INVESTIGATING INDUSTRIAL EFFLUENT IMPACTS ON MUNICIPAL WASTEWATER TREATMENT PLANT

by

EUNICE CHIZUBE ILOMS

Student no: 50841289

Submitted in accordance with the requirements for the degree of

Master of Science

In the subject

Environmental Science

at the

UNIVERSITY OF SOUTH AFRICA

Supervisor : Dr O. O. Ololade

Co-supervisor : Dr R. Selvarajan

July 2018

DECLARATION

I declare that the work presented in this dissertation is to the best of my knowledge and belief original except as acknowledged in the text and that the material has not been submitted, either in whole or in part, for a degree at this or other universities. I also certify that I have complied with the rules, requirements, procedures and policies of the university.

Candidate **:** ILOMS EUNICE CHIZUBE

Student Number : 50841289

Signature : …………………….. **Date**: 2018

ACKNOWLEDGEMENTS

My first gratitude goes to God Almighty because of his grace in my life throughout this period of my studies and the supernatural strength he bestowed on me throughout my sleepless nights of putting this thesis together.

I wish to acknowledge the support and guidance of my supervisors Dr Olusola Ololade for her patience and dedication to see that this thesis is a success and my co supervisor Dr Ramganash Selvaranjan for his assistance throughout my laboratory work, I pray that God will bless you both.

I want to specially thank my friend Kalu Chimdi Mang whom I no longer see as a friend but a brother. I believe our coming together was divinely ordained by God. You are truly God sent and your tireless effort and sleepless night you spent proof reading and inputting in my work just to make sure that my work is always on per and is well appreciated. Your labor of love will never be forgotten. Also to my friend Grace Ijoma who is like a sister to me, who just like Kalu was there throughout to proof read my proposal and to make sure that it is in order, may God bless you.

I also want to thank my spiritual best friend Lebo Lekotoko and my pastor, Pastor Simon Okoye, and his family for their prayers, moral, emotional and spiritual support throughout my research work. Furthermore, I appreciate those who has supported me in different ways through advice, material provision for my laboratory work, emotional and moral support especially when I was devastated in the laboratory (Dr Tim Sibanda, Prof Khaya, Dr Stephen Taylor, Ishmail, Ntabaleng, Mpho. Late Taboga Mathiba, MaryAnn, Martha Shupping, Ogugua, Ebere,), Brandon my ex learner who helped to draw my proposal maps.

Also, I will like to acknowledge Dr Fred Kanu for his tireless support especially in my chapter 4 when it seems all hope was lost. To all those whose names I am unable to mention, who directly or indirectly has contributed to the success of my research, thank you. Also to Department of Education District D16 that approved my study leave through my principal Mr J. Motshitela, thank you all for helping me realised my dream

My thanks also goes to my kids Onyinye, Kosi, Divine, and Favour Iloms. Also to my older kids Kene Modebelu and Promise Ebuzeme and my sister inlaw Mrs G Ebuzeme. You are all indeed my joy and pride. Thank you all for being there for me especially in the most difficult times and for your patience and perseverance throughout my study. My prayer is that God will help me to repay you all for all the sacrifices you made throughout the period of my studies. To my mom Mrs Patience Onyedum and my 4 brother and 4 sisters especially Mrs Peace Modebelu for their support and prayers, most appreciated.

To my one and only darling and sweetheart Mr Chris Anayo Iloms, you are my heartbeats, my life line, and a rare gem, my reason for completing this masters because you stood by me through thick and thin, tough and difficult time. You were there to support me morally, emotionally, spiritually, financially and otherwise. I truly lack words to thank you for all your care, comfort and love you have shown me, I will never be able to pay you back, for you superseded diamond. My prayer is that God will keep us together till eternity, I love you unconditionally.

I will like to finally acknowledge the fund provider, University of South Africa, under the Emerging Researchers Support Programme.

Publications

A portion of this work has been presented in an international conference

Iloms E, Ololade O.O, Ramganesh S. 2018. Investigating Industrial Effluent Impacts on Municipal Wastewater Treatment Plant. In Burke ., Maphanga, R, van Rensburg J.J, Bogaers A, Das S. Proceedings of the 2018 International Women in Science Without Borders (WISWB) – Indaba Johannesburg First Edition, ISBN: 978-0-620-78656-0, pp. 87-88. https://researchspace.csir.co.za/dspace/bitstream/handle/.../Burke_20974_2018.pdf?

DEDICATION

This research work is dedicated to God Almighty. I also dedicate it to my husband Mr Anayo Chris Iloms who stood by me tirelessly throughout this study in all areas and to my late father Mr. Michael Onyedum who loved education so much and said his joy will be to see me become a professor.

TABLE OF CONTENT

LIST OF ACRONYMS

WHO-------------------------World Health Organization

SAGES------------------------South African General Effluent Standard

SANS-------------------------South African National Standards

ICP-OES---------------------- Inductively Coupled Plasma Optical Emission Spectrometry

WWTW------------------------Wastewater Treatment Work

PCR---------------------------Polymerase Chain Reaction

TDS----------------------------Total Dissolved Solids

BOD----------------------------Biological Oxygen Demand

COD---------------------------Chemical Oxygen Demand

EC------------------------------Electrical Conductivity

DO-----------------------------Dissolved Oxygen

MENA-------------------------Middle East and North Africa

KVIP--------------------------Kumasi Ventilated Improved pit

USEPA----------------------United State Environmental Protection Agency

WWTP-----------------------Wastewater Treatment Plant

BLAST------------------------Basic Local Alignment Search Tool

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Industrial effluents with high concentrations of heavy metals are widespread pollutants of great concerns as they are known to be persistent and non-degradable. Continuous monitoring and treatment of the effluents become pertinent because of their impacts on wastewater treatment plants. The aim of this study is to determine the correlation between heavy metal pollution in water and the location of industries in order to ascertain the effectiveness of the municipal waste water treatment plant. Heavy metal identification and physico-chemical analysis were done using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and multi-parameter probe respectively. Correlation coefficients of the measured values were done to investigate the effect of the industrial effluents on the treatment plants. Heavy metal resistant bacteria were identified and characterised by polymerase chain reaction and sequencing. Leeuwkuil wastewater treatment plants were effective in maintaining temperature, pH, and chemical oxygen demand within South Africa green drop and SAGG Standards whereas the purification plant was effective in maintaining the values of Cu, Zn, Al, temperature, BOD, COD, and TDS within the SANS and WHO standard for potable water. This findings indicated the need for the treatment plants to be reviewed.The industrial wastewater were identified as a point source of heavy metal pollution that influenced Leeuwkuil wastewater treatment plants and the purification plants in Vaal, Vereenining South Africa. *Pseudomonas aeruginosa, Serratia marcescens, Bacillus sp. strain* and *Bacillus toyonensis* that showed 100% similarity were found to be resistant to Al, Cu, Pb and Zn. These identified bacteria can be considered for further study in bioremediation.

Key words: Industrial effluent, waste water, treatment plants, heavy metals, physico-chemical parameters, heavy metal resistant bacteria

CHAPTER 1: INTRODUCTION

1.1 Background Information

Water is important for all life because it is needed to sustain life on earth. Its crucial role in our economy, food production, health, and environment cannot be over emphasised (Halder and Islam, 2015). Humans can withstand food starvation for several weeks but may not withstand water deficiency because it is constantly required for effective functioning of cells, tissues and organs in the body (Murray *et al*., 2000, Chinedu *et al.,* 2011). Safe drinking water is vital for improvement and public health because of the association of water to a significant number of diseases (Prüss *et al.,* 2002). The finite nature of water means that it is hydrologically recycled continuously through the atmosphere (Naidoo and Olaniran, 2014). According to the World Water Organisation (TWWO 2010), this limitation poses terrible implications for nearly 7 billion of the world's population with dire consequences globally. However despite this understanding the trend of pollution has continued unabated.

The increased rate of industrialisation in the world is believed to contribute to drastic pollution of water resources and South Africa is not an exception (Kamika and Momba, 2013). It is estimated that every day approximately two million tons of industrial and domestic wastes are disposed into waterbodies worldwide (Pacific Institute 2010; UN WWAP, 2003). This is as a result of the increasing industrialisations of most developing countries which have contributed significantly to water and land pollution (Alam, 2010; Kraemer *et al.,* 2001).

Industrial wastewater pollution is a notable problem in South Africa, where fresh water resources is in short supply. With just over 1200 m^3 of fresh water provided per person in a year for a population closed to 50 million, the country is on the verge of being classified as water stress according to internationally definition (Savenije and Van der Zaag, 2000).

Effluents generated from both industrial and domestic activities occupy the second position with respect to sources of water. Presently, the above mentioned activities are believed to contribute to chemical and microbial pollution of South Africa's sources of water (Van Vuuren, 2009; Momba *et al*., 2009; Kamika and Momba, 2013).

Most of the research conducted globally focus on the impact of industrialisation within the developed countries. However, many studies are now pointing out the impacts of environmental pollution in developing nations showing how pollution has contributed annually to the deaths and disabilities of millions of people especially in heavily populated urban areas (Mills-Knapp *et al*.,, 2012).

The rapid growth in the population of the world, and urbanisation including the expanding intensity of food production has taken its toll on water resources. Water abstraction for agricultural, mining, industrial, and domestic use has led to decline in the quality and quantity of water that affect organisms in the water bodies and also potable water available for human consumption (UNEP, 2008).

It has been realised that release of incompletely treated or untreated wastes loaded with non-biodegradable organics, heavy metals , algal nutrients and other toxicants will accelerate the decline of the quality of receiving water bodies (Olaniyi *et al*, 2012). Chindah *et al.* (2004) also affirm this finding by stating that poor water quality is principally caused by inadequate waste disposal methods used by most industries and untreated effluents from industries being discharged carelessly. The problem is compounded by the increased rate of unregulated and illegal discharge of effluents that contaminate water across national borders (Corcoran *et al.*, 2010). Therefore, there exist the need to treat wastewaters before discharging them into the environment in order to reduce pollution.

Many developing countries are faced with huge debts, population explosion, and ever-increasing premature urbanisation due to increased industrialisation which have resulted to the understanding of the relationship between the environment, public health, and pollution (Adebisi and Fayemiwo, 2010). While most pollution and production of waste are caused by activities of the industries, high environmental pollution results from industries with little or no control to pollution and lack of facilities for treating generated waste (WHO, 1982). Water treatment facilities that is supposed to treat effluents produce from these industries are not efficient and improved upon due to technical and financial constraints (Snyman *et al.*, 2006; Ujang and Buckley, 2002). Leeuwkuil Wastewater Treatment Plant in Vereenining, South Africa, cannot be exempted, as treated wastewater has not been tested to ascertain quality standard from June to September 2016 due to financial constrains by the municipality. In view of satisfying the increasing demands of the people, individuals have no alternative but to rely primarily on water of poor quality that are not considered safe for use. This situation at Vaal is in accordance with what is reported by Okonkwo (2010), Markandya (2004) and Aina and Adedipe (1996) in the case where there are insufficient resources to treat wastewater with the aim of providing quality drinking water needed by the people; resulting in their dependence on poor quality water for survival.

1.2 Problem Statement

There are several notable incidences in South Africa of direct discharge of industrial effluent into water bodies leading to pollution and in some cases heavy metal contamination (Ahmad *et al*., 2012; Akpor *et al*., 2014, Ntuli, 2012). Even in cases where the effluent water passes through the waste water treatment facilities, these treatment plants are ill-equipped to remove large quantities of biodegradable waste and recalcitrant heavy metals (Ntuli 2012; Mema, 2010; Morrison *et al*., 2001).

The occurrence of heavy metals in the soil and water is observably a recurrent problem with potential high toxicity to flora and fauna (Fonseca *et al*., 2006). Heavy metals are both carcinogenic and toxic (Krishnani *et al*., 2008). Despite the harmfulness of heavy metals to both macro and micro-organisms at high concentration, they have proved to accumulate over time, unlike other organic pollutants that can be degraded chemically or biologically (Fonseca *et al*., 2006).

The Vaal Triangle is notably a major industrial region of South Africa with industries such as the Iron and Steel, petroleum and coal oil companies, and gold mine industries. These industries fall under the catchment areas of the Leeuwkuil waste water treatment facility (Mahlaka, 2015; Tempelhoff *et al.,* 2007). All these industries use chemicals in their industrial processes that can be considered to be persistent and non-biodegradable. The Vaal triangle is historical known as a heavily polluted region as a result of significantly large quantity of industries sited in this region (Tempelhoff *et al.,* 2007) which informed its choice as the study area for this research. There is no sufficient studies to show the correlation between heavy metal pollution in water and the location of the industries.

