IMPACT OF WASTEWATER EFFLUENT DISPOSAL ON SURFACE WATER QUALITY IN MAHIKENG, SOUTH AFRICA

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Dissertation accepted in fulfilment of the requirements for the degree *Master of Science in Environmental Sciences and Management* at the North West University

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Graduation ceremony: October 2018
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DECLARATION

I, **Mercy Akoth** (student number: 25805657), do hereby declare that this research is my own and that all the contents presented here are original, and that the same work has not been submitted for the award of a degree at this or any other University or institution of higher learning. Information sources and the work of other authors cited in this research have been duly acknowledged.

Signed.................................. Date 19/10/2018...............................

Supervisor: Prof L G Palamuleni

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ABSTRACT

The deterioration of surface water quality is of great global concern since water is a crucial resource for all aspects of life. In South Africa, water scarcity continues to plunge the country and this has led to the damming of major rivers in order to cater to the acute demand for water. The South African constitution stipulates that wastewater effluent from wastewater treatment plants (WWTP) could be discharged into surface water, as one of the alternatives to combat issues of water scarcity in the country. Such is the case in Mahikeng, the capital of the North West Province, South Africa. The town's main wastewater treatment works (Mmabatho WWTW) receives both domestic and commercial wastewater from Mahikeng, treats it using secondary wastewater treatment processes, and discharges its treated effluent into surface water (Setumo dam).

Setumo dam serves as the town's main source of water, which is abstracted by the Mmabatho water treatment works (WTW), purified and supplied to the urban and peri-urban areas of Mahikeng. The communities surrounding the dam also utilise the raw water from the dam for domestic purposes. It is on this account that this study assessed the impact of wastewater effluent discharges onto the quality of water in Setumo dam. Two hypothesis were formulated in order to achieve the overall aim of the study, where the first hypothesis (H0) stated that the wastewater effluent discharged by Mmabatho WWTW has no significant impact on Setumo dam water quality whereas the second hypothesis (H1) stated that the wastewater effluent discharged by Mmabatho WWTW has a significant impact on Setumo dam water quality.

Wastewater effluent and dam water samples from Mmabatho WWTW effluent discharge pipe and Setumo dam respectively were collected during the wet and dry seasons. The collected samples were then analysed for physicochemical (temperature, pH, EC, TDS, nitrates and phosphates, and heavy metals – arsenic, cadmium, copper, iron, lead, manganese, nickel, and zinc) and bacteriological parameters (heterotrophic bacteria, total and faecal coliforms). Results from the wastewater effluent analysis were compared with the DWA (2013) wastewater effluent quality standards while results from the dam water analysis were compared with the SANS:241 (2015) and WHO (2011) drinking water quality standards. Polymerase chain reaction (PCR) analysis was used to detect the presence of *Escherichia coli* (*E. coli*) and *Klebsiella*. One-way ANOVA was used to examine the statistical seasonal and spatial differences in the analysed dam water parameters. The analysis of the health risks associated with the consumption of water from Setumo dam was done using the risk quotient equation and the water quality index (WQI) was computed to determine the overall quality of the dam water. Pearson correlation coefficient was used to determine the association between the pollutants in the wastewater effluent and the dam water.

During the wet season, pH, EC, nitrates, phosphates, arsenic, copper, and lead were found to be above the DWA (2013) wastewater effluent quality standards while phosphates, lead, and zinc were above the permissible wastewater effluent limits during the dry season. In the dam water, all the physicochemical parameters were within the SANS:241 (2015) and WHO (2011) drinking water quality standards during the dry season except for nitrates, arsenic, lead during the wet season. The bacterial counts were significantly higher in both the wastewater effluent and the dam water during both sampling seasons except for heterotrophic bacteria in the dam water. As expected, the results from the PCR analysis confirmed the presence of *E. coli* in both the wastewater effluent and the dam water during both seasons. No *Klebsiella* was detected in the wastewater effluent and dam water during both sampling seasons. The detection of *E. coli* indicates that inadequately treated wastewater effluents may have the potential impact of disseminating pathogenic bacteria to the surface water intended for both human and animal use and this could, in turn, result in an outbreak of water-borne diseases.

The one-way ANOVA results suggest that there exists a statistically significant seasonal variation in the dam water quality ($0 \geq p \leq 0.04$) in all analysed parameters except for the EC, TDS, and phosphates, whereas EC, TDS, and total coliform bacteria yielded significant spatial variations ($0 \geq p \leq 0.09$). The risk assessment analysis revealed that nitrates, arsenic, and lead presented significant health risks to Setumo dam water consumers during the wet season (RQ > 1) and the faecal coliform bacteria during both seasons. Water quality index results revealed that the dam water quality varied between the categories "bad" in the wet season to "medium" in the dry season which would be expected given the changes in season. The Pearson correlation coefficient demonstrated strong significant correlations ($r = 0.05$) between the pollutants in the wastewater effluent and in the dam, and across the dam sampling points. The study, therefore, recommended that there should be a continuous assessment of the wastewater effluent from the Mmabatho WWTW in order to establish whether it conforms to the DWA wastewater effluent quality standards, so as to protect the quality of the surface water resource that serves as a disposal basin and in turn, mitigates the health issues arising from poor surface water quality.

ACKNOWLEDGEMENT

I would like to express my gratitude to the following people for their valued contribution to this research:

My supervisor Prof L.G. Palamuleni for her commitment and guidance during the entire course of this study. Under her supervision, I have been groomed into an independent researcher and I am therefore highly indebted to her. I also appreciate her financial contribution especially during my final year of study.

I wish to acknowledge the financial assistance that I received from the Dean of the Faculty of Natural Sciences and Agricultural Science, Prof H. Drummond who awarded me some funds towards the payment of my final year tuition fees and Prof T.A. Kabanda who also arranged for access to some of his research funds.

I would like to thank Prof C.N. Ateba for granting me full access to his molecular microbiology laboratory where I performed all the microbial analysis. Prof and the colleagues in his laboratory patiently guided me throughout the entire microbial analysis and hence I acquired a lot of knowledge in conducting microbiology tests.

I would also like to acknowledge Prof T.M. Ruhiiga for assistance in purchasing the required consumables for the sample analysis, Prof E. Mukwevho, for granting me access to the lab equipment's and Mr Sizwe Loyilani for the kind assistance he offered me at the various stages of my research work.

I acknowledge all my friends in the Departments of Geography and Environmental Sciences and Biological Sciences for their support in one way or another. I also express my gratitude to Mr S.K. Bett and Mr S.C. Nde for accompanying me during the field trips and assisting me with sample collection. I wish to specifically thank them for their unwavering support and encouragement that helped me to complete this research project. They were my pillar of strength.

To the strong women in my family, particularly my mom Mrs M.N. Katiti and Mrs N. Kibet, your profound love and support saw me through this journey. Above all, I thank the Almighty God Jehovah for giving me the strength to go on through His holy spirit that cultivated the needed fruitages in me.

DEDICATION

I dedicate this research to my family.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Water is a crucial resource for all aspects of life with the major global users being agriculture, industrial and domestic sectors. According to UN-Water (2014), the agricultural sector accounts for 70% of global water withdrawals whereas the industrial and domestic sectors account for the remaining 20% and 10% respectively - although these figures vary considerably across countries. Although water is an essential resource for facilitating these various sectors, the world today is faced with water scarcity issues. Compounding the situation further is the deterioration of its quality as a result of water pollution, which is noted to be one of the threatening environmental problems and is of global concern (Afroz *et al.*, 2014). Causes of water pollution globally have been attributed to rapid population growth, urbanisation, increasing food production, and the unregulated and illegal discharge of contaminated water, and hence putting pressure on water resources (Corcoran *et al.*, 2010).

Globally, an estimated 2 million tons of sewage, industrial and agricultural wastewater is discharged into rivers, lakes and oceans, leading to the death of at least 1.8 million children under the age of 5 years from water-related diseases (Aahman *et al.*, 2008). Surface water has the potential to assimilate certain levels of pollution; however, when the wastewater discharged into surface water is of a higher concentration, it degrades the quality of the surface water, thus rendering it unsafe for human use. Wastewater from agriculture, industrial and domestic sources contains organic chemicals, heavy metals and microbial pathogens, which can be toxic to human health, aquatic ecosystems and further degrade the environment (Drechsel *et al.*, 2010). Therefore, the focus of this study was to explore the impact of wastewater effluent disposal on surface water quality in Mahikeng, South Africa.

South Africa, like many other countries globally, is faced with the problem of scarcity of freshwater resources. In order to manage its scarce water resources, surface water resources have been well developed and utilised to supply water to the majority of the urban, industrial and irrigation sectors (CSIR, 2010). However, scarce water resources have been compounded by deterioration of their quality due to water pollution. Oberholster *et al.* (2008) attributed the pollution of water to domestic and industrial wastewater effluents that are continuously being discharged into the country's limited surface water sources.

In Mahikeng, which is the capital city of the North West Province - South Africa, water scarcity issues are prevalent given the location of the town. The town is located in the semi-arid region of the country. In order to manage the water scarcity, the town extensively depends on both surface and groundwater resources for domestic, agricultural and industrial uses (Van Vuuren, 2013). Setumo dam, previously known as Modimola Dam, is the main source of surface water whereas groundwater is extracted from the Molopo and Grootfontein well-fields (Mulamattathil *et al.*, 2014). The Mmabatho Water Treatment Works (WTW) abstracts water from Setumo dam, purifies it and supplies to the Mahikeng and Mmabatho urban and peri-urban areas. However, upstream of this dam is the Mmabatho WWTW, which directly discharges its treated wastewater effluent into the dam.

1.2 Problem statement

Mmabatho WWTW receives domestic wastewater, commercial wastewater, storm water and runoff water which undergoes secondary wastewater treatment, after which it is discharged into Setumo dam. Although the wastewater undergoes various secondary wastewater treatment processes, DWA (2010) reports that the Mmabatho WWTW discharges effluents that are of poor quality into Setumo dam. Pathogens such as *Aeromonas* and *Pseudomonas spp* have been isolated from the dam water and these have been attributed to the discharge of insufficiently treated effluents by the Mmabatho WWTW (Mulamattathil *et al.*, 2014). Apart from pathogenic microbes, wastewater effluents could contain pollutants such as heavy metals, hydrocarbons, organic matter and eutrophic nutrients, which can disrupt the eco-balance of the aquatic life and affect the human health (Drechsel *et al.*, 2010; Davies, 2005). Although surface water bodies undergo natural self-purification, the continuous discharge of wastewater effluents of poor quality increases the number of pollutants in receiving water and hence could hinder the dam's natural treatment processes, thereby leading to the deterioration in its water quality. Due the fact that the communities around Setumo dam directly utilise the water for drinking, washing, fishing and livestock watering (Dikobe *et al.*, 2011), and the nature of wastewater effluents, a continuous assessment of the quality of the wastewater effluent is therefore essential in protecting the quality of the dam water that serves as potable water for the surrounding communities.

1.3 Justification

Significant quantities of wastewater are generated from surface run-off, household wastes and industrial discharges, agricultural and mining activities among others. This has, therefore, prompted an establishment of the WWTW to treat wastewater from the different sources. Given their different origins, the wastewater is often loaded with pollutants, which could be harmful to human and animal health and contributing heavily to the degradation of water resources (Abdelkader *et al.*, 2012). A general assumption is that pollutants in wastewater are eliminated through wastewater treatment. However, not all polluting agents are removed through the standard wastewater treatments (Teijon *et al.*, 2010). Studies have shown that the consumption of water polluted by wastewater effluents puts the health of its consumers at risk (Olaolu *et al.*, 2014; Mohod & Dhote, 2013).

In Mahikeng, the Mmabatho WWTW was established to receive and treat wastewater generated from the city and to discharge the treated effluent into Setumo dam as one of the alternative solutions to combat water scarcity. Although the re-use of wastewater effluent is an important component in addressing water scarcity, this could not only affect the quality of drinking water if proper treatment procedures are not implemented but also the health of the direct consumers of the dam water. Identifying the pollutants that remain in treated wastewater effluents could help provide insights into the most suitable wastewater treatment process and this, in turn, will improve the overall quality of water disposed in the dam. In addition, this could protect the health of the communities utilising water from this dam.

1.4 Aim and objectives

The aim of this study was to determine the impacts of wastewater effluent disposal on Setumo dam and the risks associated with the consumption of water from this dam. The following specific objectives were followed in order to accomplish the aim of this study;

1. To examine the levels of heavy metal concentration.
2. To analyse the presence of nutrients and microbes.
3. To establish the seasonal and spatial variations of the pollutants in Setumo dam.
4. To assess the risks associated with the water in Setumo dam.

1.5 Hypotheses

In line with the above-mentioned objectives, the following hypotheses were put forward;

H_0 – The wastewater effluent discharged by Mmabatho WWTW has no significant impact on Setumo dam water quality.

H_1 - The wastewater effluent discharged by Mmabatho WWTW has a significant impact on Setumo dam water quality.

1.6 Description of the study area

The study took place in Setumo dam, previously known as Modimola Dam in Mahikeng, North West Province, South Africa (Figure. 1.1).

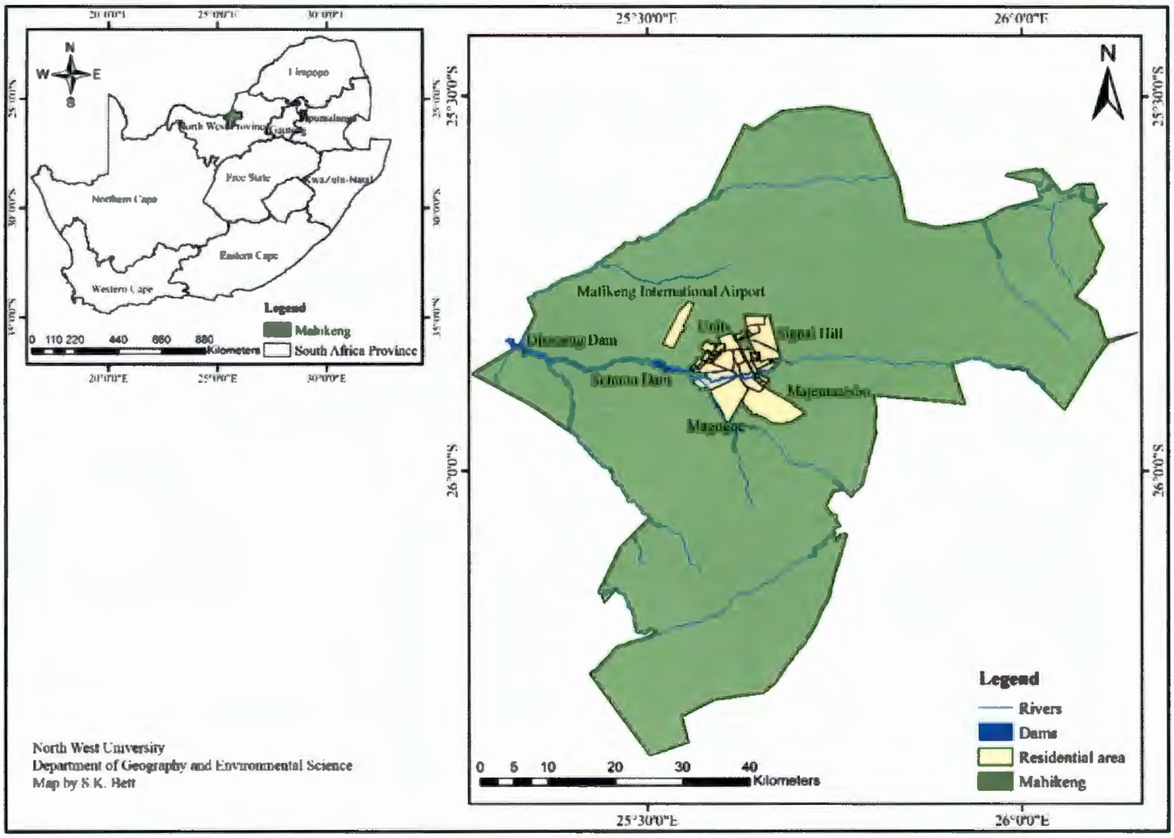

Figure 1.1: Map of the study area within South Africa

1.6.1 Location

Mahikeng (previously known as Mafikeng) is the administrative capital of the North West Province. The town is located on coordinates 25°16′20″E and 25°40′47″S, 20 km south of the Botswana border (Figure 1.1). The town is part of the Ngaka Modiri Molema District. Setumo dam, is located along coordinates 25°30′1″E and 25°51′30″S (Figure 1.1); situated in Unit 14, a residential area in a suburb called Mmabatho.

1.6.2 Population

Mahikeng has a population of 291,527 people with 82% of the population living in urban areas while 13% are scattered around the rural areas (StatSa, 2012). Population growth brings about an increase in urbanisation and industrialisation and this, in turn, exerts pressure onto natural resources such as land and water to facilitate the human activities. Increased human activities as a result of an increase in population could generate large quantities of wastewater, which if not properly managed, leads to the pollution of surface water sources (Juma *et al.*, 2014).

1.6.3 Climate

Mahikeng has a typical semi-arid climate with low rainfall intensity (Maraka, 1987). The climate varies from very hot during summer temperatures of 40°C to as low as 4°C during winter. The area receives an annual rainfall ranging from 200 mm to 600 mm, which occurs as a heavy thunderstorm or light rain. Rainfall occurs mainly during the summer season (October to March) and the winter months accounting for 5% of the annual total rainfall (Tessema, *et al.*, 2012). Hence the summer season is regarded as the wet season whereas the winter season is the dry season. Dust storms are also prevalent in Mahikeng and the surrounding areas. These seasonal changes in rainfall and temperature greatly influence the quantity of water in Setumo dam and other surface water sources in Mahikeng. For instance, the heavy rainfall received during 2016/2017 resulted in a high water storage in Setumo dam, however, such variations in climate could affect the quality of its surface water sources (Whitehead *et al.*, 2009).

