideline values for BOD is 3 – 6 mg/L while the WHO is 50 mg/L, however, this level was exceeded by most of the sampling months except for August 2020 and March 2021. This is detrimental as continuous discharge of the WWTP effluent will impact the receiving waterbody to some degree and may negatively impact the aquatic organism, especially those downstream.

4.2.7 Chemical Oxygen Demand

Figure 4.8 shows the monthly variation in COD for both the WWTP and the Veldwachters River (2020 – 2021). As previously mentioned, COD is known as an alternative test to BOD to determine the concentration of organic matter in wastewater samples. The COD results ranged between 0.83 – 912.15 mg/L, where high values were found at the influent.

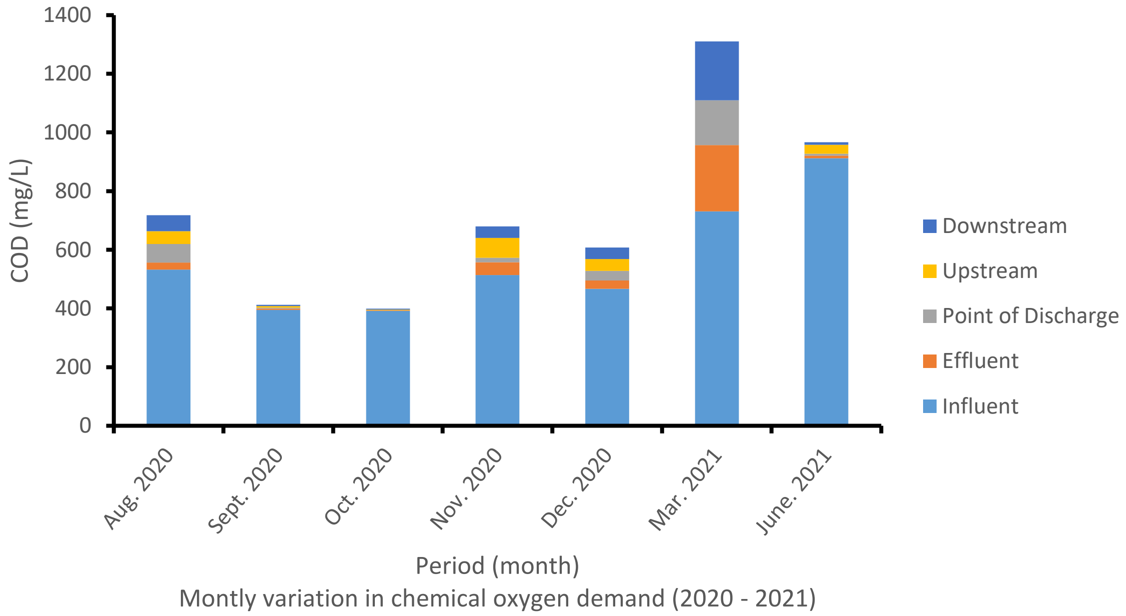

Figure 4.8: Monthly variations in COD 2020 - 2021

According to Aniyikaiye *et al.*, (2019); Baharvand and Daneshvar (2019), COD values are usually higher than BOD due to organic materials found in water that are resistant to microbial oxidation. A similar trend was observed in this study, except for some months (Sept, Oct, Nov, and June), similarly as reported in other research by Akan *et al.*, (2008); Agoro *et al.*, (2018);

Aniyikaiye *et al.,* (2019); Baharvand and Daneshvar (2019). Similarly, to BOD, high levels of COD observed in wastewater are caused by chemicals which are organic or inorganic and possess oxygen demanding traits. Furthermore, elevated levels of both BOD and COD in water cause drastic oxygen depletion and highly affect aquatic life (Odjadjare and Okoh, 2009). The recorded values suggested that the WWTP effluent complied with the set limits ≤ 75mg/L by DWAF and ≤100 by WHO for COD for all sampling months. Nevertheless, the COD values for March (226 mg/L) were above the required standards, which could be attributed to seasonal variation. Moreover, this high COD value is an indication that due to these seasonal variations, the effluent contains materials that are resistant to microbial degradations and can detrimentally affect the receiving river.

4.3 Mean and standard deviation values of the physicochemical parameters

The mean values of the physicochemical parameters are further shown in table 4.2 below. The values for temperature, pH, DO, EC, TDS, ORP, BOD and COD were in the range of 14.2 – 29.5 ^{o}C; 4.7 – 9.75; 1.7 – 9.5 mg/L; 559 – 1241 µm/cm; 376 – 840 ppm; -350 – 273 mV; 0.9 – 175.6 mg/L, respectively. The mean effluent values of all the parameters show no significant difference to those on the points along the river. This further proves that based on the physicochemical parameters, the effluents may be discharged into the river.

Table 4. 2: Mean (±SD) values of physicochemical parameters obtained at the sampled sites

	Sampling Points				
	Influent	Effluent	Point of Discharge	Upstream	Downstream
Physicochemical Parameters					
Temp (^{o}C)	22.6±3.2	23.9±2.98	23.8±3.46	16.7±8.04	24.1±3.17
pH	8.1±1.1	7.8±1.36	8±1.13	7.1±3.29	8.3±0.89
DO (mg/L)	4±1.69	4.5±1.93	5±2.13	4.2±2.56	4.4±2.11
EC (µS/cm)	1096±74.77	646±44.25	653±47.04	660±301.45	650±47.06
TDS (mg/L)	730±50.55	431±31.14	434±28.09	437±199.08	435±23.72
ORP (mV)	-40±201.29	143±82.96	151±73.71	111±71.28	143±57.04
BOD (mg/L)	102.6±44.97	19.3±27.07	13.7±10.41	12.4±11.22	9.8±5.33
COD (mg/L)	563.38±191.52	47.9±79.93	39.35±54.65	31.38±24.79	50.16±69.08

Table 4.3: Correlation coefficient between physicochemical parameters

Correlations

	pH	DO	Temperature	EC	TDS	ORP	COD	BOD
pH	1.00							
DO	.468**	1.00						
Temperature	-.450**	-.515**	1.00					
EC	.003	-.212	-.056	1.00				
TDS	.015	-.211	-.060	.997**	1.00			
ORP	-.196	.150	.087	-.540**	-.536**	1.00		
COD	.011	-.334	.068	.822**	.830**	-.186	1.00	
BOD	.003	-.161	-.016	.793**	.790**	-.174	.652**	1.00