The persistence occurrence of heavy metals in municipal effluent and their ability to bio-accumulate after treatment, emphasises the need for easy, cost-effective and biological methods to determine and control toxicity levels of industrial effluents and help minimise domestic households receiving polluted water. It is on the basis of the above reason that this study will be carried out to investigate the correlation between an increase in heavy metal pollution in water and the location of industries and to ascertain the effectiveness of the municipal water treatment.

1.3 Rationale of the Study

Industrial pollution is a continuous source of environmental degradation that affects land, water and air (Adebisi and Fayemiwo, 2010). Some industries often deliberately discharge untreated effluents into water bodies without adequate treatment (Akaninwor *et al.,* 2007). In other cases their reliance on waste water treatment facilities has proved ineffective. In both cases the effects are the same on water bodies and the end-users are affected by the consequences of industrial pollution.

It has become imperative that routine analysis for heavy metal presence and development of protocols that can readily determine the occurrence of recalcitrant heavy metals and other non-biodegradable compounds, be prioritise to enhance the tests for quality of water so as to ensure that end-users of water are provided with safe potable drinking water (Longe and Omole, 2008). For this reason this research is proposed as a means to, not only highlight the limitations of the wastewater treatment facilities in the removal of persistent heavy metals but also to elucidate the presence of the metals as a direct consequence of industrial pollution and to offer empirical data that can lead to changes being made to the existing treatment processes.

It has become necessary to ascertain regularly the quality of water because of the growing trend of poor individuals in the society locating informal settlements and farm steads along the river banks and downstream (Matowanyika, 2011; Chikoto, 2009). This is applicable in Leeuwkuil Treatment Plant where the final treated water from final effluent is used as a water source for animals in the farm as an alternative. It is obvious that any pollution of these water bodies directly impacts first-hand on these individuals living close to the polluted river (Matowanyika, 2011). The Vaal River is no exception although the final effluent is used for agricultural purposes including small scale farming and cattle rearing, the bioaccumulation of heavy metals within crops and livestock cannot be ignored as ultimately such produce will be consumed by people (Bhagirath and Ratna, 2002).

1.4 Significance of Study

This study will enhance the knowledge of the influence of industrial pollution on surface water, wastewater treatment plant and possible impacts on human health, especially in developing countries. Furthermore, it serves as a point of reference for industries with the aim of remedying problems of water pollution and provide effective ways of treating waste in their respective industries especially in South Africa industries.

1. 5 Research Questions

i. Do waste water released by the industries have an impact on municipal wastewater treatment plants effluent quality in the Vaal area?
ii. Is there a correlation between the quality of potable water and the effectiveness of treatment plants in the Vaal area?

1.6 Aim and Objectives

1.6.1 Aim

The aim is to determine the correlation between heavy metal pollution in water and the location of industries in order to ascertain the effectiveness of the municipal wastewater treatment plant.

To achieve this goal, the following specific objectives will be pursued:

1.6.2 Objectives

- Identify and quantify the presence of heavy metals and physico-chemical parameters in effluents from the Leeuwkuil Plant and Industries in the Vaal area.
- Identify and quantify the presence of heavy metals and physico-chemical parameters from Vaal River and potable water samples from the Vaal areas.
- Compare the results of heavy metal and physico-chemical parameters obtained from the effluents, Vaal River and potable water samples to current national and international standards.
- Isolate and characterise bacteria resistant to heavy metals found in the effluents, river and potable water samples from the Vaal area.
- Determine the correlation between the quality of potable water and the effectiveness of the Wastewater Treatment Plants in the study area.

1.7 Thesis Outline

This study investigated industrial effluent impacts on Leeuwkuil Municipal Wastewater Treatment Plant and identified the presence of heavy metals in effluent, potable water and five (5) industries that discharges effluent into Leeuwkuil Sewage Plant using physico-chemical and microbiological assessments. Also, to isolate and characterise bacteria resistant to heavy metals found in different effluent, river and potable water in the Vaal areas.

Chapter one covers the background study, problem statement, justification of the study, aim and objectives of the study.

Chapter two review relevant literature to the study aim from the global to the local context.

Chapter three explains methods and procedures employed in conducting this study the effect of effluent discharged on surface water quality is evaluated in terms of physico-chemical study, microbiological and molecular characterisation. It also contain the identification and quantification of the presence of heavy metals in the effluent from five (5) industries that discharges at Leeuwkuil Sewage Plant in the Vaal areas which were analysed using spectrophotometric techniques and supported by microbiological analysis of heavy metal resistant bacteria test.

Chapter four presents results of all the test carried out including physico-chemical, microbial and molecular characterisation in form of tables and graphs to ascertain the effectiveness of the water treatment strategy used in the study area and determine the underlying factors/causes that may lead to failure of the Vaal Municipal Water Treatment Plant.

Chapter five which is the final chapter, includes the summary, significance of the study, future research and conclusion.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Water resources on the planet have been affected greatly over the past few years as a consequence of human activities, which have affected basic water supply and drinking water quality globally (Martin, 2009; Jaishankar *et al.,* 2014). The faster deterioration of water quality mostly results from the discharge of ever increasing quantities of untreated or partially treated effluent that contains different contaminants resulting to water pollution (Baloyi *et al,* 2014; Morrison *et al.,* 2004). Polluted water affects different uses such as household water use, recreation, fishing, transportation, and commerce. Most health problems and diseases in different parts of the world are believed to be caused by untreated or inadequately treated wastewater discharged into water bodies resulting in the spread of diseases, and death of fishes and other forms of aquatic life (Sibeya, 2016).

The population of the world in urban centres are experiencing high rate of increments. This increment is intense in developing countries, where over 2 billion people are anticipated to inhabit by 2030 (United Nations 2012; Mateo-Sagasta *et al.,* 2015)**.** The cities found in those developing countries are believed to produce billions of tons of waste every year with sludge and wastewater inclusive. Wastewater from industrial and domestic activities in the world's view are regarded as the major sources of effluents due to high rate of industrialisation, and increase in the number of people (Akpor *et al*., 2014). Sewage discharges and industrialisation could be considered a main source of water pollution in both developed and developing countries as they contribute to eutrophication (Keller, 2012; Water Pollution Guide, 2008). The problem is more predominant in areas that use simple, inefficient and ineffective wastewater treatment systems. Eutrophication potential which results from the presence of nutrients in the effluent has been considered to be a relatively important environmental issue when performing an environmental evaluation of wastewater treatment plants (WWTPs) (Sibeya, 2016). Wastewater Treatment Plants discharge directly into water bodies and are therefore considered point sources of possible pollution. Therefore, regulating effluent is important as water pollution results to negative effect on the environment as a whole, including its effects on biotic and abiotic organisms. Naidoo and Olaniran (2014) pointed out that wastewater treatment plants are experiencing difficulties in treating wastewater resulting in the discharge of improperly treated effluents into the waterbodies. They further suggested the need to develop strict methods for monitoring effluents discharged into the water bodies. Hence water need to be treated in order to be free of microorganisms and chemical substances that can cause disease. Water purification is an important linkage between the promotion of public health and safety. The purification process consists of different stages and the stages are dependent on the kinds of contaminants present in the raw source of water. Sedimentation, flocculation, filtration and disinfection are the crucial stages involved in water purification. Generally, Municipal Wastewater Treatment Plants undergo stages of wastewater treatment and purification so as to ensure that clean and safe drinking water is provided to the population.

2.2 Wastewater Treatment Plant: The Global View

Waste from industries, domestic activities, storm water runoff, and commercial activities carried by water is termed wastewater. The number of people, and the combination of industrial and domestic activities determines the quantity and nature of wastewater generated (Naidoo and Olaniran, 2014). Naidoo and Olaniran (2014) further indicated that these activities affect their patterns of discharge and the chemical constituents of the wastewater. Treatment of wastewater has been in existence since the knowledge of humans and it is continuously under improvement (Angelakis and Snyder, 2015). Furthermore, the use of household wastewater on land is an ancient practice that has undergone different developmental stages. This has contributed to a better understanding of process, treatment methods and technology and the eventual development of better treatment methods resulting to good water quality (Paranychianakis *et al.*, 2015; Angelakis and Snyder, 2015).

Natural cycling was the predominantly used method of waste treatment by the first human communities as the waste produced by them were returned to the land and decomposed naturally (Lofrano and Brown, 2010). There were little disposal problems simply because they were small communities of nomads scattered over wide areas. The permanent settlements of people about 10,000 years signalled the establishment of a new era where agrarian way of life accompanied by ecological impacts were adopted (Lofrano and Brown, 2010). During the early civilisation, the use of dug holes as means of waste disposal were the prevailing means of waste disposal however, the health implications were not determined.

In Europe, 71% of most of the waste water produced are treated as a result of public health awareness, protection law governing the environment, advancement in technology, and most importantly, because most treatment plant were funded by most government (United Nation, 2012). Furthermore, the laws governing water and wastewater management plays essential role in enhancing treatment of wastewater in these regions for this laws are well structured and functional unlike in many parts of the world were implementation is a struggle (Sato *et al,* 2013).

In Russia Federations, the volume of wastewater treated in a year is about 14 Km^3 (UNDESA-DSD, 2004). About 28% of the treated wastewater were done in compliance to regulations that were in place while the rest is discharged into waterbodies when they were not properly treated. Sato *et al*. (2013) pointed out that poor management (60% of the treatment plants are overloaded), and dilapidated facilities (38% have been in operation for about 30 years and need rehabilitation) are the major factors for low efficiency of wastewater treatment plants. Inadequate water and wastewater management, and old wastewater treatment systems, are the major contributing factor to severe water pollution in Ukraine, Georgia (UNECE, 2003), and Caspian Sea (Stolberg *et al*., 2006). Financial provision and resource dissemination are essential for adequate management and updating of wastewater collection and treatment facilities in these regions.

The original approach adopted in the United States to dispose wastewater from urban homes was through septic tank or cesspools with underground tiles that drains wastewater into the ground via percolation (USEPA, 1992). This resulted in the pollution of groundwater used for water supply to the populace. In order to control the pollution, sewerage systems were constructed to drain wastewater from houses and other buildings into the water bodies that is at close proximity (USEPA, 1992). Sewerage lines, drainage systems and other infrastructure were constructed by the local governments for the removal of wastewater. In the mid-1880, broad sewerage systems were constructed across the United State to enhance the drainage of waste water into water bodies. The systems were beneficial to the country in the removal of waste water from homes, however, it created nuisance and health hazards in the receiving water bodies. USEPA (1992) said that receiving waters often played the roles of food and drinking water sources, and recreation centre, as a result, there was a need to pre-treat wastewater before discharge will be initiated. The first treatment of wastewater involves channelling them to the farm with the sole purpose of restoring nutrient to the soil. In the 20th century, the facilities used in the first treatment were called sewage farms.

Increase urban growth which resulted to the production of large quantities of wastewater that is required to be treated, made only sedimentation not to be sufficient, hence various improvements on wastewater treatment were introduced. Chemical precipitation was one of the improvements employed to boost sedimentation, however, creation of sludges was its drawback (USEPA, 1992). Biological treatment with trickling filters was also introduced after sedimentation to enhance the treatment of wastewater (USEPA, 1992). Various treatment methods involving biological processes were introduced with the sole purpose of reducing cost and space requirement, and increasing their efficiency. Current biological processes and activated sludge remove between 95 to 98% organic matter, bacteria and suspended solids (USEPA, 1992).

Secondary treatment was made a requirement in the United States for all treatment plants (passage of the Clean Water Acts in 1972). As a result of this decision by the state, Federal Construction Grant Program was introduced to provide additional funds as an incentive for innovative practices. The grant from the state became a motivating factor for the construction of wastewater treatment plants. However, secondary treatment was also observed to be insufficient in sustaining receiving water for swimming and fishing purposes (as stipulated by Clean Water Act in 1972), therefore, larger elimination of exact constituents such as phosphorus and nitrogen was then required which is termed as “advanced treatment”. Tertiary treatments which adds sand filtration process was also introduced in many states to enhance better treatment of wastewater. The United State Environmental Protection Agency recommend tertiary treatment followed by chlorine disinfection under the safe Drinking Water Act and Clean Water Act (USEPA, 1992). United States has more drinking water system than public wastewater systems. Approximately 19,739 wastewater pipe systems and 14,780 wastewater treatment facilities since 2008 were available. In 2002, 98% of treatment plants were owned by municipalities, while access to centralised treatment plants is general, the state of many of these plants is also bad, with old pipes and inefficient capacity leading to a release of about 900 billion gallons of untreated sewage per year.