1.6.4 Water

Mahikeng relies on both surface and groundwater sources although the majority of the people in the peri-urban areas rely on groundwater (Van Vurren, 2013). The surface water is sourced from dams, springs and streams whereas the groundwater is abstracted through boreholes. Due to the semi-arid nature of the area, an acute demand for water has resulted in the damming of the Molopo river (a major river in Mahikeng) at several places, resulting in four large reservoirs namely; Cooke's Lake, Setumo dam, Lotlamoreng dam and Disaneng dam (Munyati, 2015). Of these dams, Setumo dam serves as the main source of water in Mahikeng. Although damming of rivers may help to cater for the varying human water needs, it alters the environmental conditions of river ecosystems and hence affecting the self-purification process of the dammed river (Wei *et al.*, 2009). Due to the long residence time, dams lead to the accumulation of heavy metals and alter the dynamics of oxygen transfer mechanism in the river water, hence affecting its quality (Sharma, 2015).

1.6.5 Soils

Mahikeng is underlain by the Kalahari sand that consists predominantly of Aeolian deposits. Underneath the Aeolian sand, are limestones that have progressed into Hardpan calcrete. The Aeolian sand and limestones are further underlained by rhyolite, lavas, and schists (Geotechnical Investigation Report, 2012). Differential weathering of these rocks leads to the formation of soil within the area. Materechera (2011) characterized the resultant soil profiles in Mahikeng to be well-drained for extensive periods. The surface soil (0 to 20 cm) contains red loam soil with 56% sand, 33% silt and 11% (Materechera, 2011). During rainfall, these soils are subsequently washed away as run-off, and which may be deposited into the WWTP, and/or surface water bodies hence leading to water quality deterioration (Issaka & Ashraf, 2017; Huang *et al.*, 2013).

1.7 Research ethics

In order to uphold the various codes of ethics such as honesty, integrity, respect, and confidentiality that govern research, application for ethical clearance was made to the North-West University ethical clearance committee. This was a requirement in the fulfillment of a Master's degree with the North-West University. Permission to collect both water and wastewater effluent samples was granted and the findings from the study were intended for research purposes only.

1.8 Chapter summary

Surface water is of paramount importance in facilitating various human activities, however, anthropogenic factors continue to greatly affect its quality. Increased population growth has led to an increased generation of wastewater, which is subsequently discharged into the scarce water sources. This, therefore, means that there is a need to continually monitor the quality of wastewater in order to protect surface water sources that serve as effluent disposal basins from pollution. It is on this basis that this study aimed to identify some of the pollutants present in the wastewater effluent discharged by the Mmabatho WWTW and the impacts they have on the quality of water in Setumo dam.

1.9 Outline of the dissertation chapters

This dissertation is divided into five chapters. Chapter One has outlined a general overview of the research project, study objectives and the description of the study area. The following chapter reviews the pollutants in wastewater effluent while highlighting studies that have demonstrated the impacts of wastewater effluent discharge on the surface water quality. Chapter Three gives a detailed description of the sampling sites, laboratory procedures undertaken to achieve the main aim of this study. Chapter Four presents the results obtained from the analysis while comparing and discussing the results based on other literature findings. The conclusions and recommendations from this study are discussed in Chapter Five.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

In this chapter, reviews related to the literature on pollution of water resources that appears in international and local studies are examined critically. The literature review assists in identifying the gaps that could be filled with this study and positions this research within the context of the impacts of wastewater disposal. It has been predicted that by the year 2025, at least 1.8 million people will be living in areas of water scarcity due to heavy pressure imposed on the existing water resources (UN-Water, 2012). Rapid population growth coupled with economic development has resulted in the increased allocation of water for domestic, agriculture and industrial sectors, hence intensifying pressure on the resource. The rising demand for water to cater for the various uses has, in turn, resulted in increased worldwide pollution of surface water with wastewater and is of a serious concern.

2.2 Surface water resources

Water is essential for all socio-economic development and for maintaining a healthy eco-system. As the human population increases, there is an increased demand for water for domestic, agricultural and industrial sectors, hence putting pressure on the existing surface water resources. The ever-rising demand for water by the various sectors, as well as the worldwide growing water pollution has led to the scarcity of the available water resources. Although access to clean and safe water is a fundamental human right, studies have shown that people living in the near East and North Africa suffer from acute water scarcity, as do people in countries such as Mexico, Pakistan, South Africa, and large parts of India and China (Mekonnen and Hoekstra, 2016; UN-Water, 2007).

According to UN-Water (2007), water scarcity is defined as the point at which the aggregate impact of all users impinges on the supply or quality of water under prevailing institutional arrangements to the extent that the demand by all sectors, including the environment, cannot be satisfied fully. It can simply be said that water scarcity is a relative concept that is dependent on supply and demand. For example, large water consumption relative to water availability could result in decreased river flows, mostly during the dry period. Water scarcity stems from water shortage and this is mostly visible in arid and semi-arid regions which are often affected by droughts and wide climatic variations. One such region is South Africa, which is a semi-arid region facing water scarcity issues. South Africa faced severe and prolonged drought from 2014 to 2016, with 2015 registering a total annual rainfall of 403 mm, making it the lowest annual total rainfall ever recorded since 1904 (Donnenfeld *et al.*, 2018). As a result, the level of its surface water resources such as rivers and dams greatly reduced.

Aside from climatic variations, other factors affecting water scarcity in different countries include population growth and economic development. As the population increases, there is an increased demand for water to sustain life, for sanitation and industries, agriculture, and to generate energy among others. In South Africa, for instance, more than 60% of the country's surface water resources are currently being overexploited by the agricultural, industrial and municipal sectors, leaving only one-third of the country's surface water resources in good condition (Donnenfeld *et al.*, 2018). It is reported that about 3.5 million people in the country do not have the access to safe drinking water, especially those in rural communities (Heleba, 2012). The low and unpredictable supply, coupled with high demand, in turn, make South Africa a water-constrained country.

The concept of water scarcity may also refer to not only the difficulty in obtaining fresh water sources due to increased demand and climate change but also the deterioration of water resources as a result of increased pollution. According to WWAP (2015), pollution as one of the largest causes of water scarcity across the world. Pollution can be through oils, chemicals, industrial or human wastes that are either dumped into surface water sources without proper treatment, or that seeps through underground aquifers hence polluting the water (Owa, 2014). This, in turn, affects the surface water quality thereby rendering the water unfit for human consumption. The relationship between water quality and quantity has been recognised (Zeng *et al.*, 2013), addressing the importance of managing water quantity so as to ensure a good water quality status. The consumption of water from the existing scarce water resources that are of poor quality has led to the death of millions of people from water-borne sicknesses like cholera, typhoid fever and diarrheal diseases especially in Africa, the Middle East and large parts of Asia (WHO, 2011).

It can, therefore, be noted that the worldwide water crisis facing many countries nowadays is no longer how to acquire water, but rather, how to manage the available water resources. In an attempt to combat the problem of water scarcity in South Africa, for instance, the South African Water Act of 1956 (Act 54), encourages the discharge of wastewater effluent into surface water

(Eddy, 2003). Although this may be viewed as an alternative solution to the issues of water scarcity in the country, questions arise on the quality of the wastewater effluent being discharged into the surface water and the impact with which such an activity has on the overall water quality of the receiving surface water bodies. The following section discusses how wastewater has been considered as a viable water resource to combat water scarcity.

2.3 Wastewater as a water resource

Wastewater is a viable water resource, which when effectively managed, is crucial for future water security. The re-use of wastewater for many purposes such as agricultural and landscape irrigation, industrial processes, toilet flushing among others, is receiving increasing attention globally due to the rising demand for water (Garcia & Pargament, 2015). Reclamation and re-use of treated wastewater have been implemented as an important strategy to alleviate water shortage especially in arid and semi-arid regions (Al-Shammari & Shahalam, 2005; Du Pisani, 2005). For example, Australia as one of the driest continents on earth adopted wastewater recycling as a strategy to supply water to power stations, for industrial applications and the remaining directed to the main drinking water supply storage (Apostolidis *et al.*, 2011). This constitutes a significant component of integrated water resources management, which has been a big challenge for a long time. In addition to planned water recycling and reclamation programs, un-intentional indirect potable re-uses of wastewater have been recognised (Rodriguez *et al.*, 2009).

The indirect potable reuse of wastewater has been largely developed due to the advancement in wastewater treatment technologies. The wastewater undergoes various treatment processes before it is discharged into a surface water body, which serves as an environmental buffer. The use of surface water sources (rivers, dams, lakes) as environmental buffers has long been recognised as the world's best practice given that natural systems have a high capacity to further purify water (NRC, 1998). Surface water helps to not only dilute the recycled water, but also the retention time of the recycled water in the surface water allows for the degradation of any remaining contaminants through physical and biological processes thereby minimizing any potential risks (Rodriguez *et al.*, 2009). The following subsections discuss some of the wastewater treatment methods that have been adopted over time before the wastewater from WWTW is discharged into the environment or reused.

2.3.1 Wastewater treatment methods

Wastewater treatment plants (WWTPs) have been designed to treat wastewater from different sectors in order to minimize the environmental impacts related to the discharge of untreated/inadequately treated wastewater into surface water bodies. Wastewater undergoes various levels of treatment with the principal aim of removing the pollutants in the wastewater. For this to be achieved, municipal WWTPs have been established to collect and treat wastewater before it is returned to the receiving water bodies, or re-used. According to Metcalf & Eddy *et al.* (2003), the stages utilised by municipal wastewater treatment involve; preliminary treatment (removal of debris), primary treatment (removal of a portion of suspended solids and organic matter from wastewater), secondary treatment (removal of biodegradable and suspended solids) and tertiary treatment (removal of residual suspended solids and disinfection, which includes nutrient removal).

A variety of wastewater treatment methods have been adopted by different countries and their different treatment options have different impacts on the environment. Few of these treatments, including secondary treatment, waste stabilization ponds, and artificial or constructed wetlands, are discussed in the following sub-sections.

2.3.1.1. Secondary treatment of wastewater

Secondary treatment processes are a type of treatment method that has been adopted to remove pollutants (biodegradable and suspended solids) in wastewater with the assumption that the effluent at that stage is sufficiently safe to be released into the environment, however, studies have proved otherwise. For instance, a study conducted by Oberholster *et al.* (2008) reported a deteriorating quality of Lake Rietvlei in South Africa as a result of high nutrient loads emanating from effluent discharged by the Kempton Park and Hartbeesfontein secondary wastewater treatment facilities. Although these two WWTPs use secondary treatment to treat their wastewater, the study revealed that secondary treatment of wastewater is not sufficient enough in eliminating pollutants such as nitrogen and phosphorus in wastewater, which cause eutrophication in the water bodies receiving these effluents. Therefore, advanced levels of wastewater treatment need to be employed in order to achieve effluents that are of good quality and less harmful to the receiving waters (Sonune & Ghate, 2004).

2.3.1.2. Waste stabilization ponds

The use of waste stabilization ponds is another method of wastewater treatment. According to Phuntsho *et al.* (2009), these ponds operate through small interconnected ponds, for example, anaerobic ponds, which encourage the growth of algae used to break down organic matter, followed by facultative ponds, which are intended to refine the organic matter treatment and finally, maturation ponds intended to remove microbial pathogens and nutrients. An example of the effectiveness of this treatment method is operational in Ghana, where a study revealed an efficient removal of 83.3%, 97.3% and 99.94% of nitrates, biological oxygen demand (BOD) and faecal coliform bacteria respectively (Bansah & Suglo, 2016). Despite the relative efficiency of this treatment process, Seanego & Moyo (2013) are of the opinion that proper maintenance of these ponds is essential so as to achieve wastewater effluents that are of acceptable quality.

2.3.1.3. Artificial or constructed wetlands

The use of artificial or constructed wetlands is another land-based method for treatment of wastewater. Constructed wetlands consist of shallow ponds with plants floating on its surface, which makes them distinctive from stabilization ponds (Kadlec *et al.*, 2017). These plants utilise nutrients in wastewater, hence suppressing the growth of algae and allowing microbial degradation, thereby increasing the decomposition process of organic matter (Vymazal, 2010). The ability of these wetlands to remove chemical and biological pollutants through a complex variety of physical, chemical and biological processes can be seen in the Czech Republic, where this wastewater treatment method has been adopted since 1989 when the first full-scale constructed wetland was built (Vymazal, 2002; Toze, 1997). These wetlands are known to be cheap, easy to maintain and operate, and have since been recognized as the ideal treatment solution for the different types of wastewater, especially in small rural communities in developing countries (Zhang *et al.*, 2014; Kivaisi, 2001). Waste stabilization ponds and constructed wetlands have been considered the most effective wastewater treatment processes in achieving effluent that is of acceptable standards especially in developing countries.

However, the selection of the most appropriate wastewater treatment process ultimately depends on the quality of the influent, the removal of parent contaminants, treatment flexibility and the potential use of the treated effluent among other factors (Oller *et al*, 2011). Sato (2013) on the other hand attributes wastewater treatment capacity to the income levels of a country that is 70% in high-income countries and 8% in low-income countries. Although it is widely known that wastewater is treated before being discharged or re-used, the UN-Water report (2012) states that only 20% of the wastewater produced worldwide receives proper treatment. This hence raises questions regarding the quality of the surface water which serves as disposal basins. The present study aimed to examine some of the potential pollutants present in wastewater and the potential impacts they could have on the receiving surface water quality. The following section highlights the wastewater effluent pollutants as per the objectives of this study.

2.4 Pollutants in wastewater effluents and their impacts

Wastewater effluent can be defined as the final product from a variety of treatment processes employed by WWTPs. WWTPs treat municipal and industrial wastewater with a primary aim of protecting water bodies from pollution by this harmful waste (Paxéus, 1996). Different national and international environmental agencies, for example, Department of Water Affairs – South Africa (DWA-SA) and World Health Organisation (WHO), have established guidelines for the acceptable concentrations of pollutants in wastewater effluent in order to ensure that the discharged wastewater effluent is of a quality that does not present harm to the environment. However, considering factors such as the costs incurred in the treatment processes, the complexity of the treatment processes and a large number of parameters that have to be tested, wastewater may be insufficiently treated.

Insufficiently treated wastewater effluents could contain pollutants such as heavy metals, microbes, nutrients and organic matter that are detrimental to both humans and the environment (Akpor *et al.*, 2014). Discharging effluents of this nature pollutes the receiving water bodies and leads to the spread of waterborne diseases (Davies, 2005). The following subsections discuss some of the pollutants present in inadequately treated wastewater effluent with respect to this study and identifies the problems arising from them both nationally and internationally.

2.4.1 Heavy metals

Various debates exist as to the best definition to describe heavy metals and as such, different authors describe heavy metals in terms of their density. Järup (2003) describes heavy metals as metals with a density of more than 5 g/cm^3. Examples of heavy metals are cadmium, mercury, lead, arsenic, manganese, chromium, cobalt, nickel, copper, zinc and iron among others. Particular interest has been on heavy metals due to their toxic nature at either low or high concentration levels, depending on the type of metal (Akpor *et al.*, 2014). Exposure of humans to heavy metals can be through the air, intake of food and/or drinking water.

Although heavy metals are naturally occurring elements that are found throughout the earth's crust, they are considered to be among the most common environmental pollutants, whose occurrence in water and biota, indicate the presence of natural and anthropogenic sources (El-Bouraie *et al.*, 2010). Examples of natural sources of heavy metals in the environment are chemical weathering of minerals and soil leaching, whereas the anthropogenic sources are wastewater effluents, urban storms, and water run-offs among others (El-Bouraie *et al.*, 2010). Heavy metals are emitted as both elements and inorganic or organic compounds with the latter reported to not only be highly toxic but also greatly affecting water quality when they get into contact with the water sources (Duruibe *et al.*, 2007).

Factors such as precipitation and anthropogenic activities play a vital role in determining the concentration of heavy metals in surface water. Studies show that during precipitation, the concentration of these metals decreases due to the diluting effect, which results from the mixture of non-contaminated run-off water with these metals, whereas high heavy metal concentration owes to high evaporation rates and anthropogenic activities (Li & Zhang, 2010; Olías *et al.*, 2004). Different anthropogenic activities such as mining, industrial production, domestic and agricultural activities, generate wastewater that contains high concentrations of heavy metals and is discharged into the environment - a common practice especially in developing countries (Gupta, 2008). The practice of discharging wastewater that is highly polluted with heavy metals is a cause of concern given the nature of these metals. Heavy metals are known to bioaccumulate in the environment over a long period of time due to their non-bio-degradable nature and are highly toxic even at low concentrations, hence resulting in environmental pollution (Ng, 2006).

In aquatic ecosystems, heavy metals are reported to hinder the proper growth of aquatic organisms. In water, heavy metals form complexes with surface water components such as carbonates and sulphates. This increases the pH of water during acid rains, hence causing aquatic organisms like fish to lose their body weight and size, and eventually the extinction of fish population (Khayatzadeh & Abbasi, 2010). Several kinds of research have been carried out on the concentration of heavy metals in fish and the results have shown that the consumption of fish from water surfaces polluted with heavy metals places the consumers at great health risks (Velusamy *et al.*, 2014; Elnabris *et al.*, 2013; Alturiqi & Albedair, 2012).

According to FAO (2012), there has been a great increase in the production of fish worldwide with a reported 8.8% average annual rate in the past three decades (1980–2010). However, countries like Uganda, Tanzania, Nigeria and Egypt, which are the leading countries in fish production in Africa, harvest fish mainly from surface waters, which unfortunately serve as wastewater effluent disposal sites (El-Moselhy *et al.*, 2014; Akan *et al.*, 2012; Naigaga *et al.*, 2011; Machiwa, 2010). Therefore, it is imperative that these toxic heavy metals are removed from the wastewater because the distribution and abundance aspects of fish species are strongly influenced by the quality of water (Naigaga *et al.*, 2011). In turn, this will protect the consumers of fish and the environment.