**. Correlation is significant at the 0.01 level (2-tailed).

4.4 Correlation Analysis of the physicochemical properties (2019 – 2021)

Correlation of the physicochemical properties was assessed to show the association and the strength of the linear relationship between two variables. Pearson's correlation coefficient (r) of the different water quality parameters of the WWTP and the effluent was calculated using the pair of variables. The results of these variables showed significant and insignificant relationships with each other, as show in Table 4.3. There is a significant ($p < 0.01$) positive correlation between pH and DO, while temperature with pH and DO indicate a negative correlation ($r = -0.450$ and -0.515 at $p < 0.01$) respectively. This suggests that the river water is bad for the ecological health of the organisms because the water is too acidic and there is less oxygen for organisms to survive in the water. The correlation between pH; EC; TDS; ORP; COD and BOD was statistically insignificant (r = 0.003; 0.015; -0.196; 0.011 and 0.003) respectively. This showed that there is no relationship between these variables. These results were different to those observed by Pereao *et al.,* (2021) where a strong positive correlation existed between pH; EC; ORP and TDS values. A strong positive correlation was observed between EC, TDS, COD and BOD, while ORP showed a negative correlation with TDS and EC. According to Osode and Okoh, (2009), knowing how the variables correlate with each other helps with understanding the nature of the physicochemical parameters and their species speciation on the receiving water body and effluent.

4.5 Microplastics occurrence in the Veldwachters River water, WWTP's influent and effluent samples

Microplastics (MPs) are polymer particles that are smaller than 5mm and are classified into primary and secondary MPs depending on how they were generated. Majority of MP research has been conducted in the marine environment and there has been less research of MPs in freshwater environments. This study looked at MP occurrence for two seasons (spring and autumn) at the influent, effluent, point of discharge, upstream and downstream. WWTPs have been identified as primary sources of MPs in the environment and these results will help assess the effectiveness of the WWTPs removal of MPs. Table 4.4 shows the results of the occurrence of MPs in the WWTP and the Veldwachters River.

Table 4.4: Abundance and distribution of microplastics in the WWTP and Veldwachters River (Mean ± SD)

	Season			
	Spring		**Autumn**	
Site name	20 µm	250 µm	20 µm	250 µm
Influent	0.77 ± 0.41	0.59 ± 0.35	0.53 ± 0.23	0.40 ± 0.15
Effluent	0.77 ± 0.25	0.52 ± 0.32	0.85 ± 0.24	0.47 ± 0.16
Point of discharge	0.58 ± 0.18	0.74 ± 0.47	0.95 ± 0.28	0.51 ± 0.10
Upstream	0.93 ± 0.18	0.60 ± 0.22	-	-
Downstream	0.56 ± 0.26	0.56 ± 0.15	0.70 ± 0.11	0.37 ± 0.27

The MP particles were found in all sampling sites, including the WWTP sludge, with a total of 1445 particles for the spring and autumn seasons, by means of visual examination (stereomicroscope) and chemical composition (Fourier-transform infrared spectroscopy - FTIR analysis). Table 4.4 shows the highest mean values of MPs extracted from the water samples were recorded in spring (upstream: 0.93 ± 0.18) while the lowest were recorded in autumn (downstream: 0.37 ± 0.27). This suggests that MPs were gradually diluted with the increase in water volume that occurred in the winter season similarly to what was observed by Wang *et al.,* (2021). The mean MP abundance increased in the effluent compared to the influent, which could be attributed with the fact that larger size MPs may break down, causing an increase of smaller sized MPs. Similarly, to what was observed by Yang *et al.,* (2021) in one of their sampled sites (W3 oxidation ditch).

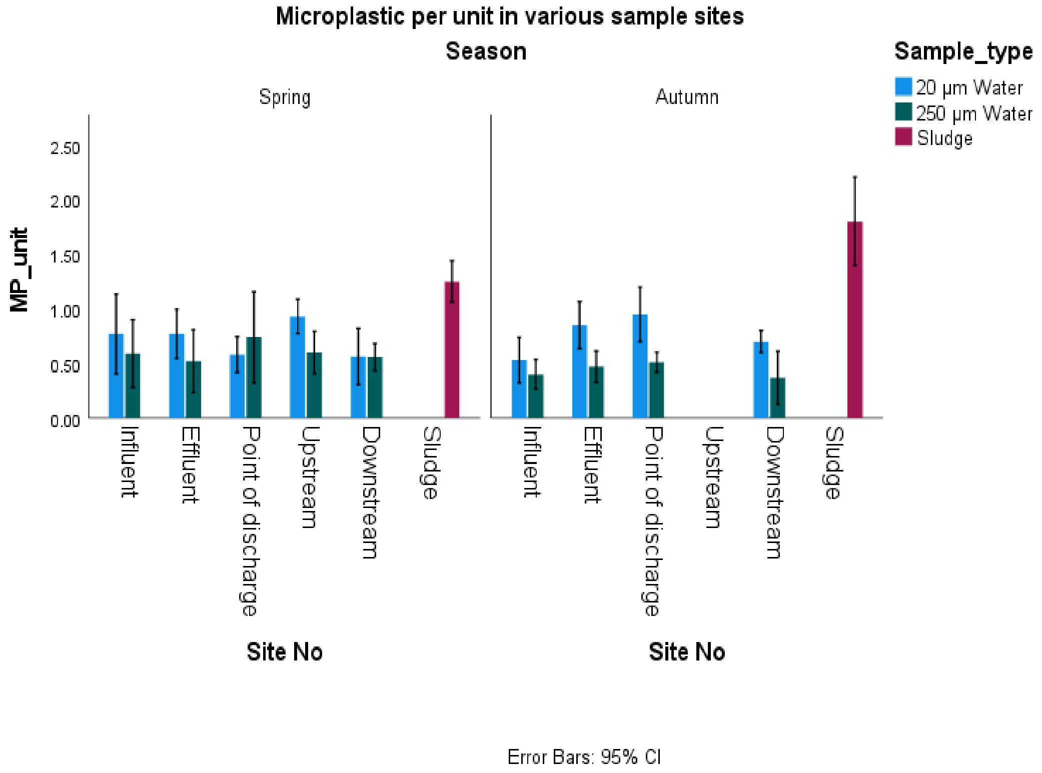

Figure 4. 9: Comparison of the extraction efficiencies of microplastic from water using 20 µm and 250 µm, as well as microplastics found in the sludge, per season

For both water and sludge samples the abundance of MP particles were found to be higher in the sludge samples as compared to the water samples as shown in figure 4.9. This suggests that MPs settled more in the sludge than in the water samples because MPs removed during wastewater treatment accumulate in the sludge (Iyare *et al.*, 2020). In the water samples, comparison of the recovery efficiencies during filtration using two mesh sizes showed that the 20 µm mesh has higher extraction efficiency than the 250 µm except in spring at the point of discharged. According to Liu *et al.*, (2019), MPs are also transported into rivers by wind, because of their low densities and small size. The point of discharge in spring had more MPs in water using the 250µm (which was done onsite) compared to the 20µm (analysed in the lab) due to that reason and this was evident on the sampling day as it was windy. The results further showed that MPs were found to be higher upstream than downstream and other sampled sites. This is not in agreement to what was reported by Kay *et al.*, (2018), where the authors observed MP quantity greater downstream than upstream and further reported that their observations confirm that treated effluent is a key source of MPs. This is different to the current study's results, suggesting that other external sources upstream could be the cause of the high quantity of MPs upstream.