In Asia, the percentage of treated wastewater is about 32. This is due to inadequate treatment facilities in several countries in the region (WEPA-IGES, 2012). Financial resources is the major drawback to the success of wastewater treatment in Asia, alongside the lack of distinct policies and the deficiency of qualified personnel in the field of wastewater management (UN, 2000). In China for example, prior to 1800s, outdoor privy was the main means of disposing human excrement (Topare *et al.*, 2011; Abbasi *et al.,* 2016). In earliest twentieth century, cistern was discovered at Yangshao Culture Ruins. Subsequently, these techniques have been used in China for thousands of years until the1970s (Angelakis and Snyder, 2015). Industrialisation and urbanisation together with increased economic growth have created great burdens on the environment, with resulting damage to natural resources including water in China (Sarah Edmonds, 2008). Maintaining an effective and efficient management of water is a difficult goal to realise in China, as the country must concomitantly fight water scarcity, increase the quality of treatment, and improve access to wastewater coverage. Lieu (2009) indicated that realising the wastewater treatment goals of the central government is challenging as discharge of wastewater has increased over the past years.

Water bodies' pollution worsen water scarcity problem. Water in most important river basins is heavily polluted, making it unfit for drinking, agriculture and use in industrial processes. Furthermore, half of the groundwater wells are also polluted, making it unfit for human use. The source of this pollution is mostly agriculture and industries. Treatment of industrial wastewater is often not sufficient to comply with water quality standards, or completely absent (Global Water Partnership (2015). Example of this industrial pollution is seen with the pollution of Huangpu River in China. This river is not only important for tourism, navigation, receiving wastewater and fishery but also provides water for almost 13 million people within Shanghai metropolitan. In mid - 1980s, partially treated or untreated domestic and industrial wastewater discharged directly through municipal sewers to the Huangpu River constitutes 70% of the effluents produced in the area. This is the principal factor contributing to the pollution of the river. The urban section of Huangpu River turned black and anoxic for about

100 days in early 1980s because of the pollution of the river and escalated to more than 200 days in the 1990s (WHO/UNEP, 1997).

The Government of Shanghai Municipal since 1979 gave much consideration to combine pollution control of Huangpu River by establishing environmental legislation and standards to control water quality and effluent discharge. Also, different bodies that enforce laws were also created to ensure standards are maintained. Environmental laws were specified for water quality and effluent in the late 1970s to the early 1980s. Institutions for implementation were also made to monitor wastewater treatment and to ensure that wastewater is properly treated and disposed (Helmer and Hespanhol, 1997).

The increasing rate of water pollution and the need to protect the environment have driven China to develop various technologies to ensure efficient treatment of wastewater (Abbasi *et al.*, 2016). The first large scale municipal wastewater treatment plant was constructed and operated two decades ago (China Environmental Protection Industry, 2008). Sewage treatment systems only emerged after scientists discovered that, water borne bacteria were the causes of many infectious diseases. Chinese government enforced the cities in China to build wastewater treatment plants. The most common treatment method used in China is secondary biological treatment processes which are usually used to treat wastewater in most plants found in municipalities, particularly in the bigger plants. The stages of treatment involves screening, primary sedimentation, conventional activated sludge, and secondary sedimentation. China has also adopted several new wastewater treatment technologies. The newer wastewater treatment technologies in China that are also used to solve problem of inadequate wastewater treatment include anaerobic-aerobic activated sludge process, absorption-biodegrading process, anaerobic-anoxic-aerobic activated sludge process, sequencing batch reactor, and cyclic activated sludge system. Now China is recorded to possess the world's second largest sewage treatment capacity immediately after United State of America. Oxidation ditch, anaerobic and sequencing batch reactor (SBR) are the most widely used processes for wastewater treatment which account for about 80% of the total treatment quantity and capacity of 29.21%, 25.45% and17.90%, (Abbasi *et al.*, 2016).

Japan has employed a broad approach for treating wastewater used in the country (Funamiu *et al*., 2008). Furthermore, 0.2 km^3 of treated wastewater in 2009 were used in the country. Out of the volume of the treated wastewater used in the country, 27% was used for landscape irrigation, 2% for recreation, and 29% river maintenance (World Bank, 2012; WRDLWB, 2012). Wastewater use in agriculture, industry, and toilet flushing is not extensive. It accounted for 7%, 1%, and 3% of the treated wastewater respectively (World Bank, 2012). Japan's use of wastewater policy is slightly unique, as it is aimed at meeting needs of water in urban areas, instead of only providing water mainly for agricultural uses (USEPA, 2004).

In Pakistan, about 32,500 ha are irrigated with wastewater (Ensink *et al.*, 2004). Van der Hoek (2004) pointed out that most of the wastewater are not treated and yet there are no clear laws in Pakistan regulating the use of the water on crops. This scenario is also observed in India where untreated wastewater is commonly used and in 1985, about 73,000 ha were irrigated with it (Strauss and Blumenthal, 1990). Since then, the volume of wastewater used for irrigation have increased significantly (USEPA, 2004). Van der Hoek (2004) further observed that large volumes of untreated wastewater are discharged into Musi River and about 40,000 ha of the land in the area are irrigated with the water from the river posing health risk to the populace that may eat the produce. Jamatia *et al*. (2014) evaluated the physiochemical characteristics of effluent discharged into a water body in India by a rubber industry and observed that the industry produces large volumes of wastewater during the processing stages. They further indicated that the processes of rubber production causes drastic water pollution because of the large effluent disposed into inland surface water thereby causing damage to the water resources.

2.2.1 Wastewater treatment in Africa

Africa is also known to be a dry continent and second to Australia in terms of dryness. According to Corcoran *et al.*(2010), African global renewable water resources that can support 15% of the global population is about 9%. Wang *et al.* (2013) pointed out that inadequate treatment of wastewater increased the deficiency of water in Africa. In Africa, wastewater treatment is reduced, as purchase of facilities for treatment is limited coupled with tenacious increase in population resulting in the increase of wastewater in many countries. Thus, large volume of the wastewater produced is not treated, with a large portion of it used for irrigation by many subsistence farmers with little effort to improve the quality of the wastewater received (Sato *et al*, 2013; Bahri *et al.,* 2008). Africa and other cities in many developing countries presently have no sewerage system (JMP, 2013). However, the countries with the systems, provides services to only a small minority of the population. This deficiency of suitable sanitation systems is now a huge challenge and will likely increase because untreated wastewater from urban areas is contaminating sources of water thereby changing irrigation with freshwater into irrigation with wastewater in and around most cities in Africa. Often the poor rural masses depend on the resource for their means of survival by preventing food scarcity. But this sometimes has dare consequences in terms of their health and the environment (Bahri *et al.,* 2008). For example, in Senegal, specifically Dakar, irrigations are done using untreated wastewater (Faruquiet *et al*., 2004), Nairobi in Kenya (Cornish and Kielen, 2004) and about 11,900 ha of land in Kumasi, Ghana (Keraita *et al.,* 2002).

Limited sewer collection system, inadequate operation and maintenance process is still a very big problem for wastewater treatment in most Africa countries because it result in poor sanitation and water quality standard. For example, in Addis Ababa (Ethiopia), the treatment plant in Kaliti was intended to meet the need of 50 000 people in 1982 (Wang *et al*., 2013) however, after 30 years of advancement, the population whose needs were met amounts to 13 000 people. Inadequate connection of houses to municipal sewerage pipelines is one of the major reasons why the target proposed could not be met. The speed at which connections is done is very slow and has not improved since 1993 (Wang *et al.,* 2013). Another example is found in the Kisumu district in Kenya, where Sunset Hotel, Kendu Bay, and Mumias road that makes up the three pumping stations in the area are worn-out and result in the run-off of sewage at manholes upstream of the pump stations, leading to undeviating release of sewage into Lake Victoria hence causing water pollution (Parkman *et al*., 2008; Wang *et al.,* 2013).

In Ghana, Keraita and Drechsel (2004) reports that infrastructure for urban sanitation is inadequate with less than 5% of the population possessing sewerage networks and only a trivial portion of the wastewater is treated. Furthermore, 20% of households have no access to any kind of toilet facility; about 31% depend on community toilets, while 22% has access to pit latrines. Kumasi ventilated improved pit are used by about 7% of households with only 9% having access to water closets. There are generally improved access to water in the rural and urban regions which causes a corresponding increase in the release of faecal sewage and wastewater leading to more waterlogging and stagnant pools of water in many towns and cities in Ghana because of lack of drains. Poor quality of water and inadequate sanitation has a consequential effect on public health and contributes to 70% of the diseases in Ghana (Water Aid, 2001). Also, in sub-Saharan Africa, wastewater goes untreated causing water contamination that activates the spread of waterborne diseases such as diarrhoea and cholera (WHO, 2007; 2008).The projected volume of wastewater released per year in the Middle East and North Africa (MENA) region is 22.3 km^3, of which 11.4 km^3 is treated (Sato *et al.,* 2013). In the MENA region, the efficiency of wastewater treatment is extremely inconsistent and a lot of treatment plants have limitations to treat a mixture of domestic and industrial wastewater. Furthermore, the treatment plants do not have the ability to put up with large capacities of wastewater as a result of increase in urban populations. The retention times for treatment of wastewater in some plants have become too little to be effective (Qadir *et al.,* 2007).

The use of treated wastewater is important in the water threatened MENA region to solve the problem of water scarcity. Presently, about 51% of treated wastewater is used for irrigation. FAO (2005) projected that 217,527 ha are irrigated with treated wastewater in Egypt whereas in Syria, 9000 ha are irrigated with treated wastewater while 40,000 ha received untreated wastewater. Similar situation was observed in Morocco where 8000 ha were irrigated with untreated or partially treated wastewater (USEPA, 2004; Sato *et al.,* 2013). In Tunisia, 240 mm^3 of wastewater is collected annually. 187 mm^3 (78%) of the 240 mm^3 are treated volume obtained from 61 treatment plants. 41 of the 61 treatment plants have capacity per day of less than 3500 $m^{3,}$ 10 of them have capacity per day of 10 000 m^3, and the rest with the largest capacity of 120 000 m^3/d. Many of the people living in the large urban centres are privileged to have good sanitation system and adequate facilities for treating wastewater. The sanitation coverage for the entire population is 85% whereas the urban and rural areas have a coverage of 96% and 65% respectively. Industries have to follow the standards established by the Tunisian government (INNORPI, 1989) before discharging their wastewater into the sewerage system. Furthermore, subsidies were given to the industries in their purchase of equipment used for pre-treating their waste before releasing into the sewerage system. Many treatment plants are positioned along the shoreline to safeguard coastal resorts and prevent pollution of waterbodies. The majority of wastewater generated in the municipality is from domestic sources (88%). The wastewater obtained in the areas is subjected to secondary biological treatment in oxidation ditches and stabilisation ponds. In many of the towns, several master plans for the sanitation processes are being established to enhance the treatment of wastewater and the safety of the populace.

The monitoring of water quality is often very poor and inadequate in most Africa countries (Re *et al.,* 2011; Wang *et al,* 2013). Most laboratories found in water works or WWTPs are not well equipped hence limiting them to testing few water quality parameter like temperature, turbidity, pH and alkalinity. This is exemplified in Nairobi (Kenya), where only few water works monitors overall organic carbon due to lack of infrastructure. In Kampala, many industries discharge their effluent illegally. Despite the fact that environmental agencies are conscious of the illegal discharge they cannot monitor these industries as a result of inadequate effective monitoring system. Another reason for poor monitoring in most Africa countries is inadequate monetary support from the government for continuous advancement and maintenance of wastewater treatment facilities. Example, the use of jar tester made since 1938 is still in practise in Nairobi. Finally, lack of power supply is also a hindrance to the treatment of waste in Africa because electricity is needed as source of energy for most plant (Wang *et al.,* 2013).

2.2.2 Wastewater treatment plant: Southern Africa development community

In Africa, about 300 million and 313 million people lack access to clean water and good sanitation respectively, and these are the root cause of many diseases (African Development Bank Group, 2015). Uncontrolled and direct reuse of wastewater is a known practice in most African countries due to limited wastewater treatment. Southern Africa is not an exception because the sanitation coverage in Southern Africa has increased from 28% to 36% in 1980 and 1990 respectively (Snyman, 2006). The number of people measured in percentage that has access to collect and dispose wastewater either in treated or untreated form is referred to as sanitation coverage. According to Snyman (2006), about 35% of the people have access to collect and dispose excreta. The methods of excreta disposal is mainly individual based comprising of septic tank system and simple latrines. Conventional and small bore sewers constituting the communal systems are scarce and accessible only in few urban high income areas (UNEP – IETC, 2002).