In plants, heavy metals such as copper, iron, manganese, zinc and nickel are rendered as trace elements or micronutrients due to their important functions in plant cells. These micronutrients become toxic when their concentrations in the plant cells are beyond a stipulated threshold (Appenroth, 2010). On the other hand, when in the required concentrations, heavy metals such as zinc, copper, nickel, iron and manganese, to mention but a few, are essential in the biochemical functions in humans and animals (Soetan *et al.*, 2010). The consumption of plants or animal meat that is highly contaminated with heavy metals has been reported to cause different biochemical disorders (Duruibe *et al.*, 2007). Diseases such as renal failure, chronic anaemia, liver cirrhosis, body cancers and physiological effects on the circulatory and nervous systems in humans have also been associated with heavy metal ingestion (Mohod & Dhote, 2013; Salem *et al.*, 2000). Even though several health effects of heavy metals have been recognized for a long time, there is still a continuous exposure to heavy metals (Järup, 2003).

Heavy metals such as lead, mercury, arsenic, chromium and cadmium have been ranked the priority metals due to their high toxicity and carcinogenicity, and are of great public health concern (Tchounwou *et al.*, 2012). Arsenic contamination, for instance, has been recognized as a big problem in several parts of the world. Contamination of drinking water sources by arsenic has been reported in a number of both developed and developing countries, with the dosage levels exceeding its stipulated standard of 10 μg/L in drinking water (Mukherjee *et al.*, 2006). Growing interest has been in Bangladesh, where it is approximated that about half of the country's population is at risk of consuming water contaminated with arsenic, a problem the country has been facing since the 1990's (WHO, 2010). Exposure to arsenic, either through drinking water, eating food or breathing air, has been known to cause skin lesions and cancer of the liver, lung, bladder and skin, neurological disorders and impaired cognitive development in children, among others (Uddin & Huda, 2011).

Ultimately, the severity of adverse health effects of heavy metals is dependent on factors such as the type of heavy metal, its chemical form, the time of exposure and the dosage ingested and/or exposed to (Tchounwou *et al.*, 2012). Although much more stringent regulations have been placed to reduce the concentration of some of the most toxic heavy metals in the environment, the permissible concentrations of these heavy metals in water, drafted by the World Health Organisation, are designated as provisional because of the difficulties encountered during measurement and their removal in drinking-water and the environment as a whole. It is on this account that heavy metals largely remain environmental pollutants that cause the most serious environmental problems, hence requiring urgent attention.

2.4.2 Nutrients

Nitrogen and phosphorus are naturally occurring elements found in soil and water and they are considered essential for healthy plant growth (Uchida, 2000). Nitrogen in water occurs in the form of organic nitrogen and ammonia with the latter being best utilised as a nutrient by microorganisms, whereas phosphorus exists as ortho-phosphorus, a form commonly found in wastewater effluent and as particulate phosphorus, which is contained in organic matter, plant and animal tissue and in waste solids (Wasley, 2007; Uchida, 2000). The occurrence of these nutrients in the environment is as a result of both natural and anthropogenic activities such as atmospheric deposition, reclaimed water for irrigation and fertiliser applications (Badruzzaman *et al.*, 2012).

Carpenter *et al.* (1998) are of the opinion that wastewater effluents from municipal and industrial WWTPs are point sources of nutrients found in surface water. The concentration of these nutrients in surface water bodies is driven by factors such as temperature as a result of seasonal variations, oxygen concentration, light, source output, location and mode of loading among others (Van Ginkel, 2011; Withers & Jarvie, 2008). Although water bodies require some of these nutrients in order to sustain aquatic life, an excess of these nutrients in water becomes detrimental and harmful to human health. Eutrophication, which occurs when there is a high concentration of these nutrients in the water, has been a great environmental challenge facing water bodies for a long period of time (de Jonge *et al.*, 2002).

The excessive nutrient load has resulted in a wide range of problems such as toxic algal blooms, depletion of dissolved oxygen and loss of aquatic life (Shock & Pratt, 2003), thereby degrading water quality and interfering with the use of water for industrial and agricultural activities, recreation, drinking, and other purposes. According to OECD (2012), an estimated one-third of the global biodiversity in surface water is reported to have significantly reduced with the largest losses recorded in China, Europe, Japan, South Asia and Southern Africa. This has been attributed to the deterioration in water quality as a result of eutrophication in surface water. OECD (2012) predicts that factors such as climate change and increased water temperatures are projected to aggravate harmful algal blooms by 20% in the first half of this century (2050).

South Africa has been faced with issues related to excess nutrients in its water resources. Van Ginkel (2011) states that eutrophication has been one of the major factors affecting the quality of the country's limited water resources, hence affecting the potential to supply clean and safe water to all people. A study by Matthews & Benard (2015) found that the majority of the surface water bodies in South Africa have been heavily impacted by eutrophication and cyanobacterial blooms, with 62% of them being hypertrophic and 54% invaded by cyanobacteria surface scum, hence posing a high health risk to the consumers and aquatic organisms. The discharge of inadequately treated wastewater as a result of the failing sewage treatment plant infrastructure has been reported to be the primary origin of eutrophication in South Africa (Harding & Thornton, 2014). These authors are of the opinion that even in situations where wastewater has been efficiently treated, the concentration of phosphorous in wastewater is not reduced to levels that may be non-detrimental to water quality because the wastewater treatment processes do not focus on the removal of phosphorus.

According to Petterson (2016), out of 72 sewage treatment plants that were tested for their performance by the Department of Water and Sanitation - SA, 27 of them did not meet the quality standards for wastewater treatment and this, therefore, concurs with Harding and Thornton (2014) and hence negatively impacting onto the quality of surface water within the country. Furthermore, in South Africa where availability of freshwater is limited, eutrophication resulting from excess nutrients could not only lead to the death of aquatic organisms as a result of dissolved oxygen depletion, but also increased costs of purifying water for the consumers as a result of bioaccumulation and biomagnification of contaminants released into the water bodies receiving wastewater (Akpor & Muchie, 2011). This, therefore, means that the management of eutrophication as a result of poorly discharged effluent in South Africa's limited water resources is of critical concern and requires urgent attention.

2.4.3 Microbes

Inadequately treated wastewater effluents introduce a wide range of microbial pathogens into surface water bodies. This is due to the fact that WWTW analyses for indicator organisms in wastewater in order to determine the possible presence of pathogens instead of further isolating and identifying the different types of microbial pollutants in the wastewater. The lack of identification and isolation of the possible microorganisms is due to challenges such as time and costs incurred for the procedures (Akpor *et al.*, 2014).

When wastewater containing high microbes is discharged into surface water bodies, the health of both humans and animals is put at risk. WHO (2006) reports that an estimated three million people, with a majority of whom, are children under the age of five, die yearly from water-related diseases as a result of ingesting water polluted with a variety of micro-organisms such as bacteria, viruses, and protozoa. Diseases such as cholera, typhoid, Salmonellosis, Hepatitis A, ulcerations of the liver and intestines, and gastrointestinal, respiratory, skin and eye infections have been associated with the ingestion of water containing microbial pathogens (Olaolu *et al.*, 2014).

Microbial pathogens potentially present in water and wastewater can be divided into three separate groups; viruses, pathogenic protozoa and bacteria, most of which are excreted in the faecal matter by humans and animals (Akpor *et al.*, 2014), contaminate the environment and then gain access to a new host through ingestion. The following sections present brief summaries on these microbial pathogens although the main focus of this study was on bacterial pathogens.

2.4.3.1 Viruses

Viruses are the most hazardous of the microbial pathogens in wastewater. According to Gómez *et al.* (2006), viruses are not only difficult to detect in wastewater but also in comparison to other microbial pathogens, they require smaller doses to cause infections. It has been reported that more than 150 enteric viruses that are excreted in faeces and urine of infected hosts have been found in different water environments, and the most common of these viruses being adenoviruses, enteroviruses, noroviruses, rotaviruses, hepatitis viruses and polyomavirus

(Wong *et al*., 2012a). Consumption of water contaminated by viruses is especially fatal to sensitive populations such as children, the elderly and the immune-compromised (Xagoraraki *et al*., 2014). Hepatitis E, for instance, has been viewed to be an endemic infection common in developing countries. It has been reported that hepatitis E virus, for instance, is responsible for over 50% of the acute hepatitis infections in India, 25% in Africa and 15-20% in Eastern-Oriental countries. This has been attributed to inadequate water supply and environmental sanitation in developing countries (Purcell *et al.*, 2008). The virus is transmitted via the faecal-oral route, principally through consumption of contaminated water. However, until recently (Lapa *et al*., 2015; Dalton *et al*., 2008), studies have also shown a widespread of hepatitis E virus in highly industrialised countries although the contamination pathways have not been fully understood.

The existence of most these viruses in contaminated water is dependent on factors such as season, the population of the geographical area and the types of viruses in circulation within the population (Lapa *et al*., 2015; Parasidis *et al*., 2013). Although wastewater treatment plants have been established to treat wastewater, the current wastewater treatment methods do not effectively remove these organisms from wastewater effluents and therefore, the development of an accurate viral indicator of wastewater contamination is needed for enhanced water quality monitoring (Symonds *et al*., 2009).

2.4.3.2 Protozoa

Pathogenic protozoa are reported to be highly prevalent in wastewater than in any other environmental sources, and the most common of these being *Giardia* and *Cryptosporidium* spp (Toze, 1997). Both parasites are reported to produce protective cysts that allow them to survive in the environment for long intervals until they are ingested by the host through direct contact with contaminated food and/or water (Health Canada, 2012). A study conducted in South Africa to assess the effectiveness of four wastewater treatment plants found that although samples were collected on a weekly basis for 4 months from the different WWTPs under the study, *Giardia* and *Cryptosporidium* species persisted in the effluents (Dungeni & Momba, 2010). This demonstrated the ability of the oocysts to survive for days or months in environmental waters even after the effluents have been discharged into the river and hence creating potential health risks to those who use water from the river for drinking, recreation and agricultural purposes.

In comparison to other protozoan parasites, *Cryptosporidium* is the smallest in size, most persistent in the environment, and highly resistant to chemical disinfection, which makes it difficult to remove from the environment and hence often chosen when referring to protozoan parasites, which use the fecal-oral route in water supplies (Medema *et al.*, 2009). The WHO (2006) reported that *Cryptosporidium* infection was highly prevalent in young adults and children under 5 years of age in developing countries and industrialised countries, whereas in developed countries, the infection was rarely seen in adults but was most common in infants of less than 1 year of age. Known symptoms of infection include diarrhoea, abdominal pain, nausea and vomiting, especially in young children (King *et al.*, 2003).

In a developing country such as Lebanon, protozoan parasitic infections are reported to be very common among children under the age of 5 years (Osman *et al.*, 2016). Osman *et al.* (2016) concluded that among other causes for the prevalence of this infection, was the consumption of untreated water most likely contaminated by faecal matter as a result of the poor sanitary system. This meant that children who drank untreated water had a 3 times higher risk of infection than those who drank treated water. Contamination of water sources by protozoa is also reported in developed countries like Spain, where a study showed a high occurrence of protozoa in all water sources with concentrations reaching 1767 *Cryptosporidium* oocysts and over 25,000 *Giardia* cysts per 100 mL (Carmena *et al.,* 2007). Causes of such high protozoan parasites in the water were attributed to run-offs from precipitation, agricultural areas and cattle farming.

Other causes of protozoan parasite infections have been attributed to the discharge of wastewater effluents in receiving water bodies that are being used by surrounding communities for various activities. A study in Poland deducted a prevalence and high concentrations of protozoan parasites in wastewater effluents from WWTPs and the results obtained were comparable to a similar study in South Africa that found that *Giardia* and *Cryptosporidium* persisted in the wastewater effluents, thereby polluting the river which served as the disposal site for the effluents (Sroka *et al.*, 2013; Dungeni & Momba, 2010). This implies a risk of transmission of protozoan parasites that are of great health risk to humans and therefore, calling for additional wastewater purification procedures.

2.4.3.3 Bacteria

Bacteria are the most common environmental pollutants that are found in wastewater and sewage, WWTP effluents, surface water, groundwater and drinking water (Zhang, *et al*., 2009). These bacterial pathogens enter the environment through faeces of both humans and animals and hence are known as faecal coliform bacteria. Examples of faecal coliforms are *E. coli* and some *Klebsiella* species such as *K. oxytoca* and *K. pneumonia* (Naidoo & Olaniran, 2013). The presence of these bacterial pathogens in wastewater is due to the high concentration of nutrients in wastewater, thereby providing a suitable medium for them to rapidly proliferate, hence increasing the risk of water-related infections (Toze, 1997). This section will mainly focus on *E. coli* and *Klebsiella* which are the microorganisms of interest in the present study.

E. coli is a type of bacteria that lives in the intestines of both humans and animals and are excreted in faeces. While most of its strains are rendered harmless, a few of them have been reported to cause infection. One such strain is O157, which is known to cause disease by producing a toxin called Shiga toxin. The bacteria that make these toxins are called "Shiga toxin-producing *E. coli*" or STEC for short, and they live in the intestines of many animals (CDC, 2017). *E. coli* infection is mainly transmitted through the consumption of contaminated water or food (Cabral, 2010). According to CDC (2017), about 265,000 people are infected yearly with STEC in the United States and STEC O157 accounts for 36% of these infections, and this mainly occurs among children (<5 years), the elderly (>65 years) and the immunocompromised. Some of the examples of the symptoms of *E. coli* infection are abdominal pain, bloody diarrhoea and hemolytic uremic syndrome (Cabral, 2010).

The determination of the microbiological quality of water used for drinking is done by testing for *E. coli*, whose presence in water indicates faecal contamination (APHA, 1995). This kind of test has shown to be the most suitable for predicting the presence of pathogens in drinking water sources (Haramoto *et al*., 2012). It can also be used to evaluate the quality of wastewater effluents, rivers, sea beaches, water used for irrigation, aquaculture sites and recreational water. In South Africa, for example, *E. coli* has been detected in surface water and this has been largely attributed to the discharge of wastewater effluents that are of poor microbial quality (Omar & Barnard, 2015; Naidoo & Olaniran, 2013; Mema, 2010; Momba *et al*., 2006). The South African water guidelines, like other drinking water guidelines, stipulate that drinking water sources should not have an *E. coli* concentration of more than 0 cfu/100 mL, otherwise communities that rely on the direct use of surface water are at a high risk of water-borne infections (SANS:241, 2013; WHO, 2011).

Klebsiella bacteria, just like *E. coli*, are found in the intestines of humans and in faeces. It has also been found to be present in different areas of the environment (Edberg *et al.*, 1986; Bagley *et al.*, 1978; Brown & Seidler, 1973). In the context of the present study, *Klebsiella* has been found to exist in wastewater. For instance, a study in the Czech Republic found *E. coli* and *K. pneumonia* strains in treated municipal wastewater effluents, thus demonstrating that wastewater effluents can be carriers of *Klebsiella* and that wastewater treatment does not necessarily eliminate all microbial pathogens (Dolejska, *et al.*, 2011). According to these authors, wastewater effluents from the WWTP are discharged into the river. This practice, which is common among WWTPs, leads to the transfer of microbial pathogens into surface water sources, thereby increasing the risk of exposure of pathogenic bacteria to humans and animals (Miah *et al.*, 2017; Omar & Barnard, 2010; Podschun *et al.*, 2001).

Different methods have been used to determine faecal contamination of water sources. Douterelo *et al.* (2014) describe culture-dependent and culture-independent methods for the detection, identification, quantification and characterization of microorganisms within a water system. According to these authors, examples of culture-dependent techniques are heterotrophic plate count and membrane filtration, which can be used to detect and enumerate faecal coliforms in water while a culture-independent technique such as Polymerase Chain Reaction (PCR), is used to amplify the DNA fragments of microorganisms. Of the 2 techniques, the authors are of the opinion that although culture-independent techniques are costly, time-consuming and require specialized equipment and training, they are rendered a much more effective method for confirming the identity of the isolated microorganisms, an opinion that had earlier been suggested by Bai *et al.* (2010). However, for effective understanding of the microbial community and the overall quality of a water source, a combination of both techniques needs to be applied (Douterelo *et al.*, 2014).

The presence of bacterial pathogens in wastewater effluent indicates their insufficient removal during wastewater treatment. The discharge of such effluents into surface water leads to their transfer into the water. As a result, several severe human health effects associated with the consumption of water containing high bacterial counts have been reported in both developing and developed countries. This, therefore, indicates that there is an urgent need to improve wastewater treatment processes so as to mitigate microbial pollution of surface water sources.

2.5 Chapter summary

Wastewater has been viewed globally as a viable water resource. The re-use of treated wastewater has been regarded as an important strategy in the alleviation of water scarcity especially in arid and semi-arid regions of the world. In South Africa, WWTWs are mandated by the constitution to reclaim their treated wastewater effluent by discharging it in surface water. WTWs, in turn, abstract water from these surface water sources (rivers, lakes, dams), treat it and supply to the various sectors that need water to facilitate their activities. This chapter has, however, demonstrated that although different wastewater treatment processes have been adapted both locally and internationally, insufficiently treated wastewater effluents are still being continually discharged into the environment. As a result of the wide range of pollutants present in wastewater effluent, some serious health and environmental problems have been reported. Despite the global recognition of the severe health effects arising from the contamination of surface water sources with wastewater effluent, the problems continue to exist. It is therefore essential that the quality of the wastewater effluent is assessed in order to protect the surface water serving as the disposal basins. The following chapter discusses the analytical techniques and procedures that were employed in order to achieve the main aim of this study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter outlines the research approach for the study. It gives a detailed account of the sources and types of data, the methods of collection as well as the various analytical techniques employed in the study. Rationalisation is given regarding the use of these techniques and principles underlying their application by way of equations, thus creating a basis for logic in the preceding chapters.