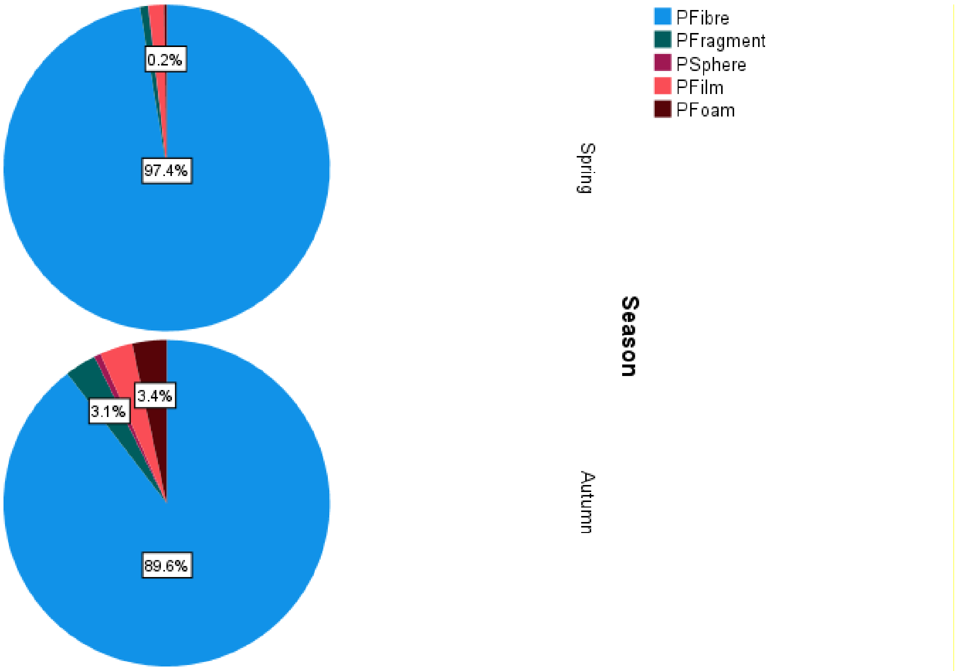

Figure 4.10: Distribution of microplastic type found at the WWTP and the Veldwachters River per season

Based on the MP type categories proposed by GESAMP (2019), fibre/filament; fragment, film, sphere, pellet, and foam; fibre was recorded as the most dominant MP particle found in spring and autumn with an average percentage of 97.4% and 89.6 respectively (figure 4.12). Fibre was one of the most dominant particles found in wastewater, which is consistent with previous research (Liu *et al.*, 2019; Conley *et al.*, 2019; Bayo *et al.*, 2019; Yang *et al.*, 2021). Furthermore, Bayo *et al.*, (2019), states that influents entering a WWTP often come from washing machines which could result in fibres being most dominant. However, in other studies different shapes dominate, and this difference is based on the source and composition of the WWTP studied, including the amount of sewage entering the WWTP.

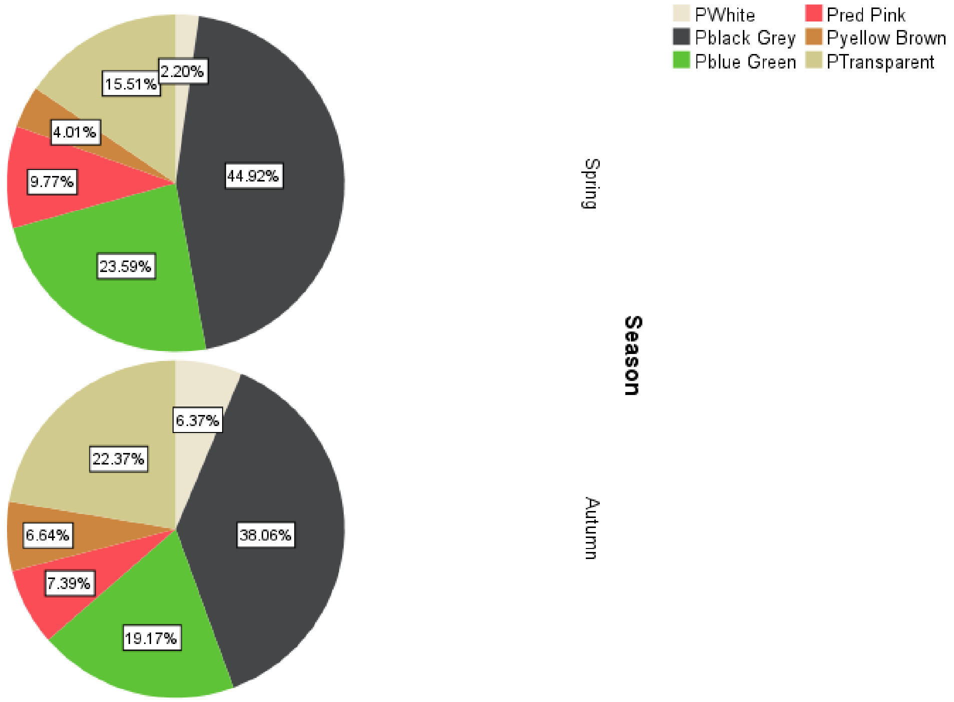

Figure 4.11: Percentage distribution of colours of MPs found at the WWTP and the Veldwachters River per season

The distribution of MPs based on colours is shown if figure 4.11, six colours were observed. Among them, in both spring and autumn black/ grey was mostly found 44.9% and 38.1, followed by blue/ green 23.6% and 19.2% respectively, while the contents of white were relatively low.