Zimbabwe, a semi-arid country is dependent on steady rain with their average yearly rainfall as low as 657mm that varies with locations. The country has a good sanitation coverage of 97% in their main towns making it possible for wastewater to be used as a water resource (Thebe and Mangore, 2012). Conventional sewerage systems are used for the collection and transportation of sewage to the treatment plants. The quantification of industrial and domestic effluents are made difficult because of the inter-connected sewer drains. Zimbabwe government do not allow the use of combine sewers (Nhapi and Gijzen, 2002). This implies that storm water flows unswervingly into rivers, streams, and reservoirs in the juxtaposition of the cities. Among the137 wastewater treatment plants in Zimbabwe, 101 are waste stabilisation ponds (Madyiwa, 2006). The largest volume of sewage in Harare and Bulawayo is treated by improved activated sludge systems with biological nutrient removal followed by the conventional trickling filter system. These systems are used to make sure that most plants conform to effluent discharge regulations (Thebe and Mangore, 2012). In Zimbabwe, the municipalities in the urban areas are charged with the responsibility of treating wastewater. Some industries and wastewater treatment plants release partially treated or untreated wastewater directly into the storm drains leading to the immediate pollution of reservoirs and streams with wastewater. The rate of wastewater treatment sometimes fails to comply with the standards stipulated as a result of malfunctioning facilities at the treatment plants. The councils in urban regions have been financing the rehabilitation of existing treatment plants instead of constructing new plants. The major limitation is financial deficiency in the replacement of old wastewater collection and treatment facilities and improper disposal systems since treated wastewater is disposed directly on farmland. This is the reason behind the non-adherent of wastewater disposal guidelines specified in the Statutory Instrument 6/2007 of the Environmental Management Act of Zimbabwe since wastewater is applied excessively on land (Chiris *et al*., 2017; Thebe and Mangore, 2012).

Namibia is one of the most arid countries in the world bordered by two deserts (the Namib Desert in the west and Kalahari Desert in the east). The rainfall per year in Windhoek is almost 370 mm (Department of Water Affairs, 1988). Soon after the independence of Namibia in 1990, the Water Act of 1956 was reviewed (Sibeya, 2016). The subdivisions of the revised Water Act of 2013 that address the release of industrial wastewater include Section 21(1), which states that the treatment of wastewater shall form an integral part of water usage. It goes further to specify that treated wastewater shall comply with the General Standard Quality restrictions (Sibeya, 2016). In addition, this Section 21(2) stipulates that treated wastewater be discharged as close as possible to the original water (Sibeya, 2016). In 2013, Namibia reviewed the then existing Water Act of 1956, and catered for the prohibition of release into the sewerage system, of any industrial effluents from tannery, abattoir, brewery, dye-house or any other intolerable industrial waste which might constrain the biological activity of the wastewater treatment facility of a local authority (Sibeya, 2016). This led to industries being made responsible for treating the wastewater they produced, (on their premises) before releasing effluent into the environment (Lahnsteiner and Lempert, 2005).

Botswana is a landlocked country with water scarcity that is severe. The average rainfall is about 450 mm/year. In Botswana, the main source of water supply is groundwater which serve about 80% of the masses. The restrictive water resource in Botswana has put a strain on efficiency of water (Opelo and little, 2004). Conventional wastewater treatment (ponds, biofilters and activated sludge) is used to treat wastewater before discharging into the environment. Ponds are used for the small centres because of the ease and cost of operating them while the more compact activated sludge is used in the main centres. It was noted that pond systems evaporate or waste more water that the more compact activated sludge process and it was encouraged that these systems should be considered where and when re-use is expanded (Opelo and Little, 2004).

South Africa has between 40 to 60% water stress as a result of low average rainfall per year and high rate of evaporation (Eberhard and Robinson, 2003; Adewumi *et al.,* 2010). In South Africa, a lot of communities struggle to have access to reliable and acceptable quantities of potable water for various water requirements. This is against the backdrop of declining availability of freshwater and water demand increase. Presently, the interest in the wastewater reuse for non-drinking water requirements is increasing (Adewumi *et al.,* 2010).Most WWTPs in South Africa are relatively small systems and there are about 51 plants within the eight provinces processing waste between <500 m^3/day – 10000 m^3/day depending on the size of the plant. It is believed that for any wastewater plant to function effectively, it must be upgraded or maintained continuously by competent operations staff to avoid unnecessary breakdown that may cause pollution to water bodies leading to outbreak of water borne diseases.

Morrison *et al.* (2001) in their study investigated the physicochemical properties of receiving water bodies versus treated wastewater final effluents in rural areas, and it revealed that the Keiskammahoek treatment plant in the Eastern Cape has poor water-borne sanitation. Their investigation showed that the levels of biological oxygen demand (BOD) and dissolved oxygen (DO), surpassed EU standards for the safety of the aquatic ecosystem and that the river Keiskamma located in the Eastern Cape where the treated water is discharged is eutrophic as a consequence. Igbinosa and Okoh (2009) did a study on a water treatment plant within the rural community in the Eastern Cape and observed that the effluents after treatment did not meet the required standard in terms of organic waste, dissolved oxygen, COD, orthophosphate, nitrates and nitrites critical for the provision of water that is clean and safe. These research revealed that effluent generated from the treatment used in the studies represent a significant health and environmental hazard to rural communities who are dependent on the receiving waterbodies as their source of domestic water use.

The lack of effective treatment would not only affect the health of humans but also be detrimental to aquatic organisms. Therefore there is a need for constant regulation of pollution of the surface waters in rural Eastern Cape Province. It was recommended that provincial government and all environmental agencies should have measures to certify that released wastewater after treatment conform with standard rules and regulations (Igbinosa and Okoh, 2009). Similar investigation was carried out by Mema (2010) in which he identified poor operations and maintenance as an underlying cause of inadequate effluent treatment in four municipalities used in his case studies which included the waste water treatment plants found in the Eastern Cape, Western Cape and Kwa Zulu Natal. Mema (2010) emphasised that such discharges and consequential leachates into ground water has led to several disease outbreaks in the different provinces that can be directly linked to inadequate waste water treatment.

Several researchers have asserted that industries can be regarded as a major contributor to water and land pollution due to the magnitude of environmental degradation they cause in the environment, (Dan' azumi and Bichi, 2010; Asia and Ademoroti, 2001; Amoo *et al*., 2004). However, the existence of these industries is inevitable and indispensable. Industrial wastewaters have a hazardous impact on quality of water, quality of habitat, soil and flowing waters (Ibrahim and Tayel 2005; Ethan *et al*., 2003), which if not properly controlled, will put human health and the environment in serious danger (Odjegba and Bamgbose, 2008). These wastes and emissions produced from most of these industries comprise of both hazardous and toxic substances that are detrimental to health of humans once they enter the food chain (Rajaram and Ashutost, 2008; Jimena *et al*., 2008; Ogunfowokan *et al*., 2005; Setyorini and Ipinmoroti, 2001). Of these hazardous substances, heavy metals (e.g. Pb, Cd and Hg), and toxic organic chemicals (e.g. pesticides, PCBs, dioxins, polyaromatic hydrocarbons (PAHs), petrochemical and phenolic compound) have been highly reported (Gbadebo *et al*., 2010; Njoku *et al*., 2009).

Bhagirath and Ratna (2002) stated the association between the development of industry and the changes in the local surrounding could cause harm to animal husbandry, crops and humans. Bhagirath and Ratna (2002) reported the links between industrial development and changes in the micro (local) environment that caused damage to crops, humans and animal husbandry. A community within the patancheru industrial belt was used in their study. These authors found out that most people in the village suffered from various diseases arising from water pollution. Some of these diseases include diarrhoea, skin infection, abdominal pain, joint pain, defective vision and respiratory diseases.

A study by Ntuli (2012) on industries located in South Africa which included industrial sectors such as tanning, textile finishing and food processing industries (edible oil and sugar refinery industries) demonstrated the inadequacy of the effluent treatment processes that produced treated water that was within the toxic range. This study highlighted that the best method for monitoring industrial effluent discharges should be systematic and automated using water meter reading devices (Ntuli, 2012). Sandeep and Shweta (2008) also noted that the high exploration and recharging rate, unsuitable dumping of both liquid and solid wastes combined with the lack of firm implementation of law and loose governance have immensely contributed to the worsening of the quality of ground water not only in southern Africa but globally. The alternative method of effluent waste disposal that relies on spillage on land areas has the detrimental effect of polluting ground water due to seepage as soils generally have maximum absorption capacities. Such strategies if continued unrelenting could cause severe challenges in the future (Mukherjee and Nelliyat, 2006).

2.2.3 Wastewater treatment/water quality in the Vaal

The Vaal River is the most vital river in South Africa because it is the ever busy river that forms the backbone of economy in South Africa since it provides services to the economic hub of South Africa, the Gauteng Province (Tempelhoff, 2006). Quality of water in the Vaal River alongside with some tributaries to the river is extremely affected as a result of mines, farmers, urban and dense settlements, and industrial users. Tempelhoff *et al*. (2007) pointed out that Vaal River is seemingly under pollution threat. The major source of water pollution especially in the Vaal according to Mamabolo (2012) include poor wastewater treatment works management, uncontrolled sewage, chemical discharges from different industries, leakage and spillage of petroleum, old mines and pits waste dumping, human settlements, and agrochemicals that are washed off or seep down from farm fields.

According to Water wheel (2009), many wastewater treatment works (WWTW) situated in Vaal area do not comply with the stipulated standard by the present legislation that addresses appropriate treatment of water. This results in diverse issues affecting the quality of water in this catchment. Department of Water and Environmental Affairs (DWEA, 2009), stated that poor wastewater treatment works management, and uncontrolled sewage in the Vaal is due to the absence of skilled contractors who render services and meagre construction of treatment plant that reduces the life expectancy of facility; shortage of municipal staff to operate and maintain water services facilities; and absent or weak municipal systems for the management of facilities.

This is true as this situation is also applicable in Leeuwkuil Wastewater Treatment Works where this research was carried out. Rand Water Analytical Laboratory were contracted to carry out all their analytical test, monitor treatment process through daily supervision and give full report on compliance of Leeuwkuil plant. However, their contract were no longer effective from June 2016 due to lack of finance leading to noncompliance by the municipality. This affected the quality of water discharged to the Vaal River since no laboratory test was carried out from June last year to ascertain water/effluent standard conformity. Secondly, disinfection with chlorine also stopped one month before the exit of Rand water contract due to broken/damage equipment that is used to dose the treated effluent. This caused increase in the level of physico-chemical parameter of final effluent before discharge into the Vaal River and the pollution of the river's downstream.

According to Mema (2010), many studies discovered that the reason for untreated sewage and poor quality wastewater discharges is poor operation and maintenance. In addition, poor plant designs, faulty equipments of municipal WWTWs and overloaded capacity of wastewater also contribute to poor quality wastewater discharge. This is the case in Leeuwkuil Wastewater Treatment Work. The infrastructure is designed to treat about 36 Ml/day but instead it treats 42 Ml/day in summer (the increase is partly due to storm water from rain and increasing population in the area) and 39 Ml/day in winter (decrease is partly due to the new water restriction/regulation implemented in the area during the day). Overloading of this infrastructure may be one of the reason for improper wastewater treatment leading to noncompliance in terms of discharge of treated wastewater. This has also affected/damaged the bio-filters and core screen that is supposed to be used to remove solid particles hence resulting to using mechanical/ manual bar screen which takes more time and in some case does not remove all the solid causing more damage by blocking the effluent flow passage (Mema, 2010).

The release of wastewater that was not properly treated results in algal blooms. Based on that, most rivers in South Africa are faced with the threat of eutrophication (de Villiers and Thiart, 2007). According to the Mamabolo (2012) eutrophication is caused by runoff from agricultural and urban activities, septic tank leak, and municipal and industrial wastewater. All these contribute to accumulation of phosphorus and nitrogen compounds especially in surface water causing eutrophication. Eutrophication has been considered to be a relatively important environmental issue when performing an environmental evaluation of WWTPs (Sibeya, 2016). Wastewater treatment plants discharge directly into water bodies and are therefore considered point sources of possible eutrophic pollution based on previous studies according to Mamabolo (2012). Akpo and Muchie (2011) said that the reduction in dissolved oxygen (DO) caused by eutrophication increases fish mortality and results in the most frequent occurrence of toxic phytoplankton. The continuing reductions in DO levels may bring about changes in the composition of species and eventually lead to their death. Through direct contact and consumption of contaminated water, humans may also be exposed to toxins. Animals and humans are affected by toxins at a molecular level affecting cells, tissues, and organs. Other researcher who studied eutrophication in the Vaal are Mostert (2009) and De Villiers (2009) who conducted research to identify variation in the costs associated with eutrophication in the Vaal River System. Similarly, Sibanda (2014) in his research reiterated the economic impact on agriculture and water treatment that eutrophication had. Gray (1997) and Seanego (2014) stated that the death of algae leads to increase in organic waste causing reduction in oxygen levels by decomposition. This causes a decline of aquatic ecosystem diversity.