3.2 Water sampling procedures

3.2.1 Description of Setumo dam

Setumo dam, previously known as Modimola Dam, is situated in Unit 14, a residential area in a suburb called Mmabatho (Figure 1). The dam has a full storage capacity of 20.7 million cubic meters (DWA, 2010). This reservoir not only serves as the main source of water for Mmabatho WTW but also for industries in Mahikeng, with large industrial abstractions registered at 5.3 million m^3/a (DWA, 2010). Due to its proximity to the village and easy access, the surrounding communities use this water for drinking, washing, fishing and livestock watering. It should also be noted that Setumo dam is used as a disposal site for wastewater effluent by the Mmabatho WWTW, which is located upstream of this dam (Dikobe *et al.*, 2011).

3.2.2 Sampling sites

Stratified random sampling was employed in the selection of the sampling sites. The strata of particular interest in the study area were Mmabatho WWTW and Setumo dam. Setumo dam serves as one of the reservoirs of the Molopo River. It was therefore, essential to collect water from both strata in order to determine the overall quality of water within each stratum and to determine whether the wastewater effluent discharged from the Mmabatho WWTW had any impact on the quality of water in Setumo dam. However, due to the erratic rainfall at the beginning of the 2015/16 rainy season, there was flow in the Molopo river and hence the collection of water samples to cater for the wet season was done in 2017 when heavy rains were received countrywide (SAWS, 2017). A total of nine sampling sites were randomly selected from the selected strata as follows (Figure 3.1);

- Point B - the end of the wastewater effluent discharge pipes of the Mmabatho WWTW
- Point C – intersection point between the wastewater effluent and the dam water
- Points A, D, E, F, G, H, and I, randomly selected in order to determine the overall water quality of the dam

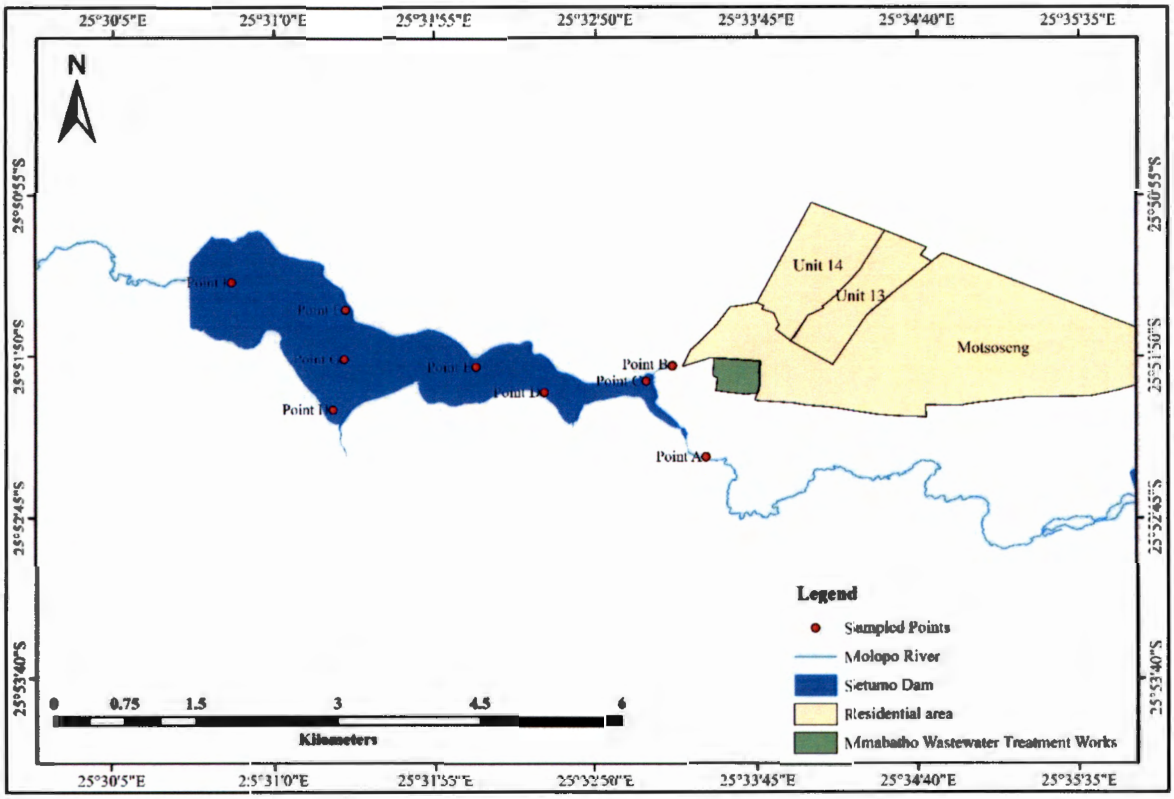

Figure 3.1: Wastewater effluent and dam water sampling sites

3.2.3 Collection of wastewater effluent and water samples

The wastewater effluent and dam water samples were collected on a seasonal basis i.e. during the wet (February) and the dry seasons (July). This was done in order to accomplish objective three of this study which was to establish the seasonal and spatial variations of the pollutants in Setumo dam. . The wastewater effluent and dam water samples were collected in sterilised 1 litre HDPE bottles. The HDPE bottles were used because they minimise contamination and are favourable for sample preservation (Hall, 1998). At each sampling point, the sampling bottles were rinsed with the sample, filled up and tightly capped. Sampling at each point was done in triplicate sets and the bottles were kept in a cooler box with ice and transported to the laboratory for further analysis. The parameters – temperature, pH, electrical conductivity and total dissolved solids that required on-site analysis were analysed immediately at each sampling point.

3.3 Wastewater effluent and water analysis

3.3.1 *In-situ* analysis

The on-site analysis was done using a multimeter that measures temperature, pH, electrical conductivity (EC) and the concentration of total dissolved solids (TDS) in the collected samples. The meter probe was rinsed with distilled water and immersed in each sample for about one minute such that the meter could come to equilibrium. When the readings of each parameter were constant, the measurements were then recorded on the field data sheet. Rinsing of the meter probe with distilled water was done repeatedly for each sample and between samples to avoid contamination. The same procedure was followed for each of the collected samples per sampling point. Analysis of these parameters was done to provide an insight on the concentrations of the heavy metals, nutrients and microbes in both the wastewater effluent and dam water.

3.3.2 Laboratory analysis

3.3.2.1 Sample digestion and heavy metal analysis

This sub-section indicates the procedure undertaken for heavy metals analysis in order to address the first objective of this study, which was to examine the level of heavy metal concentration. Analysis of heavy metal concentrations was done for both the wastewater effluent and dam water samples. Each sample was filtered through a 0.45 µm membrane filter in order to remove silicates and other insoluble materials, and approximately 2 mL of hydrochloric acid and 6 mL of concentrated nitric acid were each added to every 5 mL of the sample in a test tube. The prepared mixture of each sample was digested using the Anton Paar Multiwave 3000 microwave to ensure the removal of organic impurities from the samples and thus preventing interference in the analysis. The digested samples were then quantitatively transferred to a 50 mL volumetric flask and made up to the 100 mL mark using distilled water and allowed to settle overnight. The analysis of heavy metals in the digested samples was then carried out using an inductively coupled plasma optical emission spectroscopy instrument (ICP-OES).

The following sections 3.3.2.2 to 3.3.2.4 describe the methods used to address objective two which was to analyse for the presence of nutrients and microbes. This was done for both the wastewater effluent and the dam water in order to accomplish the main aim of the study.

3.3.2.2 Nitrates

The analysis of the nitrate levels in the samples was done according to the manufacturer's instructions (Merck, South Africa). One level micro-spoon of NO_3^{-1} was placed into a dry test tube and 5 mL of NO_3^{-2} reagent (15-25 °C) was added into it using a pipette. The mixture was then shaken vigorously for 1 minute until the reagent NO_3^{-1} was completely dissolved. An aliquot of 1.5 mL of the water sample was very slowly and carefully allowed to run from the pipette and into a test tube containing the reagent. The sample was immediately mixed briefly and allowed to stand for 10 minutes (reaction time). The sample was then filled into a 10 mm rectangular cell (measuring range 0.5 - 20.0 mg/L NO_3-N) and measured using a spectrophotometer. This step was followed for each of the collected samples.

3.3.2.3 Phosphates

Following the manufacturer's instructions (Merck, South Africa), 10 mL of each sample was pipetted into a dry and clean test tube. This was followed by an addition of 10 drops of reagent PO_4^{-1} and 2 level blue micro-spoons of PO_4^{-2}. The mixture was shaken vigorously until the reagent was completely dissolved, and left to stand for 5 minutes (reaction time). The sample was then filled into a 50 mm cell (measuring range 0.005 – 1.000 mg/L PO_4-P) and measured using a spectrophotometer. This procedure was followed for each of the collected samples.

3.3.2.4 Determination of coliform bacterial load using selective agar

This section outlines bacteriological analysis of the wastewater effluent and dam water samples and the identification of the bacterial isolates using preliminary and confirmatory biochemical tests. The membrane filtration technique was used in the determination of heterotrophic bacteria, total and faecal coliform counts using a previously described technique (APHA, 1995). Aliquots of 50 mL of each sample were filtered through 0.45 µm membrane filters using a Gelman Little Gaint pressure/vacuum pump machine (model 13156, Gelman Sciences, MI, USA). Membrane filters were aseptically transferred onto m-Endo agar, m-FC agar and heterotrophic plate count agar (Merck, South Africa) to selectively isolate total coliforms, faecal coliforms and heterotrophic bacteria respectively. Samples were analysed in triplicate.

The m-Endo and heterotrophic agar plates were incubated aerobically at 37°C for 24 hours while m-FC agar plates were incubated aerobically at 45°C for 24 hours (Paruch & Maehlum, 2012; Rompre *et al.*, 2002). After incubation, all metallic sheen and typical blue colonies on m-ENDO and m-FC agar plates were considered to be total and faecal coliforms respectively, while yellow colonies on nutrient agar were counted as heterotrophic bacteria. The colonies were enumerated and results were recorded. The m-FC agar plates were stored at 4°C for further identification of faecal coliforms.

3.3.2.4.1 Bacteria identification

Preliminary test

In order to screen for characters of bacteria belonging to the family *Enterobacteriaceae*, the presumptive faecal coliforms from the m-FC agar plates were isolated and sub-cultured onto m-FC agar using the streaking plate method. A total of 23 and 26 isolates with different colonial morphologies in the wet and the dry seasons respectively, were individually revived by sub-culturing onto m-FC agar and the plates were incubated aerobically at 37°C for 24 hours to obtain pure colonies (Salle, 1954). The obtained pure colonies were then subjected to PCR identification assays.

3.3.2.4.2 Confirmatory tests

DNA extraction and PCR amplification

Individual pure bacteria colonies were inoculated into 3 mL of Luria-Bertani broth (Merck, Germany) and incubated at 37°C for 24 hours. Aliquots of 1.5 mL from each overnight culture were transferred into microcentrifuge tubes and vortexed for 2 minutes to acquire a pellet. Genomic DNA was then extracted using a genomic DNA extraction kit and according to the manufacturer's instructions (Zymo DNA extraction kit). The eluted DNA was stored at -20°C and later used for PCR identification tests. Presumptive *E. coli* and *Klebsiella* isolates were screened using the *uidA* and *ntrA* specific primers respectively.

PCR reaction mixtures were prepared in 25 μL volumes comprising 1 μL of the template DNA, 12.5 μL of the PCR master mix, 0.5 μL of both oligonucleotide primers (forward and reverse) and 11 μL of RNase free distilled water. Thermal cycling for *E. coli* was performed as follows: initial denaturation at 95°C for 10 minutes, followed by 35 cycles of 95°C for 45 seconds, 59°C for 30 seconds, 72°C for 90 seconds and a final elongation at 72°C for 10 minutes. For *Klebsiella*, the following conditions were used: initial denaturation at 95°C for 10 minutes, 35 cycles of 95°C for 45 seconds, 55°C for 30 seconds, 72°C for 90 seconds and a final elongation at 72°C for 10 minutes. Primers used in this study are indicated in Table 3.1.

Table 3.1: Oligonucleotide primers used for the detection of *E. coli* and *Klebsiella*

Primer	Primer sequence (5' to 3')	Targeted gene	Amplicon size (bp)	Reference
uidA	(F) CTGGTATCAGCGCGAAGTCT (R) AGCGGGTAGATATCACACTC	*uidA*	600	Anbazhagan *et al.* (2011)
ntrA	(F) CATCTCGATCTGCTGGCCAA (R) GCGCGGATCCAGCGATTGGA	*ntrA*	90	

F = Forward; R = Reverse

Agarose gel electrophoresis

Amplified DNA fragments were resolved by electrophoresis using 2% (w/v) agarose gel containing 0.1 µg/mL ethidium bromide. About 1 µL of the extracted DNA samples were loaded onto the gel and electrophoresed for 40 minutes at 80 V. In each gel, a 100 bp DNA molecular marker was also included and used to determine the sizes of amplicons. The amplicons were visualised under UV light (Sambrook, 1989). A Gene Genius Bio-Imaging System was used to capture images using GeneSnap software (version 6.00.22). Images were analysed using GeneTools software (version 3.07.01) to determine the presence or absence of the targeted bands and the images were saved as tagged image file format (TIFF).

DNA Sequence analysis of PCR amplicons

The amplified gene fragments were sequenced by Inqaba Biotec, Pretoria. The identities of the isolates were confirmed using a Blast Search with the NCBI Search Tool: (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3.4 Statistical analysis

Statistical analyses were performed on the results obtained from the laboratory analysis. The mean and standard deviation were calculated and tabulated since the samples were collected in triplicate set per sampling point. The results obtained from analysis of the wastewater effluent were compared to the South African DWA wastewater effluent discharge guidelines for wastewater discharged into a water resource (DWA, 2013). The wastewater effluent guidelines selected were the "special limit" guidelines since the wastewater effluent is discharged into surface water used for domestic purposes by the surrounding communities. The results from the dam water analysis were compared to both the South African and World Health Organisation (WHO) drinking water quality standards (SANS:241, 2015; WHO, 2011).

In order to determine the validity and reliability of the results obtained with respect to the aim of this study, the following statistical tests were performed;

1. One-way analysis of variance (ANOVA) was used to examine the seasonal and spatial variations of all the parameters analysed in the dam water and hence addressing objective three of the study which stated a need to establish the seasonal and spatial variations of the pollutants in Setumo dam.

2. Pearson correlation matrix was used to determine the significant difference between the wastewater effluent pollutants (nutrients, heavy metals and microbes) and the pollutants in the dam water. The Pearson correlation matrix was also used to evaluate the overall significant difference in the concentration of these pollutants across the different sampling points within the dam.

3.5 Risk assessment

The risk assessment of this study was done using the risk quotient (RQ), which is a ratio of exposure and effect (Peterson, 2006), in order to answer objective four which was to assess the health risks associated with water from Setumo dam. The RQ is useful in assessing whether the pollutant concentrations exceed threshold levels. In the circles of risk characterization and assessment, this is termed a tier 1 risk assessment approach. Determination of the risk quotient was done for all the analysed pollutants in Setumo dam to determine any potential health risks associated with the consumption of the water. The following equation was used;

$$\textit{Risk quotient (RQ)} = \frac{\textit{Concentration of pollutant}}{\textit{The regulatory limit of the pollutant}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{Equation 1}$$

Whereby if the risk quotient was less than 1, it meant that there was none or acceptable health risk but if it was equal to or exceeds 1, then it meant that there were a significant health risk and measures to reduce exposure were necessary.

3.6 Water Quality Index

Further analysis was done using a Water Quality Index (WQI) that was developed by Brown *et al.* (1970). The WQI numerically summarizes water quality parameters from certain sites within a surface water body into a single value, thereby categorising the quality of water as excellent, good, medium, bad or very bad (Table 3.3). The standard water quality parameters used in the index are temperature, pH, dissolved oxygen, biochemical oxygen demand, total solids, turbidity, nitrates, phosphates, and faecal coliforms (Malathi *et al.*, 1999). This helps to determine the suitability of the water for human consumption. The parameters are then given weights based on their importance in water quality (Table 3.2) (Basin, 2001).

Table 3.2: Water parameters and their relative weights

Water Parameters	Weights
Temperature	0.10
pH	0.11
Dissolved Oxygen	0.17
Biochemical Oxygen Demand	0.11
Total Solids	0.07
Turbidity	0.08
Nitrates	0.10
Phosphates	0.10
Faecal coliforms	0.16

The water parameters in this study that were entered into the WQI calculator were the dam water; temperature, pH, total dissolved solids, nitrates, phosphates and faecal coliforms. The WQI was calculated from the standard formula (Brown *et al.,* 1970) Equation 2.

$$WQI = \sum_{i=0}^{n} QiWi \text{ .... } \textit{Equation 2}$$

Where; Q_i = sub-index for i^{th} water quality parameter

W_i = weight associated with i^{th} water quality parameter

n = number of water quality parameters

Q value is an indication of water quality relative to 100 of one parameter. The WQI is determined as the weighted average of all water quality parameters of interest.