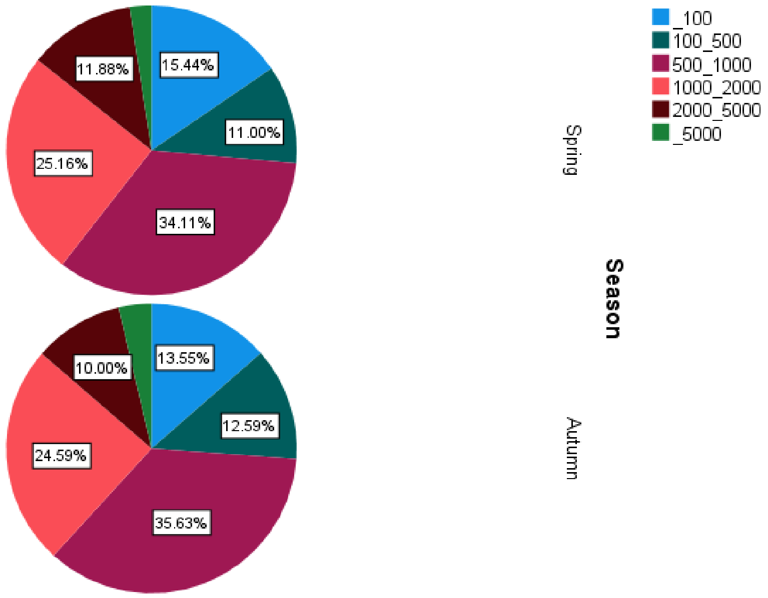

Figure 4.12: Percentage distribution of sizes of MPs found in all sites per season

Analysis of MPs based on size indicated that most of the MPs recorded were less than 2000 µm and both seasons show similar observations. The MP size did not vary with the seasons, was an indication that MPs remain in water for a long time and have continuous impacts on the environment (Wang *et al.*, 2021). This observation is consistent with what was reported by Sparks and Awe (2021), in a study of MPs found in retail mussels. Selected images of MPs found in the different study sites are presented in Figure 4.13.

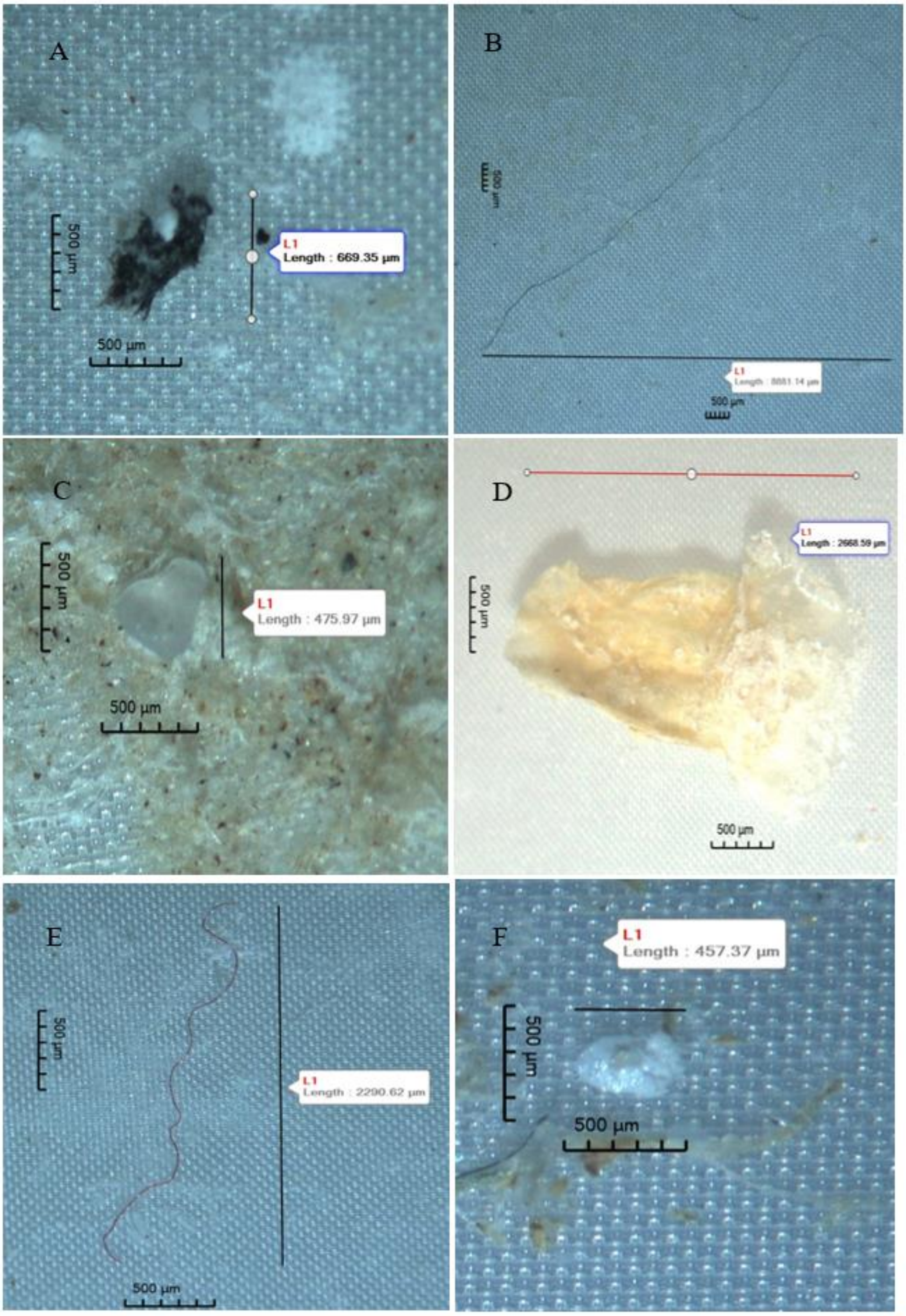

Figure 4.13: Figure 1: Microplastic particle types found at WWTP and the Veldwachters river (a) fragment, (b) fibre, (c) pellet, (d) foam, (e) fibre and (f) foam.