Mamabolo (2012) is another researcher who carried out a study in the upper Vaal water management area (WMA) to find out the effect of cooperative governance in the sewage treatment works. He found out that the problem faced by the upper Vaal WMA is acid mine drainage (AMD). For more than 130 years, this basin is been mined with some of the companies not existing presently, yet they are responsible for costly environmental and socioeconomic impacts. Mamabolo (2012) said since the government cannot trace the owners of this mines which poses health risk to both human and the environment, then they should take measures to remedy the pollution. Department of water affairs and forestry (DWAF, 2004) observed the threat faced by the water quality of the Grootdraai Dam (in the Vaal) as a result of current mining activities. Therefore, regulating effluent is important as water pollution has a serious effect on the environment as a whole, including effects on biotic and abiotic organisms (Okoh *et al.,* 2007).

Leeuwkuil Wastewater Treatment Plant discharges their final effluent to the Vaal River which in some case do not comply with the standard of discharging wastewater. Inspite of the important role played by the Vaal River in providing water to the South African economic centre, the river remain polluted, hence the reason for this research at Leeuwkuil to find out reasons for some of the inefficiencies in the treatment of wastewater.

Due to the pollution of the Vaal River, the routine valuation of water and wastewater quality is very vital in the Vaal in order to safeguard public health safety, aquatic organism and the surroundings (Okoh *et al*., 2007). However, water quality data on fresh and marine waters in South Africa are still infrequent and uncoordinated. As a result, there exist a need to provide a good and effective wastewater treatment process locally and globally.

2.3 Treatment of Sewage

Treatment Efficiency (TE) refers to the measure of effectiveness to which a treatment plant reduces concentrations of pollutants in wastewater, also known as removal efficiency (Khanijo, 2002). The performance of wastewater treatment plants is vital and needs consistent monitoring due to the fact that the water bodies where the treated effluent is discharged support life. The effectiveness of sewage treatment plants are usually measured by evaluating the level of pollution in the influent and the effluent at the plant discharging into the environment (Sukumaran *et al.,* 2015).

The effectiveness of treatment and qualities of its parameters in terms of the specified standards determines the performance of each treatment phase. Therefore, WWTP performance is dependent on state of WWTP's facilities and the strength of treatment of each treatment units (Qasem, 2011). Environmental regulations are continuously focussed on the quality of the effluent released from treatment plants. These regulations include sets of restrictions on the effluent quality that must be adhered to by any WWTP. There is daily and seasonal variation that exist between wastewater treatment properties of various WWTP making standardisation of assessment procedures for all plants very challenging. Base on this, special attention is needed to evaluate the environmental influences of existing facilities of wastewater treatment (Kumar and Pinto, 2010).

There are three principal methods of wastewater treatment applicable in most countries: The first being the preliminary treatment of wastewater involving the removal of large solid materials that can hamper the easy movement of sewage through the plants or damage the equipment. Examples of these large solid materials are wood, heavy grit particles, rags and faecal materials. According to Tebbutt (1983), most of the huge floating materials can be sieved using bars that are spaced at 20-60 mm and the sieved solid materials are collected from the bars at particular intervals.

Grit and silt are made to settle at the bottom of the plant through the reduction of the velocity of flow to a range of 0.2-0.4 m/s while the organic matters are left in suspension (Gray, 1997)

Primary treatment involves pumping and containment where the water is directed from the source through the necessary pipes to the holding container. The water in the holding container is screened using mechanical coarse grid to eliminate bulky debris like garbage, leaves, papers, sticks and other particles that are capable of interfering with the next treatment step. The screened water can be stored in the appropriate storing containers for a few days to allow natural biological purification to take place. The water in the storage container is subjected to a pre-conditioning process where the water that has a lot of hardness causing salt is treated with sodium carbonate to precipitate the calcium carbonate. Finally, pre-chlorination is done to reduce the growth of foul producing organisms on the pipe-work and tanks (Metcalf and Eddy, 1972; United State Environmental Protection Agency, 2004). Removal of pathogens during primary treatment varies with different rates of removal for different pathogens (Gray, 1997; IAWPRC study group, 1991). The commonly used method of treatment in the United States before the implementation of the Clean Water Act in 1972 was primary wastewater treatment however the Act enforced the application of secondary treatment.

Secondary treatment involves pH adjustment where the acidic water is treated with soda ash or lime to raise the pH. This process enhances coagulation and flocculation. Coagulation, being the next process in this stage involves the use of chemicals (aluminium sulfate also known as alum) to glue or stick with the small suspended particles causing them to settle at the granular media filter. Flocculation is the joining of the particles to form large settable particles which can be settled out. This is done in a flocculation basin and water leaving this basin flows into the clarification or settling basin. The settling basin is a huge tank with low flow rate which allows the floc or large particles to settle at the bottom. This process is followed by filtration which is the last step in the secondary treatment where the floc is separated from the water (USEPA, 1992). The major aim of secondary treatment is to decrease the biological oxygen demand caused by reducing organic matters (USEPA, 1992). To achieve this, a great quantity of biological unit operations are made accessible which consists of bacteria that cannot manufacture their own food and make use of the organic materials for energy production and development in aerobic environments. These operations are classified into fixed film processes based on the microbial population. Fixed film reactors consists of biofilms adhered to a fixed surface where organic matters are adsorbed and broken down aerobically. In suspended growth reactors, the organisms mixed freely with the sewage and are maintained in suspension by mechanical agitation or mixing by air diffusers (Horan, 1990). Several studies have shown that biological oxidation systems can remove more than 90% of pathogenic bacteria from sewage. However, there is a variation in the removal of viruses and the major mechanism for removing viruses is by adsorption (Gray, 1997; IAWPRC study group, 1991; Kott *et al*., 1974; Lloyd and Morris, 1983). Historically, the first trickling filter used in secondary treatment was fixed at Salford near Manchester, England in the year 1895. Since that year, many other filters were installed and used in the treatment of wastewater (Stanbridge, 1976).

The next stage of treatment after secondary treatment is the tertiary treatment in the municipal water treatment plant which involve the disinfection of the filtered water using chlorine a process called chlorination or ozone or ultraviolet radiation (United State Environmental Protection Agency, 2004; Metcalf and Eddy, 1972). Increase in the development of industries in the world has resulted in drastic water pollution and South Africa is not an exception. This can be traced to increase in a high load of waste water the treatment plants are receiving. This increase can also impact negatively on the municipal water treatment plants because there will be an increase demand for resources needed to buy equipment and relevant chemical for the purification of water. In the situation where the resources are not provided, there exists a possibility of discharging incomplete treated water to the citizenry and water bodies. This will result in water pollution which can be devastating (Kamika and Momba, 2013; Yi *et al*., 2011).

Leeuwkuil Wastewater Treatment Plant also has three treatment methods consisting of firstly primary treatment involving the removal of materials such as rags, plastics, sand, metal particles using mechanical screens. The second one is secondary treatment which involves an organic and inorganic solids removal by sedimentation while floatable material are removed by skimming. Also 25-50% COD reduction, 50-70% of suspended solids and 65% of oil and grease is achieved by the use of microorganisms to decompose sewage and break it down to simpler organic and inorganic form. It means separation of solids and water takes place at this stage. The final stage is the treatment at the tertiary level involving the disinfection of effluents that are treated with chlorine before discharging the supernatant/clear water into the river.

Biological sewage treatment is among the advance techniques of sewage treatment involving microorganism application in the breakdown of pollutants (Abraham *et al*., 1997) and this method is also applicable in Leeuwkuil WWTP. This is a secondary process but Leeuwkuil treat up to tertiary level. This method of wastewater treatment process is directed towards the highest reduction of BOD of wastewater with a minimal reduction of biological solids. According to Abraham *et al.* (1997), the reduction in the BOD of wastewater is attained by eliminating materials with high demand for oxygen from the system through the metabolic activities of the microorganisms, elimination of surplus microorganisms from the system, microorganisms collection and recycling in the system, and the separation and settling of activated sludge solids to make an acceptable quality of wastewater effluents. This biological treatment of wastewater is divided into on-site and off-site treatment system. On-site treatment of wastewater involves treating wastewater from home or business and returning of the treated wastewater back into receiving environment. Off-site treatment of wastewater involves the treatment of wastewater that has been conveyed using sewerage system. The treatment methods adopted in treating sewage by the wastewater treatment plants also depends on the composition of sewage in the treatment plant.

2.4 Composition of Sewage

Wastewater and other forms of waste which can be carried in a liquid medium that are produced by industries in most countries are channelled mostly to municipal water treatment plants where they are treated for recycling purposes (Gray, 1997; Tebbutt, 1983). The composition of wastewater reflects the way of life of people and the technologies applied in the community (Gray, 1997). A typical example of the wastewater obtained from domestic activities is sewage. Sewage is a composite mixture of inorganic and natural organic substances. Most of the organic compound in sewage occurs as fats, protein, amino acids, volatile acids and carbohydrates. The mineral components of sewage include Ca, K, Mg, Na, S, Cl, phosphate, ammonium salts, bicarbonates and heavy metals (Tebbutt, 1983; Horan, 1990; Lim *et al*., 2010). Sewage also consists of a wide range of organisms like viruses, bacteria and protozoa. Most of these organisms are believed to be mild in their action and can also be applied in biological treatment of sewage. However, sewage also comprises of harmful microorganisms which are released by sick individuals and symptomic carriers (Tebbutt, 1983).

Heavy metal concentrations in drinking water are higher than some internationally accepted standards in most parts of the world. The presence of heavy metal in drinking water and its bioaccumulation in the food chain are of serious concern, thus routine environmental monitoring and tracking of such contamination is of utmost importance to researchers (Martin, 2009; Jaishankar *et al.,* 2014). Research has shown that there are a million individuals with unending heavy metal poisoning which has become a global public health problem, while about 2 million children die every year from infections for which polluted drinking water is the main cause (US Department of Health and Human Services, 2008). Heavy metals in drinking water have been found to cause negative impacts on the health of human through food chain contamination. It has been proven that heavy metal toxicity is a major threat with several health risks associated with it. The major risks to human wellbeing from heavy metals are linked to lead, cadmium, mercury and arsenic contact. These metals have been broadly examined and their adverse consequences frequently audited by worldwide bodies, such as the World Health Organisation (WHO, 1982). Heavy metals are being discussed extensively in this study based on their impact in the environment (Naidoo and Olaniran, 2014).

2. 4.1 Heavy metals

The term "heavy metals" refers to any metalloids or metallic elements with high density that is greater than 5 g/cm^3 and are said to be toxic at minute concentration (Lenntech, 2004). These metals collectively account for less than 1% of the composition of the earth's crust (Ogunleye and Izuagie, 2013) and occur in dissolved form in water, particulate and colloidal phases (Adepoju-Bello *et al*., 2009).Their existence in water can be traced to mineral erosion within sediments, ore deposits leaching and volcanism extruded products, domestic effluents, harbour channel dredging, solid waste disposal and industrial effluents. These are believed to be the anthropogenic origin of heavy metals in water bodies (Marcovecchio *et al*., 2007).

Heavy metals are broken down into essential metals such as copper, manganese, zinc, and iron, and non-essential metals such as cadmium, lead, and mercury (Fazli *et al*., 2015). Copper (Cu), Zn, and Ni at low concentrations have been reported to perform an essential role in several physiological functions of living being by providing vital co-factors for metallo-proteins and enzymes (Fazli *et al*., 2015). Furthermore, according to Adepoju-Bello *et al*. (2009), manganese, cobalt, copper, iron, molybdenum and zinc are required at minimal levels as catalyst for enzymatic functions. However, there is the likelihood of toxic reaction in the instance of excessive exposure of these metals to organisms. The complex formation of these metals with the proteins in the organisms which involves the carboxylic acid (–COOH), amine (–NH_2), and thiol (–SH) functional groups of the proteins enhances the production of toxic effects by the metals. These reactions between the metals and the proteins results in the formation of modified biological molecules which have lost their ability to function effectively resulting in the malfunctioning of the cells and consequently, the death of the cells. The binding of these metals to the functional groups of these proteins have the tendency to affect the protein structure and inactivate important enzymatic systems that is connected to the catalytic attributes of the enzymes. There is a possibility of this type of toxin resulting in the production of radical, dangerous chemicals, capable of causing oxidation of biological molecules (Fazli *et al*., 2015).

Olafisoye *et al*. (2013) reported that human beings exposed to these heavy metals either by consuming food or drinking of liquid substances (ingestion) infested with these metals experienced harmful effects over time as a result of the metals accumulation in the body. It has been observed that people living or working in an industrialised area that utilises these heavy metals and their compounds, and disposal sites where these metals are not properly disposed or incinerated where applicable, have higher risk of exposure with significant effects thereafter (Martin and Griswold, 2009). Based on the environmental and human impact of these heavy metals, a brief discussion of their sources, biological importance and negative effect of over exposure becomes necessary in this study.