Table 3.3: NSFWQI water quality classification

Designation	Index value
Excellent	91 -100
Good	71 - 90
Medium	51 - 70
Bad	26 - 50
Very bad	0 - 25

Adopted from Riszi *et al.*, (2016)

3.7 Chapter Summary

The sampling procedure and techniques have been presented in this chapter. Wastewater effluent and the dam water samples were collected in triplicate sets at each sampling point. Sampling was done during both the wet and the dry seasons. The physicochemical parameters of the samples were analysed on-site during sample collection whereas the chemical and bacterial parameters were analysed in the laboratory, following specific procedures. The mean and standard deviations were calculated for each of the results. One-way ANOVA was used to examine the statistical significant seasonal and spatial variations in the mean results obtained from the dam water analysis. Pearson correlation matrix was used to determine the significant difference between the wastewater effluent pollutants and the pollutants in the dam water and to evaluate their overall significant difference among the dam sampling points. The results were further used in the risk quotient equation to determine the significant health risks associated with the dam water. The WQI was used to determine the overall quality of water in the dam. The following chapter presents the results of the analytic procedures presented in this chapter. The results and discussion of these results are presented concurrently.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter presents the results obtained from the analysis of the wastewater effluent and the dam water samples collected from the Mmabatho WWTW and Setumo dam respectively. The wastewater effluent and dam water samples were collected in triplicates during both the wet and the dry seasons and the average values obtained after analysis are presented in tables. The results from the analysis of the wastewater effluent (point B) are presented in one table and compared to the DWA wastewater effluent quality standards. The results from the analysis of the dam water (point A, C, D, E, F, G, H, & I) are presented in another table and are compared to the SANS:241 and WHO drinking water quality standards. The tables are then followed by a comprehensive discussion of the results based on existing literature.

The results from the one-way ANOVA test at 95% confidence level ($\alpha = p<0.05$) and the Pearson correlation coefficient ($r = 0.05$) are also presented and discussed. The evaluation of the seasonal and spatial variations of the analysed parameters in the wastewater effluent using one-way ANOVA was not done because the wastewater effluent was collected from one exit point. This was the only discharge point for wastewater effluent from the Mmabatho WWTW.

4.2 Physicochemical analyses

Tables 4.1a and 4.1b show the results recorded for the wastewater effluent and dam water samples during both the wet and the dry seasons. The data obtained from the wastewater effluent reveals that pH and EC during the wet season did not comply with the DWA wastewater effluent quality standards, but were in compliance during the dry season (Table 4.1a). There are no standards for temperature and TDS in wastewater effluent. The data obtained from dam water during both seasons indicates that all of its physicochemical parameters were within the SANS:241 and WHO drinking water quality standards, except for pH during the wet season which was above WHO pH threshold value (Table 4.1b). One-way ANOVA results indicated that at 95% confidence level, 50% of the physicochemical parameters analysed in the dam water varied significantly; both seasonally (Table 4.1c) and spatially (Table 4.1d).

Table 4.1a: Physicochemical parameters in the wastewater effluent samples during the wet and the dry season

	Temperature (°C)		**pH**		**EC (µS/cm)**		**TDS (mg/L)**	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Point B	23.2±0.1	22.4±0.14	**7.66**±0.03	7.33±0.04	**1055**.33±2.52	991.5±3.54	676.67±1.53	634.5±2.12
DWA	**Not stated**		**5.5 ≥ pH ≤ 7.5**		**500 ≥ EC ≤ 1000**		**Not Stated**	

Table 4.1b: Physicochemical parameters in the dam water samples during the wet and the dry season

	Temperature (°C)		**pH**		**EC (µS/cm)**		**TDS (mg/L)**	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Point A	25.7±0.1	13.25±0.07	8.57±0.06	8.45±0.07	825.33±5.69	933±1.41	528±3.61	597.5±0.71
Point C	24.2±0.1	18.05±0.07	7.88±0.01	7.85±0.51	758.33±0.58	771±4.24	485±1.0	493±2.83
Point D	24.0±0.1	14.75±0.07	8.91±0.05	8.36±0.08	428.67±1.15	481±5.66	274.67±1.15	307.5±3.54
Point E	24.0±0.1	15.7±0.14	9.33±0.01	8.29±0.06	391.67±0.58	458.5±4.95	250.67±0.58	293.5±3.54
Point F	24.4±0.1	16.0±0.28	9.29±0.03	8.43±0.06	386.33±0.58	445.5±4.95	247.67±1.15	285±2.83
Point G	24.7±0.1	16.65±0.07	9.23±0.02	8.54±0.11	386.67±0.58	444±2.83	247.67±0.58	284±1.41
Point H	24.0±0.1	14.35±0.07	9.06±0.04	8.4±0.06	395.67±1.73	434±1.41	255.33±4.04	277.5±0.71
Point I	24.4±0.1	15.2±0.28	9.22±0.04	8.09±0.07	382±1.73	448±4.24	244.33±1.15	287±2.83
SANS:241	**Not Stated**		**5 ≥ pH ≤ 9.7**		**≤ 1700**		**≤ 1200**	
WHO	**Not Stated**		**6.5 ≥ pH ≤ 8.5**		**Not Stated**		**< 1000**	

Table 4.1c: ANOVA values showing the seasonal variation of the physicochemical parameters in the dam water

Parameters	Sum of Squares	df	Mean Square	F	Significance
Temperature	319.067	1	319.067	256.028	0.000
pH	1.613	1	1.613	10.908	0.005
EC	13243.982	1	13243.982	0.375	0.550
TDS	5316.597	1	5316.597	0.368	0.554

Table 4.1d: ANOVA values representing the spatial variation of the physicochemical parameters in the dam water

Parameters	Sum of Squares	df	Mean Square	F	Significance
Temperature	6.315	7	0.9.2	0.022	0.999
pH	1.519	7	0.217	0.802	0.608
EC	491823.647	7	70260.521	35.608	0.000
TDS	200965.449	7	28709.349	35.797	0.000

4.2.1 Temperature

A general observation of the temperature of the wastewater effluent and dam water was that the wet season is characterized by warmer temperatures and cooler temperatures during the dry season. The overall average temperature of the wastewater effluent was 23.2°C and 22.4°C during the wet and the dry seasons respectively (Table 4.1a). There are no DWA standard guidelines for temperature in wastewater effluent. The temperature of the dam water averaged 24.3°C during the wet season and 16.3°C during the dry season (Table 4.1b) and it showed a statistically significant difference, p = 0.000 (Table 4.1c). This can be attributed to environmental climatic changes. However, the study found the dam water temperatures to have no statistically significant spatial variation, p = 0.999 (Table 4.1d).

Although temperature generally influences the overall quality of water (physio-chemical and biological characteristics) (Palamuleni & Akoth, 2015), there are no set temperature guideline values recommended for good quality water. However, it is important to note that increased water temperatures could encourage the proliferation of microorganisms, leading to problems related to taste and odour, decrease in the solubility of gases such as oxygen, carbon dioxide, nitrogen, and methane, and also affect the metabolism rate of aquatic organisms (Yilmaz & Koc, 2014; WHO, 2008).

4.2.2 pH

The pH of water reflects the degree of acidity ($pH < 7$) or alkalinity ($pH > 7$). Wastewater effluent had pH average values of 7.66 and 7.33 during the wet and the dry seasons respectively (Table 4.1a). The pH of the wastewater effluent samples during the wet season was noted to be slightly above the DWA wastewater effluent quality standards. The increase in pH level may be due to denitrification processes in the secondary pond, which occurs during the conventional wastewater treatment process (Chebor *et al.*, 2017). It is worth noting that the Mmabatho WWTW uses the conventional treatment processes in treating wastewater.

An overall average pH value of 8.94 and 8.3 for the dam water samples was recorded during the wet and dry seasons respectively (Table 4.1b). It was found that the pH of the dam water varied significantly between the two sampling seasons, $p = 0.005$ (Table 4.1c). The results of the pH of all the water samples collected during the wet and the dry seasons were considered to be within the allowable limits of SANS:241 drinking water quality standards, except for the pH value in the wet season, which was above the WHO drinking water quality standard. However, there was no significant variation in pH among the dam sampling sites, $p = 0.608$ (Table 4.1d).

According to Trivedi *et al.* (2010), many biological activities occurring in water are dependent on pH and any slight deviation from the stipulated standards could be disastrous to aquatic organisms. Low water pH has been reported to cause water to taste sour whereas a high pH causes water to taste soapy (DWA 2006). Low levels of pH in water have also been known to cause corrosion in galvanized pipes and also to increase the concentration of heavy metals in water (Virkutyte & Sillanpää, 2006; DWA, 1998). Although there are no human health implications related to the pH of water, pH is still considered one of the most important parameters of water.

4.2.3 Electrical conductivity

Electrical Conductivity (EC) is the ability of water to carry an electrical current. The average EC of the wastewater effluent was 1055 µS/cm and 991.5 µS/cm during the wet and the dry seasons respectively (Table 4.1a). The EC of the wastewater effluent was slightly above the

DWA wastewater effluent quality standard during the wet season. This could probably be due to the increase in the number of dissolved salts that are drained into the WWTW during the wet season as a result of runoff, among other things (Pal *et al.*, 2015).

The overall average EC of the dam water was 494.3 µS/cm during the wet season and 551.9 µS/cm during the dry season (Table 4.1b). There are no WHO drinking water quality standards for EC in water, but the SANS:241 drinking water quality standards accept an EC of either 1700 µS/cm or lower. It can be noted that although the EC of the dam water was higher during the dry season, the variations between the two seasons were not statistically significant, $p = 0.550$ (Table 4.1c). However, there existed statistically significant variations among the various sampling sites, $p = 0.000$ (Table 4.1d) and this could indicate surface water pollution from point and non-point sources (Pal *et al.*, 2015). The higher average EC results obtained in the dam water during the dry season were unexpected as the wet season is often accompanied with high EC values due to high surface runoff as a result of heavy rainfalls (Pal *et al.*, 2015).

4.2.4 Total dissolved solids

Total dissolved solids (TDS) is a measure of the number of dissolved salts in water. The wastewater effluent had TDS average values of 676.67 mg/L and 634.5 mg/L during the wet and the dry seasons respectively (Table 4.1a). Although there is no DWA wastewater effluent quality standard for TDS, the higher concentration of TDS in the wastewater effluent during the wet season can be attributed to significant loads of dissolved salts that are drained into wastewater drainage pipes and into the WWTW during rainfall events (Sheila, 2007).

The average concentration of dissolved salts in the dam water was 316.7 mg/L during the wet season and 353 mg/L during the dry season (Table 4.1b). The results indicate that TDS in the dam was within the SANS:241 and WHO drinking water quality standards. Seasonal difference in TDS in the dam water was found not to be statistically significant, $p = 0.554$ (Table 4.1c) but there existed statistically significant spatial variation, $p = 0.000$ (Table 4.1d). Depending on their concentration in water, TDS is reported to affect the quality of the surface water and the productivity of aquatic organisms (Pal *et al.*, 2015). The results obtained during the study also indicate a direct relationship between the concentrations of TDS in the dam water and wastewater effluent with their respective EC (Table 4.1a; 4.1b).

4.3 Chemical analyses

The results and discussions of the chemical parameters in the wastewater effluent and dam water samples are presented in this section. The chemical parameters that were analysed in both the wastewater effluent and dam water samples during the wet and dry seasons are heavy metals (arsenic, cadmium, copper, iron, lead, manganese, nickel and zinc), nitrates and phosphates.

4.3.1 Heavy metal analyses

The collected wastewater effluent and dam water samples were analysed for the presence of selected heavy metals as indicated in Section 3.3.2.1. Tables 4.2a and 4.2b below show the concentrations of these heavy metals in the wastewater effluent and dam water samples during the wet and the dry seasons respectively, representing objective one of this study. The data obtained from the wastewater effluent revealed that iron, manganese and zinc complied with the DWA wastewater effluent quality standards during the wet season and arsenic, copper, iron and manganese during the dry season (Table 4.2a). But, the data from the dam water indicates that copper, iron, nickel and zinc were within the SANS:241 and WHO drinking water quality standards during the wet season while all the heavy metals during the dry season, were within the set drinking water quality limits (Table 4.2b). One-way ANOVA results indicated that at 95% confidence level, arsenic, iron, lead, nickel and zinc revealed statistically significant seasonal variations (Table 4.2c) while none of the heavy metals showed any statistically significant spatial variations (Table 4.2d).

Table 4.2a: Heavy metal concentrations in the wastewater effluent samples during both the wet and the dry season

	Arsenic (mg/L)		**Cadmium (mg/L)**		**Copper (mg/L)**		**Iron (mg/L)**		**Lead (mg/L)**		**Manganese (mg/L)**		**Nickel (mg/L)**		**Zinc (mg/L)**	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Point B	**0.17**	0.01	0	0	**0.02**	0	0.02	0.04	**0.03**	**0.11**	0.04	0.04	0	0	0.04	**0.13**
DWA	**0.01**		**0.001**		**0.002**		**0.3**		**0.006**		**0.1**		**NS**		**0.04**	

Table 4.2b: Heavy metal concentrations in the dam water samples during the wet and the dry season

	Arsenic (mg/L)		**Cadmium (mg/L)**		**Copper (mg/L)**		**Iron (mg/L)**		**Lead (mg/L)**		**Manganese (mg/L)**		**Nickel (mg/L)**		**Zinc (mg/L)**	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Point A	**0.16**	0.01	0	0	0.02	0	0.01	0.01	**0.11**	0.01	0	0	0	0	0.04	0.13
Point C	**0.13**	0	0	0	0.02	0	0	0.01	**0.08**	0.01	0	0	0.01	0	0.05	0.13
Point D	**0.17**	0	0	0	0.02	0	0.01	0.01	**0.12**	0.01	0	0	0.01	0	0.05	0.13
Point E	**0.15**	0.01	0	0	0.02	0	0.01	0.01	**0.12**	0	0	0	0	0	0.04	0.13
Point F	**0.13**	0	0	0	0.02	0	0.01	0.02	**0.13**	0.01	0	0	0.01	0	0.04	0.13
Point G	**0.13**	0.01	0	0	0.02	0	0	0.02	**0.14**	0	0	0	0.01	0	0.04	0.13
Point H	**0.16**	0	0	0	0.02	0	0.01	0.01	**0.11**	0.01	0	0	0	0	0.03	0.13
Point I	**0.16**	0	0	0	0.02	0	0.01	0.02	**0.11**	0.01	0	0	0.01	0	0.05	0.13
SANS:241	**0.01**		**0.003**		**2**		**2**		**0.01**		**0.4**		**0.07**		**5**	
WHO	**0.01**		**0.003**		**2**		**NS**		**0.01**		**0.4**		**0.07**		**Not Stated**	

Table 4.2c: ANOVA values showing the seasonal variation of the heavy metals in the dam water

Parameters	Sum of Squares	df	Mean Square	F	Significance
Arsenic	0.0841	1	0.0841	567.422	0.000
Cadmium	0	1	0	65535	---
Copper	0.002	1	0.002	65535	---
Iron	1.563e-04	1	1.563e-04	6.482	0.023
Lead	0.046	1	0.046	275.383	0.000
Manganese	0	1	0	65535	---
Nickel	1.563e-04	1	1.563e-04	11.667	0.004
Zinc	0.031	1	0.031	1225	0.000

Table 4.2d: ANOVA values representing the spatial variation of the heavy metals in the dam water

Parameters	Sum of Squares	df	Mean Square	F	Significance
Arsenic	9.75e-04	7	1.393e-04	0.013	0.999
Cadmium	0	7	0	65535	---
Copper	2.168e-19	7	3.098e-20	1.55e-16	1
Iron	1.44e-04	7	2.054e-05	0.469	0.833
Lead	8.75e-04	7	1.25e-04	0.021	0.999
Manganese	0	7	0	65535	---
Nickel	9.375e-05	7	1.339e-05	0.429	0.859
Zinc	1.75e-04	7	2.5e-05	0.007	1

4.3.1.1 Arsenic

Arsenic (As) is a naturally occurring element found in the Earth's crust. However, human activities such as discharging of contaminated wastewater into surface water can increase its concentration in the environment. An average arsenic concentration of 0.15 mg/L was detected during the wet season in the dam water while in the dry season, an average of 0.004 mg/L was recorded (Table 4.2b). The one-way ANOVA results indicated statistically significant seasonal variation, $p = 0.000$ (Table 4.2c) while there was no statistically significant difference in the spatial variation (Table 4.2d). The concentration of As in the dam during the wet season did not meet the SANS:241 and WHO limit of arsenic in drinking water. High As concentration in the dam especially during the wet season could be attributed to As pollution by wastewater effluent being discharged into the dam. It can be noted that although the As concentrations in the wastewater effluent during the dry season were within the acceptable DWA standards of arsenic in treated wastewater effluent, higher As concentrations (0.17 mg/L) were recorded during the wet season (Table 4.2a). The presence of high As concentrations in the wastewater effluent indicates the pollution of wastewater influent by arsenic-containing compounds (Tchounwou *et al.*, 2012) and the inability of the Mmabatho WWTW to remove it from the wastewater.

Heavy metal pollutants in water are readily taken up by fish, and hence, the presence of As in the dam even at low concentrations could affect the fish's immune systems. According to Nayak *et al.* (2007), even a short-term exposure of fish to As hinders the fish's ability to fight and eradicate both viral and bacterial infections, thereby making consumers of such fish susceptible to opportunistic pathogenic infections. The WHO (2010) reported some examples of the human effects of consumption of water contaminated with As such as skin lesions, skin cancer and lung cancer among other cancers, disorders of the nervous system, heart attack which could subsequently lead to death. Therefore, As concentration in the dam water, especially during the wet season, renders the water unsuitable for its designated uses.

4.3.1.2 Cadmium

Cadmium (Cd) occurs naturally in the Earth's crust and its ores are closely associated with zinc, lead and copper (Järup, 2003). Natural (10%) and anthropogenic (90%) activities may lead to the introduction of cadmium in the environment (Okada *et al.*, 1997). Cd is regarded among the list of the most toxic heavy metals even at low concentrations (Kumar & Singh, 2010). SANS:241 (2015) and WHO (2011) stipulates that the minimum concentration of Cd in wastewater effluents should be <0.01 mg/L and <0.003 mg/L in drinking water, given its toxic nature. No Cd compounds were detected in the wastewater effluent and dam water during both sampling seasons (Table 4.2a and Table 4.2b) and as such, the seasonal and spatial significant differences in Cd concentrations in the dam water were not computed.