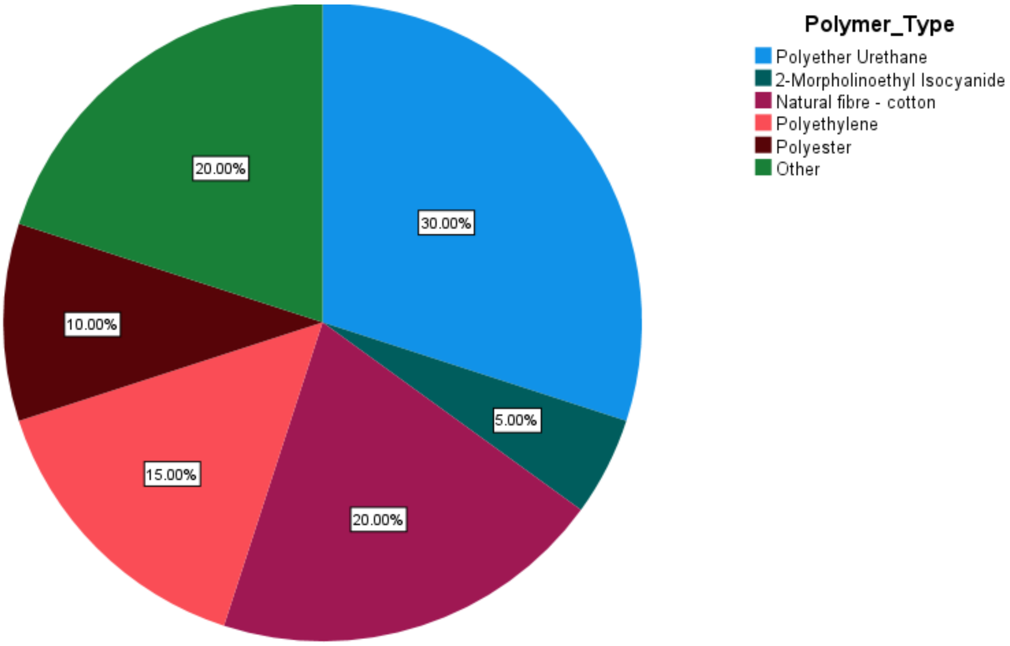

Figure 4.14: Percentage polymer types confirmed using FTIR-ATR in the WWTP and Veldwachters River

A total of 1445 MPs were recorded and 54% (n = 597) for spring and 46% (n = 499) for autumn were larger than the 500 µm required as the minimum size sampled for FTIR-ATR polymer type identification. There is a total of 51 (4.7%) MPs that have been scanned for polymer identification. The types of polymers detected were categorised into three categories, actual plastics (55%) which consists of polyether urethane, polyethylene, polyester; natural fibre – cotton (30%); and the remaining polymer material containing low frequency e.g., morpholinoethyl isocyanide (5%). Meanwhile, 20% suspected MPs such as plant material, clay, and salts were verified as non-plastic and were categorised as other, based on the FTIR polymer type results as shown in figure 4.14. Polyether urethane was the most abundant material, found at sites (b) effluent; (c) point of discharge; (d) upstream and (e) downstream, which was mainly detected as fibre and is used as synthetic rubber and might originate from tire wear, similarly to an observation by Bayo *et al.*, (2019). Figure 4.15 shows the polyether urethane peaks for the sites (b) effluent; (c) point of discharge; (d) upstream and (e) downstream, peaks are noticed at 1092, 1093, 1108, 1111, 2851, 2852, 2894 cm^{-1}. Meanwhile polyethylene peaks at the (a) influent were 1000, 1468, 1378 and 2923 cm^{-1}. Sites (d) upstream and (f) sludge showed polyester peaks around 703, 980, 1091, 1325, 1720 and 3000.

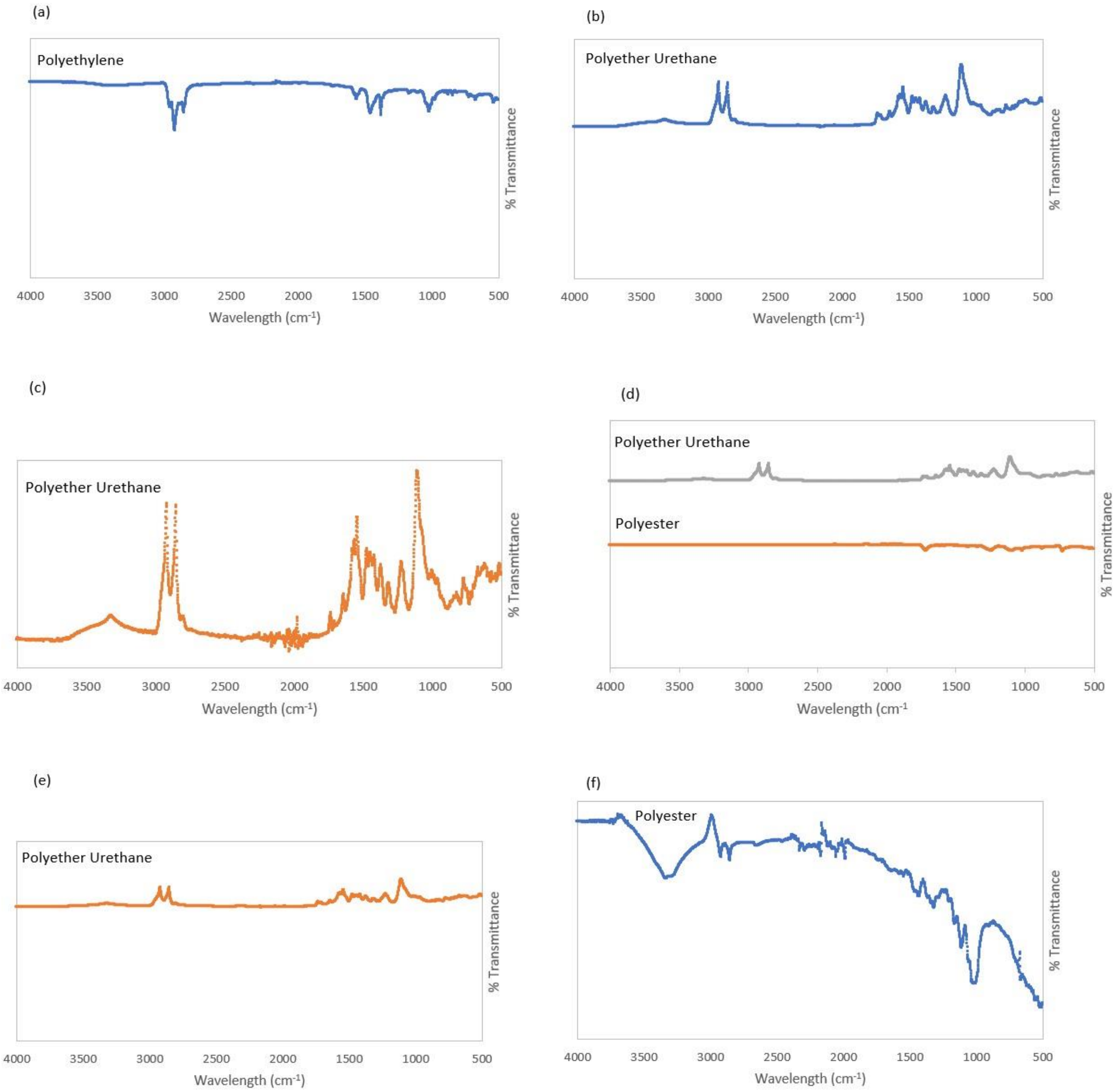

Figure 4.15: FTIR spectra of microplastics found in (a) Influent; (b) Effluent; (c) Point of discharge; (d) Upstream; (e) Downstream and (f) Sludge at the various sites in the WWTP and Veldwachters River