2.4.2 Sources of heavy metals

Heavy metals consists of single metals or compounds which are natural components of the environment, but their uncontrolled use for human purposes has altered their geochemical cycles and biochemical balance (Martin and Griswold, 2009). Heavy metals can be released into the environment by either anthropogenic or natural causes. They occur as natural components on the earth's crust, making them tenacious environmental pollutants because they cannot be degraded or destroyed. They enter the body system via food, air, and water and bio-accumulate over a period of time (Lenntech, 2004; UNEP/GPA, 2004). In rocks, they occur as ores in diverse chemical state, from which they are recovered as minerals. Some exist and can be recovered as both sulphide and oxide ores such as iron, copper and cobalt (Duruibe *et al*., 2007).

Anthropogenic sources of heavy metal pollution include those connected to fuel from fossils and burning of coal, wastewater from industries, disposal of solid waste, fertilisers, mining, sewage discharge and metal processing (Hutton and Symon, 1986).The major cause of emission for the anthropogenic sources specifically is mining operations (Hutton and Symon, 1986; Battarbee *et al*., 1988; Nriagu, 1989). In some cases, even long after mining activities have ceased, the emitted metals continue to persist in the environment. Peplow (1999) reported that hard rock mines are operated between 5-15 years until the minerals are depleted. However, metal contamination that occurs as a consequence of hard rock mining persists for hundreds of years after the cessation of mining operations.

In the last decade (January 1, 2000 – December 31, 2009), there has been an increase in both industrial activities and urbanisation in Africa. This has led to upsurge in the amount of various waste including heavy metal discharged into the environment in all parts of the continent leading to potential heavy metal pollution. Studies carried out by Alo and Olanipekun (2004) confirms mining activities as heavy metal sources of contamination in Africa. Example, arsenic in Namibia and South Africa, mercury in Algeria, tin in Nigeria and Zaire and copper in the Zambia (Alo and Olanipekun, 2004). These led to excessive discharge of metal-rich mine tailings and metal smelting which subsequently led to pollution. It has been pointed out that there could be other sources of environmental pollution in African region such as leather tanning, electroplating, emission from vehicles car exhaust, production of fuel and energy, rigorous agriculture and sludge deposition. Crude oil and hydrocarbon exploration and exploitation also pollute the surroundings with large amount of toxic metal. However, mining activities still take the first priority in heavy metal discharge into the environment. These activities result in excess release of heavy metals such as Mercury (Hg), Cadmium (Cd), Arsenic (As), Chromium (Cr), Lead (Pb,), Nickel, (Ni), Cobalt (Co), Zinc (Zn), Copper (Cu), Iron (Fe), and Manganese (Mn) into the soil and waterbodies. Persistent exposure and greater accumulation of such heavy metals can have harmful health effects on human life and aquatic biota. Eight common heavy metals are discussed in brief. Also their sources and their effects are explained (Rajendran *et al*., 2003*)*.

2.4.3 Overview of selected heavy metals for present study

Cadmium (Cd), Lead (Pb,), Mercury (Hg), Chromium (Cr), Zinc (Zn), Copper (Cu), and Aluminium (Al) among other are the heavy metals of interest in this study because of their excess release in the environment by many industrial activities (Jaishanker *et al*. 2014). An understanding of the level of toxicity of these metals to humans and aquatic animals is needed. The table below shows the uses and the toxic effects of some of the heavy metals.

Table 2.1: Application of Heavy Metals, uses and their toxic effects (Rajendran *et al*., 2003).

Heavy Metals	**Use**	**Toxic Effects**
Copper Cu)	Vitamin B12	Diarrhoea, low blood pressure & paralysis
Zinc (Zn)	Fertilizer	Vomiting, renal damage and cramps
Mercury (Hg)	Coal, electrical batteries	Tremors, birth defects, kidney damage & loss of hearing or vision
Lead (Pb)	Plastic, paint, pipe, batteries, gasoline &auto exhaust	Neurotoxic
Arsenic (Ar)	Pesticides, coal, detergents	Liver cirrhosis, mental disturbance and cancer
Cadmium (Cd)	Fertilizer, plastic and pigment	Kidney damage, injury in central nervous system and mental retardation
Chromium (Cr)	Tanning, paints, pigments, fungicide	Nephritic, cancer, and ulceration
Aluminium (Al)	Transportation, foil, casting, packaging food	Alzheimer's disease, parkinson's disease, pre-senile dementia, senility

2.4.4 Bioaccumulation of heavy metals

Bioaccumulation is described as an increase in the level of chemical substances present in organisms over time when compared to the level of the substances in the environment (Valavanidis and Vlachogianni, 2010; Lenntech, 2008).The contaminants that bio accumulate are mostly substances that cannot dissolve in polar solvent like water but can dissolve in fats and oils, such as polychlorinated biphenyls (PCBs) and dioxins. These pollutants can be found in fatty tissues, such as the liver, instead of muscle tissues (Jakimska *et al*., 2011; Shun-Xing *et al*., 2007). The introduction of toxins into the food web can result to the transfer of the toxin from one predator to another leading to higher levels of pollution in the top predators as they accumulate those toxins in their tissues (Uaboi-Egbenni *et al*., 2010; Ahmad *et al*., 2012).

Alinnor and Obiji (2010) carried out an investigation on heavy metal content of fish samples in Nworie River in Nigeria to demonstrate the effect of bioaccumulation of heavy metals. The results demonstrated that some elemental toxicants such as Iron (Fe), Cadmium (Cd), and Manganese (Mn) were identified in fresh fish species, *Tilapia guineensis*. It was also noteworthy that the same research highlighted the presence of industries in the vicinity that discharged untreated waste products into the water body as such the bioaccumulation of these heavy metals can be attributed to the presence of these pollutants released by those industries. Furthermore, these elemental toxicants will most likely be transferred to humans upon consumption of the fish from this area thereby posing a health hazard due to cumulative effects in the body (Odoemelam, 2005, Obodo, 2004; Burger *et al*., 2002).

A study carried out by Olowu *et al*. (2009) investigated the level of heavy metals in crabs and prawns in Ojo River, Lagos, Nigeria. The result of this investigation revealed that these crabs accumulated chromium and cadmium concentration that exceeded the National Agency for Food and Drug Administration and Control, Nigeria (NAFDAC) and WHO standards for consumable food. The increased quantities of cadmium are associated with inhibition of enzymes in humans who consume such contaminated crabs (Binning and Baird, 2001). Danis *et al*. (2006) demonstrated that the impacts of consuming heavy metals are not immediate but rather become visible only after a few years. Their persistent presence and ability to bio-accumulate emphasises the need for easy, cost-effective treatment and biological methods to determine and control toxicity levels of industrial effluents and help minimise domestic households receiving polluted water. However, it is necessary to initially identify the presence of heavy metals and their effects in water bodies (Ajao *et al*., 2012).

2.4.5 Effects of heavy metal pollution

Water is South Africa's most scarce natural resource due to high temperatures and seasonal rainfall (Strydom *et al*., 1997) and has been under increasing danger of contamination in current years. Its status as an emerging economy has meant that it has experienced in the past decade a rapid trend of industrialisation (Du Plessis and Smit, 2005; Ayres, 1992) with the novel pattern of industrial areas becoming integrated with domestic areas of human habitation. The vital impact of industrialisation on the growth and advancement of the country cannot be underscored. However, the fast growing industrialisation has its own direct and indirect harmful effects on the surrounding. According to Nasrullah *et al*. (2006), the development of industries is through the establishment of new industries or development of already prevailing industries. These establishments have led to the production of industrial effluents capable of causing air, water and soil pollution. Furthermore, spatially small scale cottage industries have the tendency of releasing untreated effluents into the environment and possibly cause air, water and soil pollution (Naidoo and Olaniran, 2014). The major cause of deterioration of our surroundings that affects the water we use, the air we breathe and the soil could be attributed to the pollution caused by the industries. However, water pollution is undoubtable the major threat to human wellbeing within the South African context (Du Plessis and Smit, 2005). Several industries including the Vaal area are involved in metal and coal mining processes which utilise chemical reactions that involve heavy metals as derivatives and by-products. These industries exploit fresh water usage in their industrial processes and discharge effluents that contain heavy metals such as Gold (Au), Copper. Lead, Zinc and Nickel Lead (Pb), Nickel (Ni), Copper (Cu) and Zinc (Zn) and discharge from tailings and waste rock impoundments (Banza *et al*., 2009). Increasingly, these mining activities threaten the water sources on which humans depend on.

Researchers have found a strong link between individuals residing near industrial sites, prolong contact to heavy metals and some idiopathic diseases (Duruibe *et al*., 2007; Ayres, 1992). A study done in Malaysia found patients with skin cancer symptoms after a long exposure to arsenic-contaminated well water in Malaysia (Jaafar *et al*., 1993). Another study conducted in the Democratic Republic of Congo showed about 43 % increment in cadmium, cobalt, lead and uranium concentration in the urine of human subjects in the mining regions (Banza *et al*., 2009). In eastern Nigeria, a study done by Ibeto and Okoye (2010) in which blood samples were collected from 240 people living in the urban vicinity were analysed for heavy metals and showed values that were significantly higher than WHO limits for nickel, manganese and chromium. The study area in the research is dominated by chemical and agricultural industries that specialises in the production of herbicides, pesticides, food additives and halogenated polycyclic hydrocarbons. These sample population of ordinary people showed a prevalence of heavy metal pollution most likely from this industries in eastern Nigeria. In a similar study done in different provinces in South Africa, the degree of pollution by heavy metal was established by evaluating the concentrations of heavy metals in maternal and umbilical cord blood from people living in areas ranging from rural, urban, industrial, inland, coastal, fishing and mining sites (Rollin *et al*., 2009). High concentrations of lead, mercury, selenium and cadmium were found in the umbilical cord blood samples of babies indicating the risks of heavy metal pollution in unborn babies and not just adult alone. It also emphasises that the dangers of heavy metal pollution is not an isolated incident and judging by the results of these studies, it would be dangerous to ignore the potential harm to humans.

There is a tendency for heavy metals to enter into the food chain, through food and water and accumulate through a process called bioaccumulation, causing anaemia, disorder of kidney, nervous system failure, high blood pressure and others. These heavy metals do not only damage human tissues and organs but plant tissues as well (Muneer*, et al.*, 2010; Hanif *et al.*, 2010).Works done by researchers have demonstrated that most of the heavy metals are often associated with metal poisoning in humans since most of the metals play no significant function biologically in living organisms while some of them such as Cu and Zn are essential at minute quantities but harmful when increased beyond the permissible limit (Ahmad *et al*., 2012).

There is no doubt that industries contribute enormously to the economic development of any country in both developed and developing countries. However, their industrial practices have also produced adverse health effect and environmental consequences because they have contributed to the pollution level of our surface water and ground water aquifers. In fact, many aquatic organisms have gone into extinction because of rapid industrialisation and urbanisation which led to increase in heavy metals in the surroundings as a result of waste being disposed without any appropriate treatment (Santona *et al*., 2006). The annual reports of Mills-Knapp *et al*.(2012)stated that large number of people in developing countries are more susceptible to harmful pollution from industrial processes. This is often because small-scale industries lack the knowledge of the best methods that can reduce waste being generated in their operations or may not have the knowledge of the used chemical toxicity. Poor communities where the small-scale industries are regularly located, have minute capability, either financially or culturally, to take measures to reduce potential exposure to these chemicals. Additionally, these communities have little or no health care facilities that can salvage the health effects that arises from the exposure to these toxic chemicals. Due to low overall standards of health in these poor communities, such as poor nutrition, which exacerbate the health risks arising from the impacts of the toxic substance they are exposed to, with children being the most affected because they accumulate heavy metal pollutants in their tissues twice as fast as adults (Mills-Knapp *et al*., 2012). The negative effect caused by these heavy metals has resulted to untreated wastewater impacting negatively on the surroundings and consequently human health.