The determination of the pollution level of Cd in water is crucial to the safety of both the human and aquatic health. Exposure of aquatic organisms to surface water containing various levels of Cd may lead to its bioaccumulation in fish organs and tissues and this could ultimately affect fish consumers along the food web (Perera *et al.*, 2015). Continuous ingestion of water and/or fish containing high levels of Cd could lead to a deficiency of copper and zinc, which are essential in the biochemical functions in humans and animals (Kumar & Singh, 2010; Soetan *et al.*, 2010). Several negative human health impacts have been associated with Cd exposure such as kidney failure, bone damages, respiratory failure and even cancer (Bernard, 2008; Godt *et al.*, 2006). Therefore, the results from the dam water indicate that there are potentially no health risks associated with the Cd.

4.3.1.3 Copper

Copper (Cu) is a reddish trace element that occurs naturally in rocks, soil and surface water. The presence of Cu in surface water is as a result of natural and anthropogenic sources. The concentration of Cu in the wastewater effluent was 0.02 mg/L during the wet season while no Cu was detected during the dry season (Table 4.2a). The detected Cu concentration in the wastewater effluent during the wet season was above the recommended DWA Cu limit in wastewater effluent. Its presence in wastewater effluent could be due to the contamination of the municipal wastewater stream by industrial wastewater (Biswas & Mishra, 2016). Analysis of Cu concentrations in the dam water found an average of 0.02 mg/L during the wet season and 0 mg/L during the dry season (Table 4.2b). The detected concentrations were below the stipulated SANS:241 and WHO drinking water quality limit of 2 mg/L. The analysis of the seasonal and spatial variations of Cu in the dam water could not be computed because no Cu was detected in the dam water during the dry season (Table 4.2c).

Cu is an essential micro-nutrient for the biochemical functions in humans and animals (Soetan *et al.*, 2010). However, elevated concentrations are reported to cause serious health risks such as liver, brain and kidney damage, which could subsequently lead to death (Angelova *et al.*, 2011; Soetan *et al.*, 2010). The effects of high Cu concentrations on the ecosystems as well as individual aquatic organisms in surface water have also been documented (Solomon, 2009). Given the results obtained, it can be noted that Setumo dam generally poses no potential health risks emanating from Cu.

4.3.1.4 Iron

Iron (Fe) is one of the most highly abundant metals in the Earth's crust. The presence of Fe in surface water could be as a result of geological activities or anthropogenic influences, however, its concentration in water is low given its low solubility (Xing *et al.*, 2006; Harris, 1992). Fe concentration in the wastewater effluent was 0.02 mg/L and 0.04 mg/L during the wet and the dry seasons respectively (Table 4.2a). In the dam water, Fe concentrations had an average of 0.0075 mg/L during the wet season and 0.014 mg/L during the dry season (Table 4.2b). The

Fe concentrations in both the wastewater effluent and the dam water were within their respective stipulated limits. Analysis of the seasonal variation of Fe in the dam water showed that Fe varied statistically significant between the two sampling seasons, p = 0.023 (Table 4.2c) however, there was no statistically significant difference in the spatial variation (Table 4.2d).

Fe is an essential trace element that is utilised by both plants and animals for the sustenance of life. For instance, humans require Fe for blood oxygen synthesis and enzymatic activities, making it one of the crucial elements of the human body functions. However, Fe deficiency in humans could cause anaemia, which leads to general body weakness and fatigue whereas an excess of Fe in the body could cause liver cirrhosis, heart diseases, bone ailments and premature death among others (Kotze *et al.*, 2009). High doses of Fe have been associated with the Parkinson and Alzheimer's diseases, and are also reported to interfere with Cu absorption especially in infants (Angelova *et al.*, 2011; Kotze *et al.*, 2009). In surface water, high Fe concentrations alter the colour of water to brown, affects the biogeochemical cycling of organic matter, nitrogen and phosphates, and may resultantly become toxic to aquatic biota either directly or indirectly (Kritzberg & Ekström, 2012). Based on the results obtained, it can be concluded that the dam water is safe from iron-related problems.

4.3.1.5 Lead

Lead (Pb), like other heavy metals, is a naturally occurring element found in trace amounts in the Earth's crust. The occurrence of Pb in the environment (air, soil, and water) is mainly from anthropogenic activities such as industrialisation and mining. Given the health risks associated with human exposure to large amounts of Pb, the present study analysed for its concentration levels in the wastewater effluent and dam water. The results in Table 4.2a show that the wastewater effluent had an average Pb concentration of 0.03 mg/L during the wet season and 0.11 mg/L during the dry season. These values indicate a high Pb concentration in wastewater effluent above the permissible limits. The high Pb concentrations in the wastewater effluent may be due to the contamination of wastewater influent with Pb-containing compounds (Tchounwou *et al.*, 2012) that are washed into wastewater drainage pipes during rainfall and an inability of the Mmabatho WWTW to remove the Pb from the wastewater.

Overall average Pb concentrations of 0.115 mg/L and 0.0075 mg/L were detected in the dam water during the wet and the dry seasons respectively (Table 4.2b). The concentration of Pb in the dam water during the wet season was above the SANS:241 and WHO drinking water acceptable limits and this could be attributed to contamination by wastewater effluent of high Pb concentration (Table 4.2a). The one-way ANOVA test confirmed a seasonal variation of Pb in the dam water, p = 0.0023 (Table 4.2c), however, there was no statistically significant difference spatially, p = 0.833 (Table 4.2d).

High concentrations of Pb are known to be cumulative toxins that affect humans, animals and the aquatic ecosystem. Concentrations of Pb exceeding 0.5 mg/L in surface water are reported to inhibit enzymatic functions in algae photosynthesis, hence leading to a decrease in algae growth and consequently affecting the entire aquatic ecosystem (Solomon, 2009). In humans, exposure to elevated levels of Pb is reported to cause severe health problems especially in children and pregnant women (Hanna-Attisha *et al.*, 2016; Bakhireva *et al.*, 2013). Health issues such as kidney dysfunction, neurological disorder, and severe or permanent brain damage have been associated with Pb poisoning (Duruibe *et al.*, 2007). Hence, the results obtained here show that the dam water is potentially safe from Pb poisoning except during the wet season.

4.3.1.6 Manganese

Manganese (Mn) is another naturally occurring element that can be found in soil, water, food and predominantly, in rocks (Williams *et al.*, 2012). Anthropogenic sources such as municipal wastewater discharge, sewage sludge, mining and mineral processes can also lead to the introduction of Mn in the environment (Howe *et al.*, 2004). The analysis of Mn concentrations in wastewater effluent indicated a consistency in its concentration (0.04 mg/L) during both sampling seasons and the results were within the permissible limits in treated wastewater effluent (Table 4.2a). No Mn was detected in the dam water during both sampling seasons (Table 4.2b) and hence seasonal and spatial statistical significant differences in Mn in the dam water could not be computed. The absence of Mn in the dam water indicated the dilution of the Mn in the wastewater effluent after mixing with the dam water. Although Clement Associates (1995) reported that there is a direct relationship between the concentration of nitrates and Mn in water, in the present study, the presence of nitrates during the wet season (Table 4.3b) had no effect on the concentration of Mn in the dam water.

Mn is an essential element that is required in trace amounts by all living organisms. It is utilised by living organisms for both growth and development. Although trace levels of Mn are essential for human health, elevated levels are reported to cause significant health effects such as neurological deficits in humans and animals (Bouchard *et al.*, 2011; Reaney *et al.*, 2006). At concentrations of about 1 mg/L, Mn is also known to become toxic to aquatic organisms (Howe *et al.*, 2004). Health effects on the physiological processes and fitness of aquatic organisms have been documented (Baden & Eriksson, 2006). Therefore, it can be concluded that there is no imminent risk of Mn and no significant health risks to the aquatic organisms in Setumo dam and the surrounding communities utilizing water from the dam.

4.3.1.7 Nickel

Nickel (Ni) occurs naturally in the environment with small amounts found in the air, water, soil and with the majority of it found in food (Cempel & Nikel, 2006). In the present study, no Ni was detected in the wastewater effluent during both sampling seasons (Table 4.2a). There are no DWA standards for Ni in wastewater effluent before the water is discharged into receiving surface water bodies. The overall average Ni concentrations in the dam water were 0.063 mg/L and 0 mg/L during the wet and the dry seasons respectively (Table 4.2b). These concentrations conformed to the SANS:241 and WHO acceptable limits. Seasonal variations in Ni concentrations in the dam water were found to be statistically significant, p = 0.004 (Table 4.2c) but no statistically significant spatial variations were observed, p = 0.859 (Table 4.2d).

Although Ni naturally occurs in the environment, anthropogenic activities such as the discharge of wastewater effluent, mining and industrialisation could lead to its bioaccumulation in the environment. Human exposure to high Ni concentrations could cause some severe health-related problems such as liver and lung damage, gastrointestinal, respiratory, renal and skin issues (Dhokpande *et al.*, 2013). The majority of these health issues usually occur as a result of the interference of Ni with the metabolism of other essential trace metals such as copper, Fe, Mn, and zinc (Cempel & Nikel, 2006). Due to its high solubility, Ni easily accumulates in aquatic plants hence making the aquatic ecosystem prone to the effects of Ni pollution (Cempel & Nikel, 2006). However, given the fact that the Ni concentration in the dam was within permissible limits, it can be said that the water is fairly safe from Ni pollution.

4.3.1.8 Zinc

Zinc (Zn) is also found in the Earth's crust, with its main sources in the environment stemming from natural and anthropogenic activities. Anthropogenic sources such as the discharge of domestic and industrial wastewater effluents, and mining, however, outweigh the natural sources. Zn can be found in air, water and soil with the latter being its greatest source. Zn concentration in the wastewater effluent was 0.04 mg/L during the wet season and 0.13 mg/L during the dry season (Table 4.2a). Zn concentration in the wastewater effluent during the wet season was within the permissible limit and not during the dry season. In the dam water, an overall average of 0.043 mg/L and 0.13 mg/L of Zn during the wet and dry seasons respectively were recorded (Table 4.2b). The concentrations of zinc in the dam water during both sampling seasons were within the SANS:241 and WHO stipulated standards for drinking water quality. Seasonal variation of Zn in the dam water was found to be statistically significant, p = 0.000 (Table 4.2c) while spatially, there was no statistically significant difference (Table 4.2d).

Although Zn naturally occurs in water, the physicochemical properties of water can determine its potential of becoming toxic to the aquatic environment. Even though aquatic animals require certain quantities of Zn for their growth, overexposure to Zn could lead to its bioaccumulation in their tissues and in turn, bio-magnified up the food chain either directly or indirectly (Murugan *et al*., 2008). Also, elevated concentrations of Zn in surface water are reported to increase the toxicity of Cd to aquatic invertebrates (Kumar & Singh, 2010). In humans, Zn is essentially non-toxic, however, acute exposure to it can present health damages to the brain, respiratory tract, gastrointestinal tract and the reproductive system (Plum *et al*., 2010). According to Angelova *et al*. (2011), although zinc helps balance Cu in the human body, excess intake for a lengthy period of time can lead to Cu deficiency. It can, therefore, be concluded that the dam water does not present Zn related issues but continuous exposure could be a health concern.

Overall, the heavy metal concentrations in the wastewater effluent, As, Cu, and Pb during the wet season; and Pb and Zn during the dry season were above the permissible limits. Most of the heavy metals in the dam water were within the permissible limits during both sampling seasons except for As and Pb during the wet season, whose concentrations were slightly above the acceptable standards.

The following section presents and discusses the nutrient concentrations in both the wastewater effluent and dam water samples.

4.3.2 Nutrient analyses

The analysis of the concentration of nutrients (nitrates and phosphates) in both the wastewater effluent and dam water samples was done as indicated in Sections 3.3.2.2 and 3.3.2.3. The results obtained for the first part of objective 2 of this study, which was to analyse for the presence of nutrients are shown in Tables 4.3a and 4.3b. A high concentration of nitrates in the wastewater effluent was recorded during the wet season while the phosphate concentration was above the DWA wastewater effluent quality guidelines during both seasons (Table 4.3a). The analysis of dam water indicates a high concentration of nitrates during the wet season compared to the dry season while the phosphate concentrations during both seasons were below 0 mg/L except for point C, which recorded 2.165 mg/L during the wet season (Table 4.3b). One-way ANOVA results indicated that at 95% confidence level, only nitrates have significant seasonal variations (Table 4.3c) while both the nitrates and phosphates did not have any significant spatial variation (Table 4.3d).

Table 4.3a: Nitrates and phosphates concentrations in the wastewater effluent samples during the wet and the dry season

	Nitrates (mg/L)		**Phosphates (mg/L)**	
	Wet	Dry	Wet	Dry
Point B	**15.2±0.10**	0.47±0.06	**3.82±0.013**	**3.57±0.009**
DWA	**1.5**		**≤2.5**	

Table 4.3b: Nitrates and phosphates concentrations in the dam water samples during the wet and the dry season

	Nitrates (mg/L)		**Phosphates (mg/L)**	
	Wet	Dry	Wet	Dry
Point A	10.97±0.21	1.27±0.06	0.14±0.004	0.13±0.002
Point C	**31.67±3.51**	0.4±0.1	2.17±0.001	0.15±0.001
Point D	**12.03±0.15**	0.77±0.06	0.22±0.003	0.11±0.001
Point E	**11.27±0.12**	0.4±0.1	0.15±0.001	0.08±0.002
Point F	**12.17±0.12**	0.87±0.06	0.07±0.001	0.04±0.002
Point G	**19.27±0.06**	0.83±0.06	0.05±0.001	0.05±0.002
Point H	**19.03±0.06**	0.67±0.06	0.05±0.002	0.04±0.002
Point I	**19.47±0.06**	1.03±0.06	0.08±0.002	0.06±0.004
SANS:241	**≤11**		**Not Stated**	
WHO	**11**		**Not Stated**	

Table 4.3c: ANOVA values showing the seasonal variation of the nutrients in the dam water

Parameters	Sum of Squares	df	Mean Square	F	Significance
Nitrates	1050.408	1	1050.408	42.207	0.000
Phosphates	0.323	1	0.323	1.212	0.289

Table 4.3d: ANOVA values representing the spatial variation of the nutrients in the dam water

Parameters	Sum of Squares	df	Mean Square	F	Significance
Nitrates	167.981	7	23.997	0.156	0.988
Phosphates	2.014	7	0.288	1.128	0.430

4.3.2.1 Nitrates

Nitrates (NO_3) are naturally occurring elements that can be found in different forms in water, air, soil and plants (Mussa *et al*., 2009). The presence of nitrates in the environment can be as a result of natural and/or anthropogenic activities (Badruzzaman *et al*., 2012). The present study found that the wastewater effluent had an average nitrate concentration of 15.2 mg/L during the wet season and 0.47 mg/L during the dry season (Table 4.3a). The nitrate concentrations during the wet season were above the DWA recommended limit of 1.5 mg/L in wastewater effluent before discharge into surface water. These results indicate an ineffectiveness in the removal of nitrates during the wastewater treatment processes and this could affect the quality of water in the dam into which the effluents are discharged.

The overall average of nitrate concentrations in the dam water during the wet and the dry seasons was 16.99 mg/L and 0.78 mg/L respectively (Table 4.3b). Seasonal variations of nitrates were found to be statistically significant at $p = 0.000$ (Table 4.3c) while there exists no statistically significant spatial variation, $p = 0.289$ (Table 4.3d). A general observation at all sampling points was that unlike during the dry season, nitrate concentration during the wet season was above SANS:241 and WHO acceptable nitrate limits in drinking water. The high concentration of nitrates during the wet season could also be attributed to the heavy rains received during the 2016/17 rainfall season (SAWS, 2017) that drained nutrients from the surrounding land and into the dam (Kleinman *et al*., 2006). Also, it was observed during the site visits that there were heaps of fresh and dried up wastes from the cattle that graze around the dam. These cattle wastes are subsequently drained into the dam during the wet season and this may lead to an increase in the nitrate concentration in the dam water.

The notably higher nitrate concentrations at Point C during the wet season could be as a result of the summation effect of nutrients from the wastewater effluent and the decomposition of organic matter in the dam. Point C is an intersection point between wastewater effluent from the WWTW and the dam water (Figure 3.1). In water, nitrates are utilised by aquatic plants as nutrients to facilitate their growth. However, excess nutrients in the dam water could rapidly accelerate the growth of algae and hence result in eutrophication. Highly eutrophic water affects the penetration of sunlight into the water, leading to the death of aquatic organisms as result of lack of dissolved oxygen (Shock & Pratt, 2003), consequently damaging the water ecosystem and gradually degrading water functions (Yang *et al.*, 2008). In humans, the consumption of water with high nitrate concentrations may cause methemoglobinaemia which is fatal, especially in babies under the age of 6 months (Harper *et al.*, 2017). Based on these results, the water in the dam is therefore not safe for aquatic well-being and domestic purposes, especially during the wet season.

4.3.2.2 Phosphates

Phosphates ($PO^{-3}{}_4$) in surface water are essential nutrients for aquatic plant and animal growth (Mussa *et al.*, 2009). However, their build-up in surface water may accelerate the ageing process of the ecosystem (Van Ginkel, 2011). Analysis of the wastewater effluent revealed a high concentration of phosphates during both seasons; 3.82 mg/L (wet season) and 3.57 mg/L (dry season) and these concentrations are above the DWA wastewater effluent acceptable quality limit of 2.5 mg/L (Table 4.3a). The detection of high phosphate concentrations during both sampling seasons demonstrates the inefficient removal of phosphorus from wastewater before it is discharged into surface water. The findings of this study are in agreement with Harding & Thornton (2014), who reported that the WWTPs in South Africa do not focus on the removal of phosphorus in wastewater.