4.6 Ecotoxicology Bioassays

The quality of the WWTP effluent was evaluated using a battery of tests which includes *R. Subcapitata*, *D. magna*, and *T. Thermophila.* Each test was done for four seasons, summer (S1), autumn (S2), winter (S3) and spring (S4). Based on the sensitivity of the organisms used, the toxicity of the WWTP was classified according to the hazard classification system for wastewaters discharged into the aquatic environment by Persoone *et al.*, (2003) as shown in table 3.3 of chapter 3. Moreover, according to Persoone *et al.*, (2003) the toxicity response was recorded when the toxic unit (TU) of mortality (*D. magna*) or growth inhibition (*R. subcapitata and T. thermophila*) was equal to or higher than 0.4 TU.

4.4.1 *Raphidocelis subcapitata* Toxicity Test

Table 4.5: The number of cells and growth rate relative to the control of *R. subcapitata* exposed to WWTP effluent

	Exposure Time (h)	Number of Cells (cell/mL)			Growth Rate (G)			Growth Inhibition (%)		
		24h	48h	72h	24h	48h	72h	24h	48h	72h
S1	Control	20,9	101,8	284,3	6,8	4,2	3,2	0	0	0
	6,25	26,7	100,4	304,8	6,9	4,1	3,1	-0,3	3,6	2,3
	12,5	7,1	85,5	296,4	5,7	4,2	3,2	16,8	1,2	-1,2
	25	22,4	95,7	331,8	7,0	4,3	3,3	-2,3	-0,8	-3,1
	50	34,1	108,8	377,7	7,3	4,2	3,2	-6,7	0,2	-2,2
	100	36,2	126,9	423,2	6,6	4,0	3,0	3,2	6,7	4
S2	Control	58,9	70,3	67,9	7,3	3,8	2,5	0	0	0
	6,25	35,5	41,1	37,5	6,3	3,2	2,1	14,3	14,6	16
	12,5	38,5	35,9	36,8	6,5	3,2	2,1	11,8	15,1	14,7
	25	50,8	43,4	47,1	6,6	3,2	2,2	10,4	15,1	13,7
	50	34,2	30,3	25,6	6,2	3,0	1,8	15,4	19,2	27,5
	100	35,9	31,1	52,9	5,7	2,8	2,0	22,5	26,5	19,7
S3	Control	27,7	34,8	91,0	6,2	3,3	2,5	0	0	0
	6,25	16,7	27,4	117,9	6,0	3,2	2,7	3,6	0,4	-6,5
	12,5	21,5	33,3	110,7	5,8	3,1	2,5	6,5	4,1	0,5
	25	21,5	33,3	95,5	6,1	3,2	2,5	2,5	0	-1,1
	50	23,6	30,3	67,4	5,9	3,1	2,3	5,3	5,5	6,8
	100	27,2	32,6	46,7	5,8	3,0	2,1	6,9	8,1	15,2
S4	Control	9,2	17,7	80,9	4,6	2,6	2,3	0	0	0
	6,25	6,1	17,9	105,4	5,2	3,2	2,7	-13,3	-21,2	-20,2
	12,5	6,5	31,0	86,3	5,3	3,4	2,6	-13,7	-26,9	-16,6
	25	8,8	11,5	75,3	5,5	2,9	2,5	-18,6	-9	-12,4
	50	9,5	20,7	79,7	5,1	2,9	2,4	-10	-11,1	-6,1
	100	7,7	14,9	80,3	5,4	3,1	2,6	-17,6	-15,9	-15,2

Acute toxicity to *R. subcapitata* was assessed by measuring its growth rate and number of cells for a period of 72 h for four seasons, as shown in Table 4.5. There was an increase in the number of cells observed in summer (S1), winter (S3), and spring (S4) (6.25 – 100%) from 24-h to 72 h exposure. In autumn (S2), however, the increased number of cells were observed at a low concentration (6.25%) and at the control after 24 h to 72 h. Furthermore, from 12.5 – 100% the number of cells increased at 24 h, decreased at 48 h, and increased at 72 h exposure, respectively. The growth rate of *R. subcapitata* decreased from 24 h - 72 h exposure and fluctuated with each % dilution per season. The results further show that toxicity and growth inhibition was found to be highest in S2 sample (27.5%) at 50% dilution, and in S3 sample (15.2%) at 100% dilution. The growth inhibition of this study never reached 100% which was similar to an observation in some samples collected from four WWTPs by Szklarek *et al.,* (2021). In the S1 and S2 samples of the current study, at the 100% dilution, the growth of *R. subcapitata* decreased to 4% and -15% respectively, which is an indication of stimulation of bacteria as reported by Katsoyiannis and Samara (2007).