2.5 The Impact of Industrial and Domestic Effluents

2.5.1 Environmental effect

Water, a part of the environment, is vital for the survival of all forms of life. It is a unique substance, because it can be renewed naturally and cleanse itself, by permitting contaminants to settle out (by sedimentation) break down, or by diluting the contaminants to a point where they are not present in toxic levels (Mann *et al*., 2014). However, this process of nature takes time, and it is hampered when considerably large amounts of dangerous pollutants are introduced into the water (Akaninwor *et al.,* 2007). The release of energy, elements, or compounds into the environment at a level that has the potential to influence biological functions negatively or that present risk at unacceptable levels to human beings or other organisms that live and survive in the environment is termed pollution (Fernández-Luqueño *et al*., 2013). When the released compounds, elements and energy find their way into our water bodies through households or industrial activities, it leads to pollution of water. Water pollution is defined as the addition of contaminants into the water bodies such as lakes, rivers, oceans, and groundwater to a certain degree that it cannot be used for specific needs such as drinking, bathing or cooking (Owa, 2014). United States Environmental Protection Agency (1997) defined water pollution as any human-caused contamination that reduces the quality of water to human beings and other naturally existing organisms. Despite the huge importance of water, its susceptibility to human impacts such as contamination from run-off and dumping of waste from human industrial activities has devastating consequences that affects the economy and impacts human and livestock health in addition to damaging the aquatic habitat and destroying marine life. A good example is the bioaccumulation of non-biodegradable compounds and heavy metals in fish and shellfish which is considered lethal to humans when consumed in large quantities (Owa, 2014; Dan'azumi and Bichi, 2010).

Water pollution occurs when pollutants such as herbicides, fertilisers, pesticides, and hazardous chemicals make their way directly or indirectly into water bodies due to inadequate treatment to remove harmful pollutants (US EPA, 1997). Among types of water body contaminations, ground water contamination is the most difficult to rehabilitate globally (Vodela *et al*., 1997). Water pollution is a major hazard to living organisms that live both on land and in aquatic environment with humans the most affected (Mann *et al*., 2014; Adjegba and Bamgbose, 2012; Tyagi, *et al*., 2007). The major effect of this water pollution (caused by the release of these households and industrial effluents into our water bodies) is reduction in the level of dissolved oxygen. This occurs due to the activities of organisms in the effluents and their competition for oxygen with the flora and fauna of the water bodies. Therefore, water may be called polluted when the physical, chemical and biological parameters reach beyond a stated level of a particular water use. The physical parameters include temperature, pH, colour, odour, salinity, total dissolved solids, turbidity, taste, and electrical conductivity which are good indicators of contamination. The biological parameters comprise of algae, fungi, viruses, protozoa and bacteria among others. The chemical parameters include sulphates, carbonates, chlorides, nitrates, fluorides, and metal ions.

The excessive nutrients discharged into the water bodies can result to eutrophication and reduction in the available oxygen that ultimately alters the biotic community structure and function. Wakelin *et al*. (2008) also reported that excess discharge of turbid effluents can cause sand and grit deposition into the aquatic system, disruption of sediment characteristics and impede the flow of natural water. Furthermore, the total physico-chemical and hydrological environment is always influenced by the introduction of poorly treated effluents affecting numerous micro and macro fauna present in the waterbodies. This can lead to the death of the vulnerable micro and macro fauna and the survival of the tolerant ones. The situation can lead to discrepancy among the cluster of organisms present and the total alterations to the surroundings in the form of light and oxygen contents, food sources in addition to loss of habitat, and nutrient modification (Wakelin *et al*., 2008).

Hydrocarbons, detergents, pesticides, nitrogen, phosphorus, and heavy metal are the major chemical contaminants of wastewater. Larsdotter (2006) reported that nitrogen and phosphorus are known to be nutrient limiting and the occurrence of nitrogen in the discharged wastewater can be unpleasant due to its ecological and public health impact. Nitrogen occurs mainly in organic form and their occurrence is linked to municipal sewage disposals and application of fertilizer to agricultural crops (Hurse and Connor, 1999). The excess nitrogen in water bodies as a result of municipal sewage disposal and fertilization application can result to the formation of ammonia which is poisonous to fish and exert an oxygen demand on receiving waterbodies by nitrifiers (Jenkins *et al*., 2003).The presence of nitrogen and other chemical contaminants have the tendency to cause algal bloom. Algal bloom disrupts the inherent composition and diversity of organisms in the aquatic communities hindering large-scale fishing and posing a problem for treatment of water.

2.5.2: Effect on human health

People dwelling near municipal sewage outfalls or polluted water sources are at a risk of being exposed to diseases as a result of increased pathogenic microorganism and worsening physico-chemical parameters (Wakelin *et al*., 2008). The recreational users of waterbodies often experience dangerous situation as a result poor visibility caused by extreme turbid effluent discharged into the waterbodies and the development of algal blooms. Furthermore, if these waterbodies are used for contact-recreational activities it can serve as a media of several diseases contracted either through body contact or ingestion of the polluted water (Department of Water Affairs and Forestry, 1996). However, the severity of the infection depends on the physical health of the individual concerned and the type of water borne disease. Odjadjare and Okoh (2010) reported that the discharge of effluents that are not properly treated often results to an increase in the microbial load of the waterbodies and consequently results to a range of water borne diseases such as giardiasis and gastroenteritis.

2.6 Laws that Exist In South Africa on Protection of Rivers

Effluent standards refer to restrictions imposed relating to quantities, rates, and concentrations of materials in wastewater discharge (World Bank, 2012). Regulating effluent is important as water pollution has a severe effect on the environment, including biotic and abiotic organisms. Many countries of the world have developed laws that help to monitor the discharge of these effluents into the water bodies thereby controlling water pollution and reducing untreated wastewater impacts. South Africa is not an exception. The country has developed many Acts and policies with respect to the control of water pollution.

The laws that exist in South Africa to protect our water resources are as follows

2.6.1 National Water Act, Act No 36 Of 1998

- This Act ensures that the water resources of the nation are protected, developed, controlled, used, managed and conserved in a way that it considers the following factors: promoting fair and impartial access to water;
- meeting the rudimentary needs of human for present and future generations;
- promoting the beneficial, sustainable and efficient use of water in the public interest;
- facilitating economic and social growth;
- redressing the outcomes of past gender and racial discrimination;
- meeting international obligation;
- promoting dam safety;
- preventing and reducing contamination and dilapidation of water resources;
- providing for growing demand for water use;
- managing floods and droughts;
- protecting aquatic and associated ecosystems and their biological diversity.

2.6.2 National Environmental Management: Air Quality Act, 2004 (Act No. 39 of 2004)

The purpose of this Act is to ensure that the environment and natural resources are secured and managed in such a way that the continuity of the resources is conserved with improved quality. The act also prohibits any activity that can lead to any harmful effect in the surroundings, including cultural heritage or social, economic, health, and ecological conditions.

2.6.3 Public Health Act of the Union of South Africa, 1919 (Act No.36 of 1919)

The purpose of this Act was to ensure that pollution through sewage disposal methods was managed, controlled and possibly prevented. This Act gave the Chief Health Officer of the Public Health Department the duty of ensuring that pollution caused by sewage disposal was controlled through any best practical method available for sewage disposal. The officer was also given the right to prevent sewage treatment works from discharging their effluents directly into the water courses without treatment.

2.6.4 Water Act, 1956 (Act No.54 of 1956)

The main aim of this Act was to control use of water in the industries and the treatment and disposal of the effluents. This Act was created to ensure that well treated water from the treatment plants are returned to waterbodies. This was due to high water demand in the country and the need to re-use effluents in order to solve water scarcity problems. Due to the negative effect of heavy metals, laws were developed to control pollution level. There is therefore a need to develop good techniques that will enhance the removal of these heavy metals. This Act is now known as the National Water Act No. 36 of 1998.

2.7 Heavy Metal Removal Techniques

2.7.1 Physical and chemical techniques of heavy metal removal

The heavy metals removal from wastewater has currently become the matter of substantial interest due to strict legislations. According to the South Africa Water Act (Act 54 of 1956), wastewater must be treated to acceptable limits and sent back to the water course from where the water was initially obtained (Morrison *et al*., 2001). However, the cost of treatment is considered a limitation to the effective treatment of wastewater. There are numerous methods used for heavy metals removal from wastewater, they include filtration and electro coagulation. This treatment is further hampered by the long duration of time required for its completion (Volesky, 1990). Research has also shown that there are various biological treatments, such as aerobic, anaerobic and biosorbents that can be used for heavy metal removal (Dhokpande and Kaware, 2013, Waisberg *et al*., 2003).

Solvent extraction, chemical precipitation, reverse osmosis, ion exchange, membrane filtration, electro dialysis, evaporation, oxidation and activated carbon adsorption are examples of conventional methods used in heavy metal removal (Volesky, 1990, Volesky and Naja, 2007) as summarised in Table 2.1

Table 2.2: Advantages and disadvantages of heavy metal removal techniques (Ghazy *et al*., 2008; Dhokpande and Kaware, 2013).

Method	**Disadvantage**	**Advantages**
Chemical precipitation	For high concentration, generates sludge, difficult separation	Simple, cheap.
Ion exchange	Sensitive to particles, expensive	Effective metal recovery
Reverse Osmosis	High pressure membrane scaling, expensive.	Pure effluent
Chemical Oxidation/reduction electrochemical	Chemicals required for high concentrations, expensive	Mineralisation, metal recovery
Evaporation	Expensive, generates sludges	Pure effluent
Hybrid Methods (floatation filtration)	Further research required	Low operating costs, high membrane fluxes

2.7.2 Microbial method of heavy metal removal

Microbes are good indicators of pollution because the extent of their pollution reduction can be evaluated using simple methodology, which is relatively rapid and the materials required for testing are readily available (Maila and Cloete, 2005). It is also regarded as an efficient method of metal removal because of its low cost, high efficiency and ecological friendly nature and method for metal clean-up (Haferburg and Kothe, 2010; Milner and Kochian, 2008; Pulford and Watson, 2003) unlike the conventional physico-chemical techniques that are expensive and may not be very effective (Hookoom and Puchooa, 2013). This physico-chemical analysis is not only complex, costly and laborious but also lacks information on the additive, antagonistic or synergistic effects of several chemicals on the biotic community in aquatic ecosystem (Tyagi *et al.,* 2007) and does not take into consideration the bioavailability of the contaminants present (Bielská, 2013).

Biological treatment methods are more appealing as a result of their cost effectiveness and environmental friendliness as well as the various metabolic pathways and versatility of microorganisms (Pandey *et al.*, 2012; Ajao *et al*., 2012) when compared with chemical oxidation and reduction electrochemical processes which are expensive due to large amount of chemicals required, although the metals are usually recovered at the end of the process (Volesky and Naja, 2007). Industrial effluent especially mine water is mostly characterised by extreme pH (acidity or alkalinity), high salinity, high concentrations of SO_4^{2-}, Al and several other toxic metals such as Fe, Cd, Co, Cu, Mo, Zn, Ni, V. The detection of microorganisms in severe surroundings of pH and metal concentrations has provided some knowledge on the understanding of microbial biosynthetic processes which enhance the bioremediation of contaminated areas (Oarga, 2009).

Studies carried out on the diversity bacteria in sites contaminated with heavy metal have shown a high diversity of microorganisms (Hookoom and Puchooa, 2013). They are native organisms that have grown and modify the environment (Hookoom and Puchooa, 2013). Microorganisms have a diverse methods to deal with high levels of heavy metals and often are specific to one or a few metals (Mejare and Bulow, 2001; Nies, 2003; Piddock, 2006). Microbes have developed methods to deal with the metals either through efflux, complexation, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration (Haferburg and Kothe, 2010). Most mechanisms described involve the efflux of metal ions outside the cell, and genes for tolerance methods which have been found on both chromosomes and plasmids. Bacteria that are resistant to and grow on metals play an important role in the biogeochemical cycling of those metal ions (Appenroth, 2010).

Recent works have been done by different researchers to understand metal – microbes' interaction, their application for metal accumulation, detoxification and their removal property (Haferburg and Kothe, 2010; Appenroth, 2010; Hookoom and Puchooa, 2013). Ilhan *et.al.* (2004) investigated the removal of chromium, lead and copper ions by microorganisms from industrial wastewater. The effect of pH, temperature, initial concentration on the metal removal was investigated. The optimum pH values were observed to be 2, 3.5, and 4.5 respectively for these three metals. At the optimised conditions the biosorption values were found to be 88.66, 100 and 44.94 mg/l respectively. It was concluded that *Staphylococcus saprophytics* was appropriate mainly for lead and chromium.

Another study on isolation, identification and characterisation of heavy metal resistant bacteria was carried out by Raja *et al*. (2008). Wastewater samples across Madurai district in India were collected then bacteria were isolated and characterised to help evaluate ideal growth conditions. The minimum inhibitory concentration was also determined. The sewage isolates showed optimum growth at 30 °C and pH 7.0 for 5 days. They observed that the growth rate of the sewage bacteria in the presence of heavy metals was consistently slower than the control (Raja *et al*., 2008).

Sharma *et al*. (2003) attempted the removal of zinc biologically using *Aspergillus* sp. They established the fungal strain in 100 ml conical flask. The initial pH was 5.6, at a temperature of 30°C. They carried out the experiments using sugar levels of 10, 15 and 20 g/l at dilution rates of 0.08, 0.04 and 0.02 per hour. They detected that there was no significant increase in the specific zinc uptake with increase in sugar level. The specific zinc uptake was found to be 120 mg/g of dry biomass at 10 g/l sugar level.