On the other hand, the average concentration of phosphates in the dam water was 0.37 mg/L during the wet season and 0.08 mg/L during the dry season (Table 4.3b). The phosphate concentrations in the dam during both sampling seasons was below 1 mg/L except for point C during the wet season and this could be due to its low solubility in water (Fadiran *et al.*, 2008). Even though there are no SANS:241 and WHO drinking water quality standards for phosphate in water, it was observed that their concentration was higher during the wet season contrary to the findings by Munyati (2015). This could be attributed to the heavy rains received during the wet season (rainfall season 2016/17) that may have drained nutrients from the surrounding land into the dam. The results from one-way ANOVA revealed no statistically significant difference between the seasonal and spatial variation of phosphates (Table 4.3c; Table 4.3d).

Although no human or animal health related issues have been reported due to the consumption of water that contains high concentrations of phosphates, their high concentrations in surface water affect the overall health of surface water ecosystems. Excess phosphate concentrations in surface water alter the composition of aquatic plants by stimulating the growth of algal blooms (Bornette and Puijalon, 2011). This accelerates the ageing process of surface water as a result of eutrophication, an issue that greatly affects South Africa's water resources (Van Ginkel, 2011).

The following section (4.4) presents and discusses the bacteria counts and isolates detected in the wastewater effluent dam and dam water during both sampling seasons.

4.4 Bacteriological analyses

Wastewater effluent and dam water samples collected for the study were subjected to microbiological analyses in order to determine the level of bacteria contaminants (heterotrophic, total coliform and faecal coliform counts) as indicated in Section 3.3.2.4. These results represent the second part of objective two, which was to analyse for the presence of microbes. Tables 4.4a and 4.4b show the results of the bacteria counts in the wastewater effluent and dam water samples analysed during the wet and the dry seasons respectively. Results for both sampling seasons indicate very high concentrations of heterotrophic, total and faecal coliform bacteria. These bacteria are seen to greatly exceed their respective wastewater effluent and water quality standards.

Table 4.4a: Bacteria counts in the wastewater effluent samples during the wet and the dry season

	Heterotrophic bacteria (cfu/100mL)		**Total coliform bacteria (cfu/100mL)**		**Faecal coliform bacteria (cfu/100mL)**	
	Wet	Dry	Wet	Dry	Wet	Dry
Point B	10000±849	1775±460	4325±318	3400±424	5900±566	2975±318
DWA	**Not stated**		**Not stated**		**0**	

Table 4.4b: Bacteria counts in the dam water samples during the wet and the dry season

	Heterotrophic bacteria (cfu/100mL)		**Total coliform bacteria (cfu/100mL)**		**Faecal coliform bacteria (cfu/100mL)**	
	Wet	Dry	Wet	Dry	Wet	Dry
Point A	5050±778	1325±318	3325±460	1750±141	5150±707	1325±460
Point C	3550±495	2050±495	1650±283	900±212	1625±460	1175±248
Point D	3575±813	1625±601	2175±318	950±212	6175±1450	1175±318
Point E	3150±849	1325±460	1875±743	725±389	2900±212	1225±248
Point F	5050±1344	3150±1061	2650±636	1675±530	3375±743	1600±212
Point G	2900±566	2700±707	2200±778	1650±283	3500±141	1800±283
Point H	5675±1096	2200±778	4825±318	3625±743	6425±177	5225±743
Point I	5075±813	1425±389	3725±460	2050±177	3500±636	1725±460
SANS:241	**< 100000**		**< 10**		**0**	
WHO	**≤ 1000**		**0**		**0**	

Table 4.4c: ANOVA values showing the seasonal variation of the bacterial counts in the dam water

Parameters	**Sum of Squares**	**df**	**Mean Square**	**F**	**Significance**
Heterotrophs	20759414.063	1	20759414.063	25.945	0.000
Total coliforms	5175625	1	5175625	5.117	0.040
Faecal coliforms	18922500	1	18922500	8.115	0.013
E. coli	3.063	1	3.063	6.726	0.021

Table 4.4d: ANOVA values representing sample point variation of the bacterial counts in the dam water

Parameters	**Sum of Squares**	**df**	**Mean Square**	**F**	**Significance**
Heterotrophs	5807773.438	7	829681.919	0.254	0.956
Total coliforms	13653593.75	7	1950513.393	2.746	0.090
Faecal coliforms	24933125	7	3561875	1.069	0.458
E. coli	4.938	7	0.705	1.254	0.376

Heterotrophic plate count, total coliform and faecal coliform bacteria are generally used to assess the microbial quality of water and to detect the presence of other pathogenic microorganisms in the water (Verhille, 2013). National and international standards stipulate that there should be zero faecal coliform bacteria in every 100 mL of water that is intended for drinking (SANS:241, 2015; WHO, 2011). Although there are no DWA guidelines for an acceptable number of heterotrophs and total coliform counts in wastewater effluent, the results shown in Table 4.4a, generally show that the wastewater effluent from the Mmabatho WWTW contains exceedingly high bacterial counts during both sampling seasons. A general trend that was identified in the study was that the bacteria counts for the wastewater effluent and dam water samples analysed in the wet season were higher than those during the dry season.

Heterotrophic bacteria count in the wastewater effluent was 10,000 cfu/100 mL during the wet season and 1775 cfu/100 mL during the dry season (Table 4.4a). In the dam water, an overall average of 4253 cfu/100 mL and 1975 cfu/100 mL of heterotrophic bacteria was found during the wet and the dry seasons respectively (Table 4.4b). Seasonal variation of heterotrophic bacteria in the dam water was found to be statistically significant, $p = 0.000$ (Table 4.4c) while there was no statistically significant difference in the spatial variation (Table 4.4d). Total coliform bacteria count in the wastewater effluent was 3400 cfu/100 mL during the wet season and 4325 cfu/100 mL during the dry season (Table 4.4a). In the dam water, the average total coliform bacterial counts were 2803 cfu/100 mL during the wet season and 1666 cfu/100 mL during the dry season (Table 4.4b). Both seasonal and spatial variations of total coliforms in the dam water were found to be statistically significant, $p = 0.040$ and $p = 0.090$ respectively (Table 4.4c; 4.4d).

The faecal coliform in the wastewater effluent had an average count of 2975 cfu/100 mL and 5900 cfu/100 mL during the wet and the dry seasons respectively (Table 4.4a). In the dam water, an overall average of 3528 cfu/100 mL and 1906 cfu/100 mL of faecal coliforms were found during the wet and the dry seasons respectively (Table 4.4b). Both the wastewater effluent and dam water faecal coliform counts exceeded their respective standards of 0 cfu/100 mL. Faecal coliform bacteria in the dam water depicted statistically significant seasonal variations, $p = 0.013$ (Table 4.4c), but no statistically significant spatial variations were observed (Table 4.4d). The high number of faecal counts in the dam water during both seasons could be attributed to faecal contamination by the faeces from the cattle grazing around the dam. Cattle wastes are subsequently washed into the dam during rainfall events and this could probably explain the presence of higher bacterial counts during the wet season.

A plausible explanation for the presence of high numbers of total and faecal coliform bacteria in the dam water during both sampling seasons could be due to the continuous discharge of wastewater effluent that is of poor bacterial quality (Table 4.4a). The bacterial results of the wastewater effluent were similar to those obtained by Hendricks and Pool (2012). These authors detected the presence of coliform bacteria of more than 1000 cfu/100 mL in wastewater effluent from a WWTP that employed the same treatment process as that of the Mmabatho WWTW. The results demonstrate that this type of wastewater treatment process is not efficient enough in the elimination of pathogenic microorganisms from wastewater.

The higher bacterial counts, especially during the wet season, could be attributed to the rainfall runoffs that drain huge quantities of nutrients from the surrounding land and into the surface water (Kleinman *et al.*, 2006), hence providing bacteria with sufficient nutrients to multiply. This is reflected by the high concentration of nutrients recorded during the wet season (average nitrates of 16.99 mg/L), (Table 4.3b). This average was noted to exceed the SANS:241 and WHO drinking water quality standard for nitrate (11 mg/L). Despite the heavy rains during the wet season, the high bacteria counts could also have been due to the higher water temperatures recorded. An average dam water temperature of 24.4°C and 15.5°C were recorded during the wet and dry seasons respectively (Table 4.1b). High temperatures in water have been reported to provide the optimum conditions for bacteria to proliferate (WHO, 2008).

Although total coliform bacteria and heterotrophs present no harm to humans and animals, their high occurrence in water indicates the potential presence of pathogenic organisms (USEPA, 2013). Besides, the detection of faecal coliform bacteria in the dam water indicates the potential of possible faecal contamination (Verhille, 2013). Therefore, water from these sources may pose severe health complications to humans if used for recreational activities and/or consumption if it is not properly treated. The following sub-sections present the results and discussions from the screening of *E. coli* and *Klebsiella* species, which were selected as the target pathogenic organisms in both the wastewater effluent and dam water.

4.4.1 Amplification of the bacterial 16S rRNA gene

As indicated under section 3.3.2.4, in order to screen for bacteria belonging to the family *Enterobacteriaceae*, a total of 49 isolates obtained during the wet and the dry seasons respectively, were selected from the m-FC media plates based on their colonial characteristics (Table 4.5). All the presumptive isolates were subjected to specific PCR designed to confirm their identities as *E. coli* and *Klebsiella* species *(K. pneumonia)* that belong to the family *Enterobacteriaceae*. As an internal control, bacterial 16S rRNA gene fragments were amplified and Figure 4.1 shows the amplified gene fragments from the representative isolates.

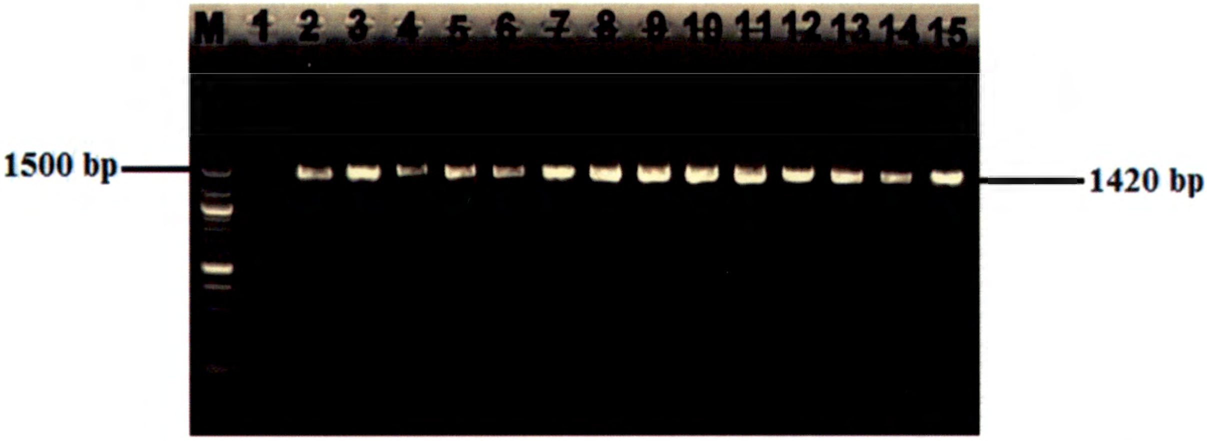

Figure 4.1: A 2% (w/v) agarose gel image depicting 16S rRNA gene fragments amplified from *E. coli* and *K. pneumoniae* isolates. Lane M = DNA marker (O'GeneRuler 100 bp base pairs DNA Ladder), Lane 1 = *Staphylococcus aureus* (ATCC 25923), Lane 2 = *K. pneumoniae* (ATCC 13883) positive control, Lane 3 = *E. coli* (ATCC 25922) positive control, Lanes 4 – 9 = 16S rRNA gene fragments from *E. coli* isolates and Lanes 10 – 15 = 16S rRNA gene fragments from *K. pneumoniae* isolates.

4.4.2 Identification of *E. coli* and *Klebsiella* using PCR analysis

The DNA samples extracted from the 49 isolates were used to confirm the identities of the *E. coli* and *Klebsiella* based on specific PCR analysis. DNA sequences that code for the *uidA* and *ntrA* genes respectively, were amplified using specific oligonucleotide primers (Table 3.1). The appropriate target sequences comprising a 600 bp *uidA* gene fragment for *E. coli* and a 90 bp *ntrA* gene fragment for *Klebsiella* species were amplified from the respective isolates. A total of 33 isolates were positive for the *uidA* gene and none tested positive for the *ntrA* gene during both sampling seasons (Table 4.5). Figure 4.2 shows the *uidA* gene fragments amplified from the isolates that were screened. The *Enterobacteriaceae E. coli* was dominant among the isolates obtained during the dry season (21/26) when compared to the wet season (12/23) (Table 4.5). Seasonal variation of *E. coli* in the dam water indicated statistical significance, p = 0.021 (Table 4.4c), whereas no statistically significant spatial variations existed, p = 0.376 (Table 4.4d).

Table 4.5: Proportion of *E. coli* and *Klebsiella* species detected using specific PCR analysis

Isolate ID	No. of Isolates from m-FC		*E. coli*		*Klebsiella*	
	Wet (NT)	Dry (NT)	Wet (NP)	Dry (NP)	Wet (NP)	Dry (NP)
A	6	4	2	2	0	0
B	2	4	2	4	0	0
C	2	3	2	3	0	0
D	3	3	2	3	0	0
E	2	2	1	2	0	0
F	2	2	1	2	0	0
G	3	3	0	2	0	0
H	2	3	1	2	0	0
I	1	2	1	1	0	0
TOTAL	**23**	**26**	**12**	**21**	**0**	**0**
	49		**33**		**0**	

NT = Number Tested; NP = Number Positive

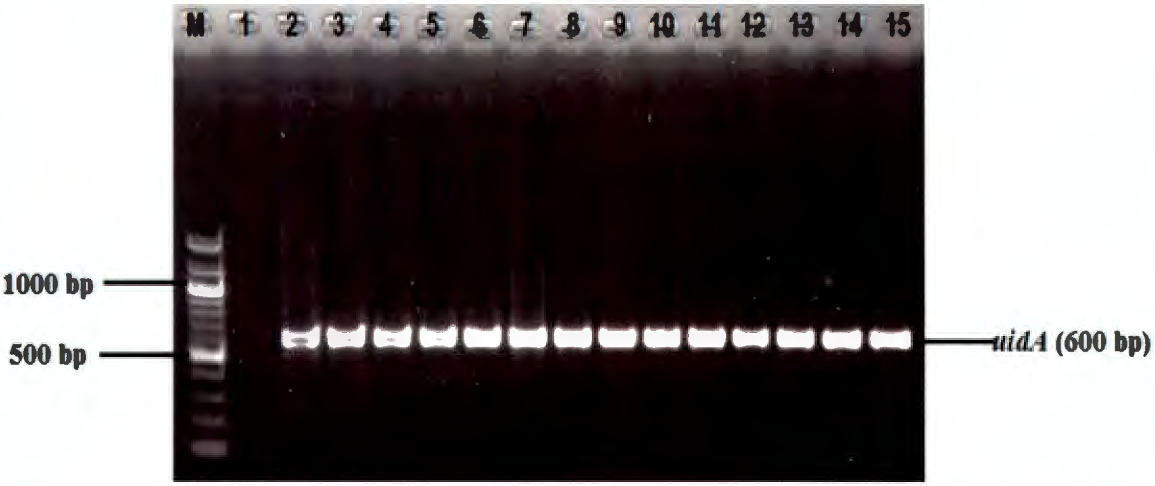

Figure 4.2: A 2% (w/v) agarose gel image depicting the *uidA* gene fragments amplified from all *E. coli* isolates. Lane M = DNA marker (O'GeneRuler 100 bp DNA Ladder), Lane 1 = *K. pneumoniae* (ATCC 13883) negative control, Lane 2 = *uidA* gene fragments amplified from *E. coli* (ATCC 25922) positive control and Lanes 3 - 15 = *uidA* gene fragments amplified from *E. coli* isolates.

Table 4.5 indicates that *E. coli* strains were isolated from the wastewater effluent (point B) that is continuously discharged into the dam. This finding indicates that the wastewater effluent is not efficiently treated to remove bacterial pathogens before it is discharged into the dam and hence a continuous source of bacterial contamination in the dam. These results are similar to those previously reported by Hendricks and Pool (2012), who found very high proportions of *E. coli* in treated wastewater effluent. The detection of *E. coli* in the dam water during both seasons is an indication of recent faecal contamination of the water (Table 4.4b; 5). The natural hosts of *E. coli* are humans and animals (Cabral, 2010). It was observed during the field visits and sample collection sessions that cattle graze around the dam and defecate around it. The cattle wastes are subsequently washed into the dam during rainfall events and this could also explain the presence of *E. coli* contamination in the dam.

Water in the dam is frequently used by the surrounding communities for household purposes, to water their cattle and also utilised for fishing. The discharge of wastewater effluent into surface water may transmit bacterial pathogens directly to humans, fish and subsequently to the fish consumers (Novotny *et al.*, 2004). The *E. coli* isolates obtained in this study may also pose severe health complications to humans and animals, especially if they harbour virulent gene determinants as was also noted by Dikobe *et al.* (2011). In addition, the *E. coli* strains may belong to recently identified pathogenic serotypes such as the *E. coli* O157:H7 and *E. coli* O104:H4 that have been reported to cause diseases in humans (Buchholz *et al.*, 2011; Ateba & Bezuidenhout, 2008). *E. coli* O157:H7, for instance, has been associated with waterborne incidences, which result in mortality in humans even in developed countries (Rangel *et al.*, 2005).

E. coli strains usually cause abdominal cramps, bloody diarrhoea and at times fever in their hosts (Cabral, 2010). Although *E. coli* has a short lifespan outside of its host, the right conditions such as temperature, pH and nutrients can provide very suitable conditions for sustained survival in the water. In addition, the pathogenicity of *E. coli* strains is usually amplified by its very low infectious dose (Lim *et al.*, 2010).