4.4.2 *Daphnia magna* Acute Toxicity Test

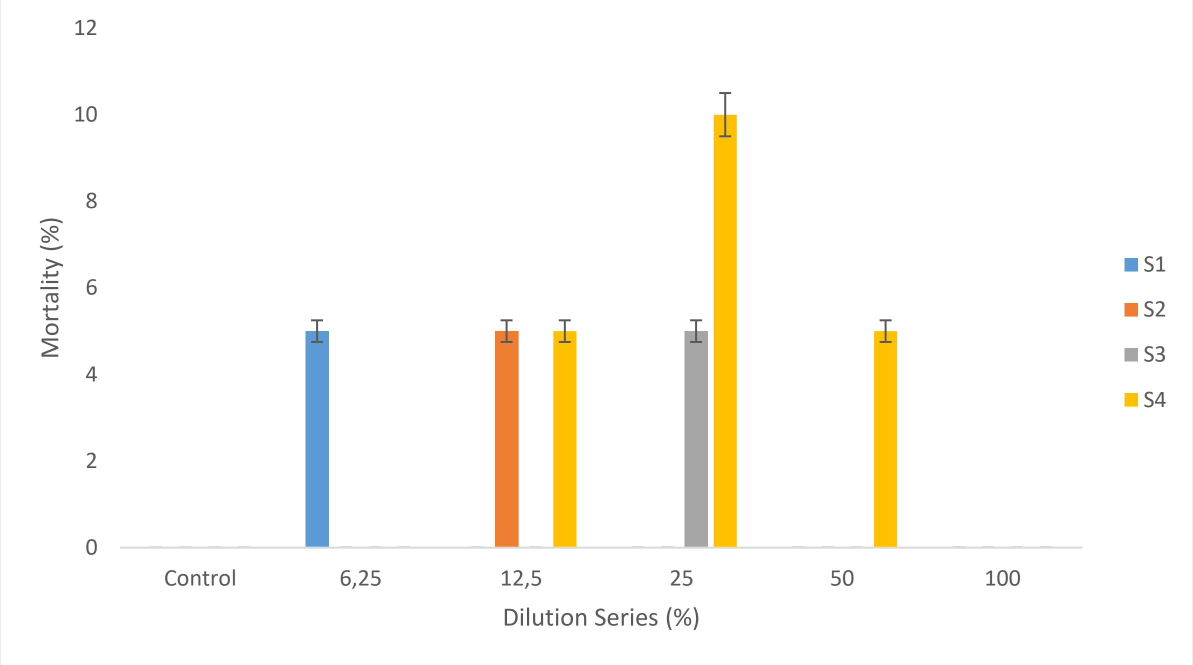

Figure 4.16: Percentage of immobile *D. magna* at the end of a 48 h exposure to the WWTP effluent period for all seasons.

As previously mentioned, *D. magna* is an excellent test model organism in aquatic toxicology and its toxicity was assessed by exposing it to the WWTP effluent over a 48 h period. The test was regarded valid when the percentage mortality or immobilization of the organisms in the controls were ≤ 10% at the end of the exposure time. The acute freshwater test results for *D. magna* exposed to different concentrations (6.25 – 100%) of effluent are shown in Figure 4.16. The total percentage mortality of *D. magna* was compared to the control in all seasons. No neonate mortality was observed in the control and the lowest concentrations of the effluent samples in all seasons except for the summer (S1) season. *D. magna* mortality rate increased in concentrations 12.5 - 50% with the spring (S4) season showing a high mortality rate between 5 – 10%. No mortality was recorded at 100% concentration of the effluent sample for all seasons which indicated that the effluent poses a risk to the aquatic organisms. This is because when effluents are discharged into waterbodies, they are diluted, and become more bioavailable to organisms. Hence it has been observed that when organisms are exposed to whole effluent (100%) they do not die, but when diluted the toxicants in water becomes more effective as seen between 12.5 - 50% dilutions.

4.4.3 Tetrahymena thermophila Toxicity Test

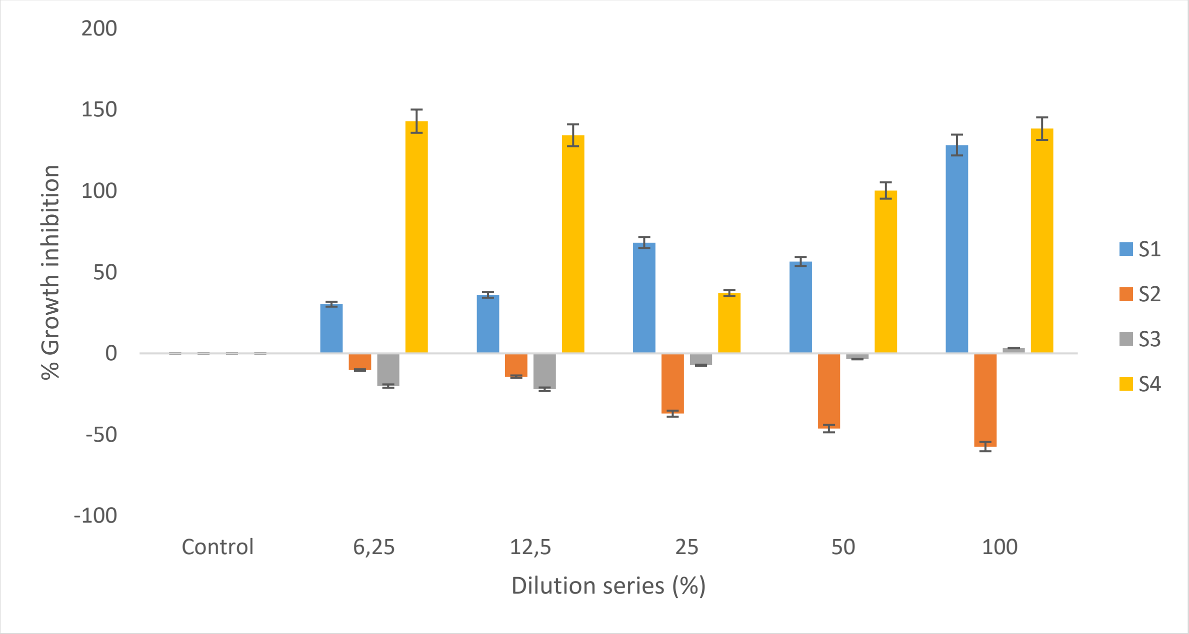

Figure 4.17: Percentage inhibition on the growth rate of *T. thermophila* after 24 h exposure to the WWTP effluent

The results of the acute freshwater tests for *T. thermophila*, is presented in Figure 4.17. The results for summer (S1) and spring (S4) showed significant growth (6.25 – 100%) dilution, where the growth rate increased as the WWTP effluent concentration increased except at the 25 – 50 % dilutions. Autumn (S2) and winter (S3) showed negative values and similarly to the algae results, this suggests stimulation of bacteria. The maximum percentage of growth inhibition for all each season (S1, S2, S3 and S4) was 128.3%; -10.3%; 3.3% and 143%, respectively. The WWTP effluent showed significant percentage inhibition in the different concentration treatments of *T. thermophila* (6.25 – 100%) when compared to the control.

Table 4.6: Probit analysis for WWTP effluent ecotoxicity tests (*R. subcapitata; D. magna and T. thermophila*).

Test Species	Season	Average EC_{10}	Average EC_{20}	Average EC_{50}
R. subcapitata	S1	104,7	110,4	122,2
	S2	0,2	15,6	n.d.
	S3	59,3	113,1	n.d.
	S4	66,6	160,2	n.d.
D. magna	S1	n.d.	n.d.	n.d.
	S2	n.d.	n.d.	n.d.
	S3	n.d.	n.d.	n.d.
	S4	n.d.	n.d.	n.d.
T. thermophila	S1	4,4	6,1	16
	S2	2,3	1,4	0,3
	S3	205,9	594,1	143,5
	S4	243,3	491,5	404,6

n.d.: not determined; EC ($_{10-50}$): Effective Concentration

Table 4.7: Toxicity classification of the WWTP effluent

	R. subcapitata		*D. magna*		*T. thermophila*					
Season	TU	Test Score	TU	Test score	TU	Test score	Class weight score	Hazard class	Toxicity	Effective percentage
S1	0.818	1	1	2	6,26	2	1,67	III	Acute toxicity	55,7
S2	1	2	1	2	384,6	4	2,67	V	Very high acute toxicity	89
S3	1	2	1	2	0,007	1	1,67	III	Acute toxicity	55,7
S4	1	2	1	2	2,473	2	2	III	Acute toxicity	66,7

The results for the probit analysis of the bioassays are shown in Table 4.6 and the toxicity classifications in Table 4.7. Persoone *et al.*, (2003) proposed that samples could be classified as non-toxic when $TU < 0.4$; slightly toxic when $0.4 < TU < 1$; toxic when $1 < TU < 10$, very toxic when $10 < TU < 100$ and extremely toxic when $TU > 100$. The EC_{50} values for *R. subcapitata* in S2, S3 and S4 could not be calculated, using the ToxRat Professional 3.2 software. However, the EC_{50} for S1 (122.2) was calculated and exhibited measurable EC_{50} values as shown in Table 4.6. Based on these EC values the TU values were determined by using the equation $TU = 100/EC_{50}$, from Ramírez-Morales *et al.,* (2020). Thus, for S1 the WWTP effluent is classified as class II, slightly acute toxicity, with $0.4 < TU < 1$. Similarly, to Katsoyiannis and Samara (2007), this study used the hazard classification method based on assumption. Assuming that samples for which EC values could not be calculated have $TU > 1$, therefore, TU is assumed to be 1, therefore the effluent in S2, S3 and S4 is classified as class III, acute toxicity with $1 < TU < 10$. This was different to the classifications made by Katsoyiannis and Samara (2007), where 7 out of 13 WWTP investigated were classified as slightly toxic. Moreover, their study also reported that different WWTP will show different results as toxicity depends greatly on the type of wastewater each plant receives. For *D. magna,* the 95% confidence limit was not detected in all seasons, and resulted in no EC_{10}, EC_{20} and EC_{50} being measured following the 48 h exposure. Therefore, if $TU > 1$ the effluent for all seasons is classified as class III, acute toxicity with $1 < TU < 10$. It is important to note that although EC_{50} was not determined in all seasons, these results do not rule out the possibility of chronic toxicity of effluent. Kocbus and Oral, (2015), investigated the toxicity of municipal wastewater treatment plant effluents, using *D. magna* as a test species. The results obtained were similar to this current study and showed TU values ranged from 3.0 – 4.2 suggesting that the WWTP is classified as Class III (acute toxicity). A chronic toxicity test with a longer exposure time, such as 21 days with the effluent, may also show different results. Therefore, chronic toxicity studies need to be evaluated to better understand the actual toxicity effects of the effluent on the aquatic environment. The respective EC_{50} values, using Persoone *et al.,* (2003), classification methods, of the S1 effluent exposed to *T. thermophila* is classified as Class III, acute toxicity, $1 < TU < 10$. The S2 effluent is classified as Class V, very high acute toxicity, $TU > 100$, while S3 and S4 effluent are both classified as Class I, no acute toxicity, $TU < 0.4$. Generally, the protozoan *T. thermophila* was the most sensitive, demonstrating a $TU > 100$, while other samples demonstrated $1\ TU < 10$. This could be attributed with the lack of cell walls that exposes *T. thermophila* to respond faster to environmental changes (Zahid *et al.,* 2014; Doerder, 2014). These results correspond with a study by Udebuani *et al.,* (2021), indicating that *T thermophila* proved to be more sensitive than other biotests used in their study.

The biotest results were further classified according to each season. The sampling for S1 is classified as acute toxicity (class III) as all three tests have a TU of 1 < TU < 10. The effective percentage is 55.7%, which shows that the WWTPs effluent contains toxic chemicals. In S2 the effluent is classified as very high acute toxicity (class V) due to its high effective percentage of 89% and the TU results of *T. thermophila* (384.6). Although other biotests (*D. magna* and *R. subcapitata*) showed TU of 1, this proves that the water poses toxic chemicals in this season. For S3 and S4, the effluent is classified as acute toxicity (class III) because all biotests had a TU of 1 < TU < 10. No other tests exhibited toxic effects and similarly to S1, these results prove that there are toxic chemicals in the water, although the physicochemical properties indicated no pollution for most of its parameters. The results proved the *T. thermophila* was more sensitive to the effluent as compared to other biotest, hence the high TU values. Similar observations were made in a study on effects of municipal wastewater treatment plant effluent quality on aquatic ecosystem organisms (Pereao *et al.,* 2021). These results further indicated that the WWTP effluent has a potential toxicological effect on aquatic organisms and that physicochemical properties alone cannot assess the quality of effluents and how they affect receiving water bodies.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study investigated the influence of a wastewater treatment plant discharge on the water quality of the Veldwachters River. The collected data, supported by literature revealed that the effluent contributes positively to the river recharge and health downstream, it has the potential to trigger eutrophication in the river system. Although physicochemical parameters were within the regulatory limits for effluent discharge, the bioassay experiments proved otherwise. This study reiterates the importance of using ecotoxicological bioassays as complementary tools to physicochemical characterisation. The MPs results showed that fibre was the most dominant type of MPs found and that MPs were not effectively removed by the WWTP technologies. The WWTP contributes to MPs burden in the receiving waterbody. However, high numbers of MPs were also found upstream and downstream the river suggesting that other sources of pollution contribute to the MPs burden of the river. The obtained results provide insights into the benefits and possible risks that must be mitigated by governments and WWTP authorities for reuse and optimal utilization of freshwater resources for sustainability.

5.2 Recommendations

The recommendations provided below suggest improvements that can be made by the government and WWTP authorities and in future developments of WWTPs in South Africa to ensure effective removal of pollutants in wastewaters. The recommendations are as follows:

- Consistent monitoring of effluent and river water physicochemical characteristics is required to ensure human health due to exposure and ecological health of receiving waterbodies.
- Ecotoxicological tests should be included in monitoring strategies of WWTPs as they have added value to effluent hazard characterization.
- There is a need for further research on ecological risks of substances in watercourses a well as different trophic level organisms’ responses WWTP effluents’ exposure.
- More effective removal technologies should be implemented in the future. There is also the need for additional research that will focus on the removal of MPs and other emerging contaminants from wastewater.

- For a long-term and sustainable strategy plastic waste management, it is imperative that organizations, governments, businesses, and society collaborate to find strategies to reduce plastic pollution.

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