4.4.3 DNA sequencing

The identities of the isolates were further confirmed by the bacterial 16S rRNA sequence analysis and the results are presented in Table 4.6 below. All the sequences obtained from the study belonged to three *E. coli* strains as is found in the NCBI Gene-bank with accession numbers MH197072 (5/28), MH197073 (17/28) and MH197074 (6/28). However, variations in the percentage similarities were observed, with the majority of the sequences revealing 85% to 99% similarity with these *E. coli* strains except for sequence 24 that registered the lowest percentage similarity (75%). The results obtained, therefore, indicate that the water from Setumo dam serves as a potential risk factor for the transmission of bacterial pathogens especially, *E. coli* to individuals in the surrounding communities and thus poses severe public health threats.

Table 4.6: Identities of isolates based on bacterial 16S rRNA sequence data

Sequence ID	Sample Point	Organism	Strain	Accession No.	Percentage similarity	Isolation-Source
Seq1	A[a]	*E. coli*	NR_112558.1	MH197073	90	Water
Seq2	A[a]	*E. coli*	NR_114042.1	MH197074	88	Water
Seq3	D[a]	*E. coli*	NR_112558.1	MH197073	91	Water
Seq4	D[a]	*E. coli*	NR_112558.1	MH197073	90	Water
Seq5	B[a]	*E. coli*	NR_114042.1	MH197074	89	Wastewater effluent
Seq6	B[a]	*E. coli*	NR_024570.1	MH197072	89	Wastewater effluent
Seq7	F[a]	*E. coli*	NR_112558.1	MH197073	86	Water
Seq8	E[a]	*E. coli*	NR_112558.1	MH197073	89	Water
Seq9	H[a]	*E. coli*	NR_112558.1	MH197073	90	Water
Seq10	I[a]	*E. coli*	NR_114042.1	MH197074	96	Water
Seq11	C[a]	*E. coli*	NR_024570.1	MH197072	99	Water
Seq12	C[a]	*E. coli*	NR_114042.1	MH197074	90	Water
Seq13	A[b]	*E. coli*	NR_112558.1	MH197073	91	Water
Seq14	A[b]	*E. coli*	NR_112558.1	MH197073	85	Water
Seq15	B[b]	*E. coli*	NR_112558.1	MH197073	94	Wastewater effluent
Seq16	B[b]	*E. coli*	NR_114042.1	MH197074	90	Wastewater effluent
Seq17	D[b]	*E. coli*	NR_112558.1	MH197073	87	Water
Seq18	D[b]	*E. coli*	NR_114042.1	MH197074	90	Water
Seq19	E[b]	*E. coli*	NR_024570.1	MH197072	90	Water
Seq20	E[b]	*E. coli*	NR_112558.1	MH197073	87	Water
Seq21	F[b]	*E. coli*	NR_024570.1	MH197072	90	Water
Seq22	F[b]	*E. coli*	NR_112558.1	MH197073	87	Water
Seq23	G[b]	*E. coli*	NR_112558.1	MH197073	87	Water
Seq24	G[b]	*E. coli*	NR_112558.1	MH197073	75	Water
Seq25	H[b]	*E. coli*	NR_112558.1	MH197073	87	Water
Seq26	I[b]	*E. coli*	NR_112558.1	MH197073	91	Water
Seq27	I[b]	*E. coli*	NR_112558.1	MH197073	87	Water
Seq28	C[b]	*E. coli*	NR_024570.1	MH197072	90	Water

[a] = obtained during the wet season; [b] = obtained during the dry season

4.5 Risk assessment

A risk assessment of the pollutants in Setumo dam was done using the risk quotient (RQ) equation as indicated in section 3.5. Both the SANS:241 and WHO standards were used to determine the RQ of each of the pollutants (Table 4.7). However, the RQ of Fe and Zn with respect to their WHO water quality standards were not computed because there were no WHO guidelines proposed for them.

Table 4.7: Risk quotient of the different pollutants in Setumo dam during the wet and the dry season

Parameter	**Mean concentration**		**SANS:241 limit**	**SRQ**		**WHO limit**	**WRQ**	
	Wet	**Dry**		**Wet**	**Dry**		**Wet**	**Dry**
Nitrates (mg/L)	16.99	0.78	11	1.55*	0.07	11	1.55*	0.07
As (mg/L)	0.15	0.004	0.01	15*	0.4	0.01	15*	0.4
Cu (mg/L)	0.02	0	2	0.01	0	2	0.01	0
Fe (mg/L)	0.0075	0.014	2	0.004	0.007	-	-	-
Pb (mg/L)	0.115	0.0075	0.01	11.5*	0.75	0.01	11.5*	0.75
Ni (mg/L)	0.063	0	0.07	0.9	0	0.07	0.9	0
Zn (mg/L)	0.043	0.13	5	0.009	0.026	-	-	-
Faecal Coliform (cfu/100mL)	3528	1906	0	-	-	0	-	-

SRQ = Risk Quotient based on SANS:241 water quality standard; WRQ = Risk Quotient based on WHO water quality standard; *RQ>1 = health risk

Based on the results obtained, all the analysed pollutants carried no health risks during the dry season except for the faecal coliform bacteria. The RQ for faecal coliforms would not be computed because both SANS:241 and WHO stipulates that there should be 0 faecal coliforms per 100 mL of drinking water. The high faecal coliforms count already indicate significant health risks with the consumption of Setumo dam water. However, nitrates, As, and Pb posed significant health risks during the wet season in the order As > Pb > nitrates (Table 4.7). The RQ of Cd and Mn was not calculated because analysis of their presence in the dam water revealed that there was no Cd and Mn during both sampling seasons (Table 4.2b). The high RQ values of above 1 for nitrates, As and Pb indicate that there are potential health and ecological risks associated with these pollutants in water (Yan *et al.*, 2018). Consideration should be made in terms of the dose intake and the period of exposure to these pollutants in order to give a comprehensive health risk assessment (WHO, 2011).

4.6 Water Quality Index (WQI)

The calculation of the WQI of Setumo dam was done using the equation indicated in section 3.6 of this dissertation. The WQI values ranged from 25.04 in the wet season to 52.26 in the dry season (Table 4.8).

Table 4.8: The mean values for each dam test parameter, the WQI values and the water quality ratings for both sampling seasons

Sampling Season	Temp (C)	pH	TDS mg/L	Nitrates mg/L	Phosphates mg/L	Faecal coliforms cfu/100mL	WQI (%)	Water quality rating
Wet	24.3	8.94	316.7	16.99	0.37	3528	25.04	Bad
Dry	16.3	8.3	353	0.78	0.08	1906	52.26	Medium

It can be observed that the dam water samples during the wet season exhibited a greater percentage of poor quality compared to the dry season. Water with a WQI rating between 0 - 25% is regarded as highly polluted and unacceptable for human consumption while a WQI rating between 51 - 70% means that the water is slightly polluted and requires conventional treatment before human consumption (House & Ellis, 1987). Nitrates and faecal coliforms were identified as the main water parameters that resulted in low WQI values during the wet season, hence regarding the water quality as bad. On the other hand, faecal coliforms relatively influenced the WQI rating of the dam water during the dry season. Water with high levels of nitrates and bacterial counts cannot be considered good water because these contaminants have several health implications as discussed in sections 4.3.2.1 and 4.4 of this dissertation.

Similar observations were made by Wanda *et al.* (2015), who used WQI to assess the water quality of wastewater and drinking water sources in Mpumalanga and North West provinces of South Africa. The study showed that the quality of wastewater and drinking water sources in both provinces was either fair (23%) or medium (53%) and high levels of *E. coli*, nitrates and phosphates among others, were responsible for the low WQI ratings. The assessment of Setumo dam water quality using WQI values is the first such attempt in Mahikeng. Hence, the WQI computation shows that the water in Setumo dam is unsuitable for human consumption without prior treatment, and the continued reliance on the dam water by the surrounding communities could result in a never-ending cycle of poor health.

4.7 Pearson's correlation coefficient of wastewater effluent and dam water

The Pearson correlation matrix was used to determine if there were any significant difference between the pollutants found in the wastewater effluent and the pollutants in the dam water. The pollutants used in this matrix were nutrients, heavy metals and bacteria analysed in the present study. The values of the Pearson correlation coefficients (r) of the pollutant concentrations in both the wastewater effluent (point B) and the dam water (points A, C, D, E, F, G, H, & I) samples analysed during the wet and the dry seasons are presented in Tables 4.9 and 4.10 respectively.

Table 4.9: Pearson correlation coefficients (r) of the pollutants in the wastewater effluent and the dam water for the wet season of 2017

	Point B	**Point A**	**Point C**	**Point D**	**Point E**	**Point F**	**Point G**	**Point H**	**Point I**
Point B	-								
Point A	0.96	-							
Point C	0.99	0.93	-						
Point D	0.85	0.96	0.79	-					
Point E	0.97	0.99	0.95	0.94	-				
Point F	0.99	0.98	0.99	0.88	0.99	-			
Point G	0.93	0.99	0.89	0.97	0.99	0.96	-		
Point H	0.93	0.99	0.91	0.95	0.99	0.96	0.99	-	
Point I	0.98	0.97	0.97	0.86	0.98	0.99	0.96	0.97	-

Significance at $p \leq 0.05$

Table 4.10: Pearson correlation coefficients (r) of the pollutants in the wastewater effluent and the dam water for the dry season of 2017

	Point B	**Point A**	**Point C**	**Point D**	**Point E**	**Point F**	**Point G**	**Point H**	**Point I**
Point B	-								
Point A	0.98	-							
Point C	0.79	0.88	-						
Point D	0.86	0.93	0.99	-					
Point E	0.87	0.91	0.97	0.99	-				
Point F	0.80	0.90	0.99	0.99	0.96	-			
Point G	0.86	0.93	0.99	0.99	0.98	0.99	-		
Point H	0.97	0.92	0.77	0.85	0.89	0.76	0.84	-	
Point I	0.99	0.99	0.85	0.92	0.91	0.87	0.92	0.95	-

Significance at $p \leq 0.05$

Tables 4.9 and 4.10 indicate that there is a significant positive correlation between the pollutants in wastewater effluent and in the dam water. Strong positive correlations were derived between point B and point A (r = 0.96), point B and point C (r = 0.99), point B and point D (r = 0.85), point B and point E (r = 0.97), point B and point F (r = 0.99), point B and point G (r = 0.93), point B and point H (r = 0.93) and point B and point I (r = 0.98) during the wet season (Table 4.9). Similarly, the result of the Pearson correlation between the pollutants analysed during the dry season indicate that there is a strong positive correlation between the pollutants in point B and point A (r = 0.98), point B and point C (r = 0.79), point B and point D (r = 0.86), point B and point E (r = 0.87), point B and point F (r = 0.80), point B and point G (r = 0.86), point B and point H (r = 0.97) and point B and point I (r = 0.99) (Table 4.10).

These results agree with the findings of Edokpayi *et al.* (2017) and Momba *et al.* (2006). For instance, Momba *et al.* (2006) found a statistical relationship between the quality of wastewater effluent and that of the surface water serving as the disposal site. The authors hence concluded that the better the quality of the wastewater effluent, the better is the quality of the receiving water body. Tables 4.9 and 4.10 also demonstrate the existence of strong positive correlations in the dam water pollutants and the different dam sampling points during the wet and the dry seasons. This signifies that these pollutants emanate from the same pollution source (Khatoon *et al.*, 2013), which in this case, is wastewater effluent discharged by the Mmabatho WWTW. Given the significantly strong positive correlation between the pollutants in the wastewater effluent with those in the dam as indicated in Tables 4.9 and 4.10, it can, therefore, be concluded that the quality of any surface water serving as a wastewater effluent disposal site is highly dependent on the quality of the wastewater effluent being discharged into it.

4.8 Chapter summary

Fe, Mn and Zn in the wastewater effluent were within the DWA wastewater quality standards except for pH, EC, nitrates, phosphates, As, Cu, and Pb during the dry season; and phosphates, Pb and Zn during the wet season. No Cd and Ni were detected in the wastewater effluent during both sampling seasons. For the dam water, nitrates, As and Pb did not meet the SANS:241 and WHO drinking water quality standards during the wet season. The bacterial loads in both the wastewater effluent and dam water were exceedingly high during both sampling seasons. PCR analysis detected the presence of *E. coli* in both the wastewater effluent and dam water. All the physicochemical parameters (except for EC, TDS and phosphates) and bacteria in Setumo dam depicted statistically significant differences in their mean concentrations. Significant health risks associated with the dam water were attributed to the high concentration of nitrates, As, Pb and bacteria and hence the WQI computation regarded the dam water quality to be "bad" during the wet season and "medium" quality during the dry season. The degrading quality of the dam water was attributed to the poor quality of the wastewater effluent as per the Pearson correlation coefficient. It can, therefore, be concluded that the water in Setumo dam should be treated before consumption as it is not suitable for drinking and/or sustaining aquatic life.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Introduction

This chapter provides a summary of the main aim of this research. The significant findings from this study are highlighted, and the conclusions presented and discussed based on the study findings. The recommendations for areas of further studies are also made.

5.2 Research Summary

The principal aim of this study was to determine the impacts of wastewater effluent disposal by the Mmabatho WWTW on the quality of water in Setumo dam. The first objective of the study was to examine the levels of heavy metal concentration (As, Cd, Cu, Fe, Pb, Mn, Ni and Zn) which was done using an inductively coupled plasma optical emission spectroscopy instrument (ICP-OES). The second objective of the study was to analyse the presence of nutrients and microbes. The concentration of nutrients (nitrates and phosphates) was done using a spectrophotometer while analysis of the microbes (heterotrophic, total coliform and faecal coliform bacteria) was done using the plate culture method. Polymerase chain reaction (PCR) analysis was used for further identification of bacteria belonging to the family *Enterobacteriaceae*.

The third objective was to establish the seasonal and spatial variations of the pollutants in Setumo dam which was done using one-way ANOVA. The fourth objective was to assess the health risks associated with water in Setumo dam and this was done using the risk quotient equation. Analysis of the heavy metals, nutrients, and microbes was done for both the wastewater effluent and the dam water samples during the wet and the dry season. The Pearson correlation coefficient was used to determine whether there existed a correlation between the results obtained in the wastewater effluent and the dam water during both sampling seasons.

Based on the results obtained, it can be concluded that the effluent from the Mmabatho WWTW is of poor quality. Excluding temperature and TDS, whose DWA wastewater effluent quality standards were not available, only Fe, Mn, and Zn conformed to the DWA wastewater effluent quality standards during the wet season whereas, during the dry season, all the physicochemical parameters under study conformed to the set standards except for phosphates, Pb, and Zn.

Phosphates, Pb, heterotrophic bacteria, total and faecal coliforms did not conform to the DWA wastewater effluent quality standards during both sampling seasons. Further analysis of the identification of bacteria belonging to the family *Enterobacteriaceae* using PCR, revealed the presence of *E. coli* in the wastewater effluent during both sampling seasons, however, no *Klebsiella* was detected. The poor quality of the wastewater effluent indicates the insufficient treatment of the wastewater by the Mmabatho WWTW.

In Setumo dam, temperature, pH, EC, TDS, Cu, Fe, Ni, and Zn were within the SANS:241 and WHO drinking water quality standards during both sampling seasons except for nitrates, As, Pb, total and faecal coliforms, which were above standards during the wet season; and the total and faecal coliforms during the dry season. Identification of *Enterobacteriaceae E. coli* and *Klebsiella* revealed the presence of only *E. coli* in the dam water during both sampling seasons. An analysis of the seasonal and spatial variation of the water parameters in Setumo dam revealed statistically significant seasonal variations in all the analysed parameters except for EC, TDS, and phosphates. Spatial variations were only statistically significant in EC, TS and total coliform bacteria.

The risk assessment revealed significant health risks in relation to nitrates, As, Pb and faecal coliform bacteria during the wet season, with the later exhibiting health risks during both the wet and dry seasons. The water quality index revealed that the water quality in Setumo dam is bad during the wet season and of medium quality during the dry season. The Pearson correlation coefficient revealed that there were significantly strong positive correlations between the pollutants in the wastewater effluent and the dam water.

5.3 Conclusion

Based on the findings from this research, it can be concluded that Setumo dam water quality is poor and poses significant health risks, especially risks related to bacterial infections. Although factors such rainwater runoff and cattle grazing could have contributed to the poor quality of water in the dam, the discharge of wastewater effluent from the Mmabatho WWTW has a statistically significant positive impact on the quality of water in Setumo dam. The entrance of inadequately treated wastewater effluent into surface water sources contributes significant amounts of pollutants to the surface water and thereby posing some serious health and ecological concerns.

Additionally, it can be concluded that the water quality in Setumo dam is more deteriorated during the wet season relative to the dry season and this could be as a result of the occurrence of the heavy rains that were received in 2016/2017 rainfall season that was preceded by the drought of 2015/2016. Furthermore, this study has demonstrated that the wastewater effluent pollutants, which influence the quality of surface water in one season, may not necessarily be the same in another season. Therefore, there is an urgent need to protect surface water resources from all kinds of pollution, especially from those arising from anthropogenic activities, in this case, the discharge of insufficiently treated wastewater effluent.

5.4 Recommendation

The study, therefore, recommends that:

- There is a need for a continuous assessment of the wastewater effluent quality from the Mmabatho WWTW in order to establish whether it conforms to the DWA wastewater effluent quality standards.
- Further studies are needed to determine the main contributing types of pollutants from the wastewater effluent in Setumo dam.
- Consideration should be made in terms of the dose intake of the pollutants in Setumo dam and the period of exposure in order to make a comprehensive risk assessment.
- Studies should also be done on the perceptions and experiences of the communities utilising water in Setumo dam.
- The DWA Mahikeng can use the WQI to continually monitor the health status of Setumo dam, and in turn, inform the public of the status of their main source of water, and advice on the best management activities to improve its quality.

5.5 Limitation

Given the heavy rains that were received in 2017, and which were preceded by the drought of 2015/2016, the findings of this study present the worst-case scenario.

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