Subhashini *et al*. (2003) also conducted research on heavy metal removal from aqueous solution using *Schizosaccharomyces pombo* in free and alginate immobilised cells. Batch studies were performed by changing parameters such as pH, temperature and metal concentration. The optimal temperature was 25°C with a pH of 4. The maximum removal of 73 % was observed at the initial concentration of 100 ppm with inoculums concentration of 1%. Their investigation gave an indication that immobilisation beads are a better metal removal method than free beads.

It is evident from the literature reviewed that many studies carried out on removal of heavy metals using microbes have shown consistency within bench phases. However, it cannot be ascertained if these methods will be viable in large scale heavy metal removal. It is also noteworthy that the effluents of most industrial plants meet all physico-chemical regulations and more often than not the microbiological standards are also met; however, their toxicity remains questionable and could still negatively impact on the receiving water (Movahedian and Asghari, 2005). It is imperative that concerted measures are needed to test for toxicity of discharge effluent. However, this is a luxury very few industrial plants and wastewater treatment plants can afford in most developing countries. Equally important is the need to increase capacity to remove these persisted non-biodegradable toxicants. The major factor contributing to this impediment is costs. Thus it is a limiting factor to the overall improvement of the water quality that reaches the end users. Prioritising the determination of the presence of recalcitrant heavy metals is essential. It is possible that even with financial constraints when the levels of heavy metal pollution are determined, crucial measures that are not costly can be taken to ameliorate the problem. Hence microbiological analysis could be a simpler and cheaper method that helps identify the presence of heavy metals.

This chapter summarises information pertaining to wastewater treatment and the treatment approach and effectiveness adopted by treatment plants. The impact of improperly treated waste water and the laws instituted by South Africa to ensure that waste water is properly treated are also highlighted. Discussion on the composition of sewage and the different types of waste water treatment (primary, secondary and tertiary treatment) found in Leeuwkuil Wastewater Treatment Plant was also included.

CHAPTER 3: STUDY AREA AND METHODOLOGY

3.1: Introduction

In this chapter, methods, and procedures employed in this study are described. The effect of effluent discharged on surface water quality is evaluated in terms of physico-chemical study, microbiological and molecular characterization. The identification and quantification of heavy metals from five different industries that discharge their effluent at a Leeuwkuil sewage plant in the Vaal areas were also analyzed using modern spectrophotometric techniques and supported by microbiological assays of heavy metal resistant bacteria.

Description of laboratory analysis carried out on samples collected from the Vaal River, Leeuwkuil Sewage Treatment Works, potable water sources and the effluent discharged from five different industries are given and the results are compared with Green Drop certification and South African General Effluent Standard (SAGES), South Africa National standards (SANS-241) and the World Health Organisation (WHO) benchmark for the required standards for discharge into rivers and potable water as specified by some regulatory bodies.

3.2 Study Area

3.2.1. General description of study area

Vereeniging is a city located in the southern part of Gauteng province, South Africa as shown in Figure 3.1A and 3.1B. This city is very close to Vanderbijlpark (to the west), Three Rivers (east), Meyerton (north) and Sasolburg (south) as shown in Figure 3.1.The climate of Vereeniging is the same with that of Johannesburg (both cities are located in Gauteng province) and is mostly influenced by altitude. Although the province is at a subtropical latitude, the climate is relatively cooler, especially in Johannesburg, at 1,700 m (5,577 ft) above sea level. Precipitation occurs as brief afternoon thunderstorms and winter is cool and dry with frost occurring frequently in the southern areas (Emfuleni Local Municipality, 2013). The minimum and maximum temperature in summer is 15 °C and 30°C and in winter is between -2°C and 20°C respectively.

The city is presently one of the main industrial manufacturing centres in Gauteng, with its principal products being iron, steel, pipes, bricks, tiles and processed lime (Emfuleni

Local Municipality, 2013). Vereeniging is under Sedibeng District Municipality which is positioned on the southern tip of Gauteng Province and strategically located on the border of three provinces, such as Mpumalanga, North West and Free State (Sedibeng District Municipal, 2010). Sedibeng district comprises of three municipalities namely Emfuleni, Midvaal and Lesedi and it is regarded as the fourth-largest contributor to Gauteng economy because of high-level metal and chemicals production. (Sedibeng District Municipality, 2010, Haji, 2011). Leeuwkuil Wastewater Treatment Works (WWTW) is located in Vereeniging within Emfuleni Local Municipality as shown in figure 3.1. This municipality falls under Sedibeng District and it offers effluent treatment services to the community. The water resources profile of the municipality fall under Upper Vaal Water Management Area which is towards the centre of the country with major rivers being the Vaal River (Emfuleni Local Municipality, 2013).

The study areas (upstream and downstream) is a part of the Vaal River and is towards the centre of the country while the inflow and final effluent are located within the premises of Leeuwkuil wastewater care works as shown in Figure 3.1 and 3.2 respectively. The waste effluent from Leeuwkuil comes from both domestic and industries which include battery industries, farmland, abattoir, galvanized industry, wire industry, iron and steel industries and many more from which the five industries used in this study was selected (Emfuleni Local Municipality, 2013). These industries produce a large number of effluents which contains toxic waste and high load of organic matter, which needs to be treated by Leeuwkuil Treatment Plant before their final disposal/discharge to Vaal River.

Emfuleni Local Municipality owns three conventional activated sludge wastewater treatment works (WWTW) with varying design capacities and treatment systems +48 as shown in Table 3.1 below.

Table 3.1: Emfuleni Local Municipality Wastewater Risk Abatement Plan.

Name of Wastewater Treatment Works	**Design Capacity (Mℓ/d)**	**Type of Treatment Process**

Leeuwkuil WWTW	36	BNR and Bio-filter
Rietspruit WWTW	36	BNR and Bio-filter
Sebokeng WWTW	100	BNR

[1] 1 BNR- Biological Nutrient Removal

Leeuwkuil WWTW discharges directly into the Vaal River whereas Rietspruit and Sebokeng WWTW discharge into Rietspruit River which is a tributary of the Vaal River. The Rietspruit and Sebokeng WWTW falls under Rietspruit Catchment, this lies close to the south west of Johannesburg. The municipality's wastewater system is served by Leeuwkuil WWTW which treats sewage from Sebokeng east (Kwaggastroom), Vereeniging and Sharpeville. Sebokeng WWTW treats sewage from south of Johannesburg, Sebokeng, Evaton and Palm Springs and Rietspruit WWTW which treats sewage from Vanderbijl Park, Bophelong and Muvhango Townships and sewer pump stations.

Figure 3.1A: Map of South Africa showing Gauteng Province. (https://www.google.com/search?q=insert+of+South+African+maps+showing+Gauteng+province).

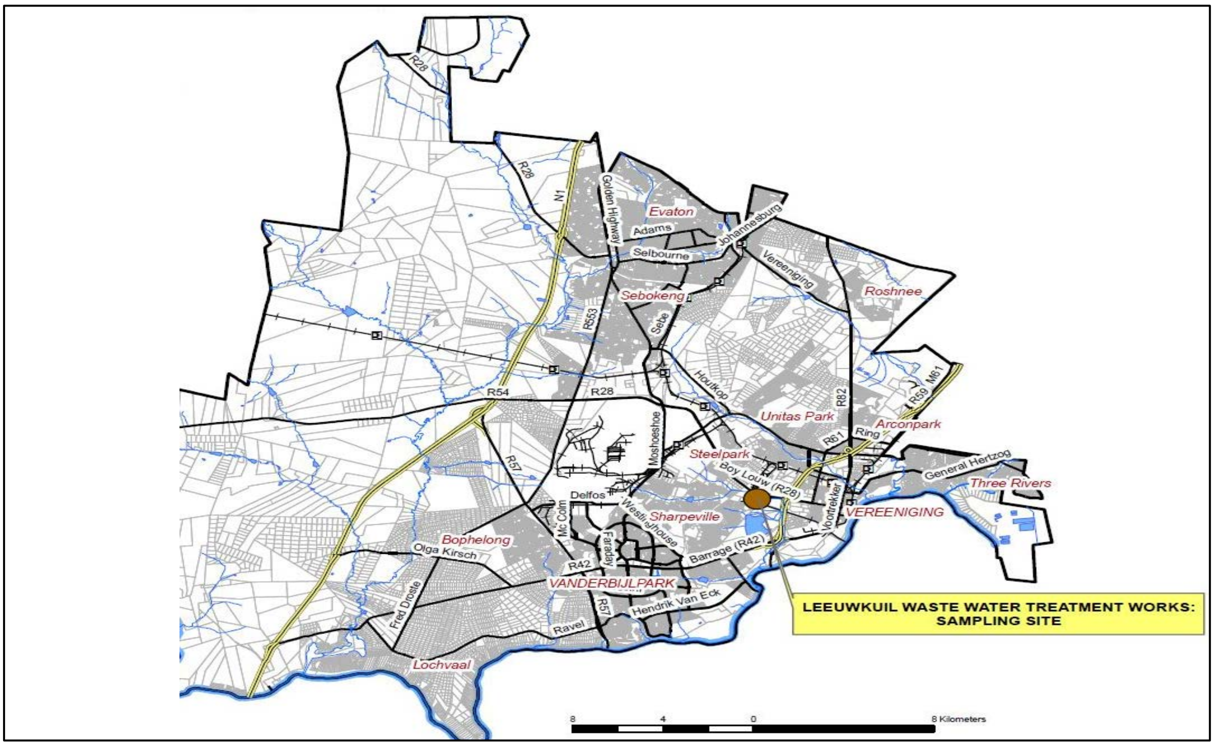


Figure 3.2B: Location of study area within Emfuleni Local Municipality

3.3 Methodology

3.3.1 Sample collection

Triplicate samples were collected monthly from all aforementioned sample sites (see the description of the sampling point in table 3.2 below). The samples were collected for a period of nine months between January and September (2017) representing the seasonal changes prevalent in South Africa (summer, autumn, winter, and spring). Water samples were collected with well-labelled 1-liter sterile glass and plastic containers separately at each sampling site and these containers were washed with the sample water before filling at the sampling point. The samples were collected midstream by dipping each sample bottle below the water surface approximately at a distance between 20-30cm, projecting the mouth of the container against the flow direction (to ensure thorough mixing).

The samples were immediately placed in cooler boxes containing ice and transported to the laboratory at the University of South Africa (UNISA) Florida Campus for chemical and microbiological analysis (after determining the physico-chemical parameter on site using the Multi-parameter) within 12 hours after collection. Before sampling potable water, tap head was sterilised by using the flaming method and then water samples were collected. The samples were collected in sterile plastic containers and similar precautions of transport and storage used in preserving other samples to prevent contamination. Microbiological samples were collected in a clean pre-sterilised 500 ml glass bottles, all the samples were immediately stored in the cooler box and transported within twelve hours after sampling. For heavy metal determination samples were acidified with 1 ml of concentrated HNO_3 in 500 ml pre- sterile acidified brown bottles during collection.

Table 3.2: Description of sampling points

Sampling point	Co-ordinates	Description
Point 1: Upstream	26º42'29.9"S 27º53'53.4"E	The upstream is from the Vaal river where fishing from local people takes place.
Point 2: Inflow	26°40'23.5"S 27°53'42.5"E	Incoming raw sewage/inffluent water from both domestic and industries that are yet to be treated
Point3: Final effluent	26°40'43.9"S 27°54'17.9"E	The final effluent discharged into the maturation pond after treatment and aeration
Point 4: Downstream	26°42'39.2"S 27°53'33.0"E	Water from the river where treated final effluent discharged at the midstream flow down to downstream
Point 5A Potable Water 1	26°41'31.4"S 27°54'44.0"E	Vereeniging (Rand Water Laboratory) sample point for first potable water
Point 5B: Potable Water 2	26°39'30.9"S 27°55'17.0"E	Duncanville – Sample point for second potable water collection (Garage)
Point 5C: Potable Water 3	26°38'20.8"S 27°56'15.9"E	Arconpark - Sample point for potable third water collection (Garrage and Car wash)
Point 6: Leeuwkuil Sewage Plant	26°40'22.1"S 27°53'45.2"E	The plant where sewage effluent is treated

Point 7A Industry 1	26º39’29.6”S 27º56’09.7”E	Company located at Vereenining road that produces lead acid batteries
Point 7B Industry 2	26º39’32.9”S 27º55’07.9”E	They produce Galvanised iron and metals for coating
Point 7C Industry 3	26º39’25.7”S 27º51’18.1”E	They produce mild steel and wires products
Point 7D Industry 4	26º40’15.2”S 27º47’41.3”E	Tank cleaning services that specialises in washing trunks.

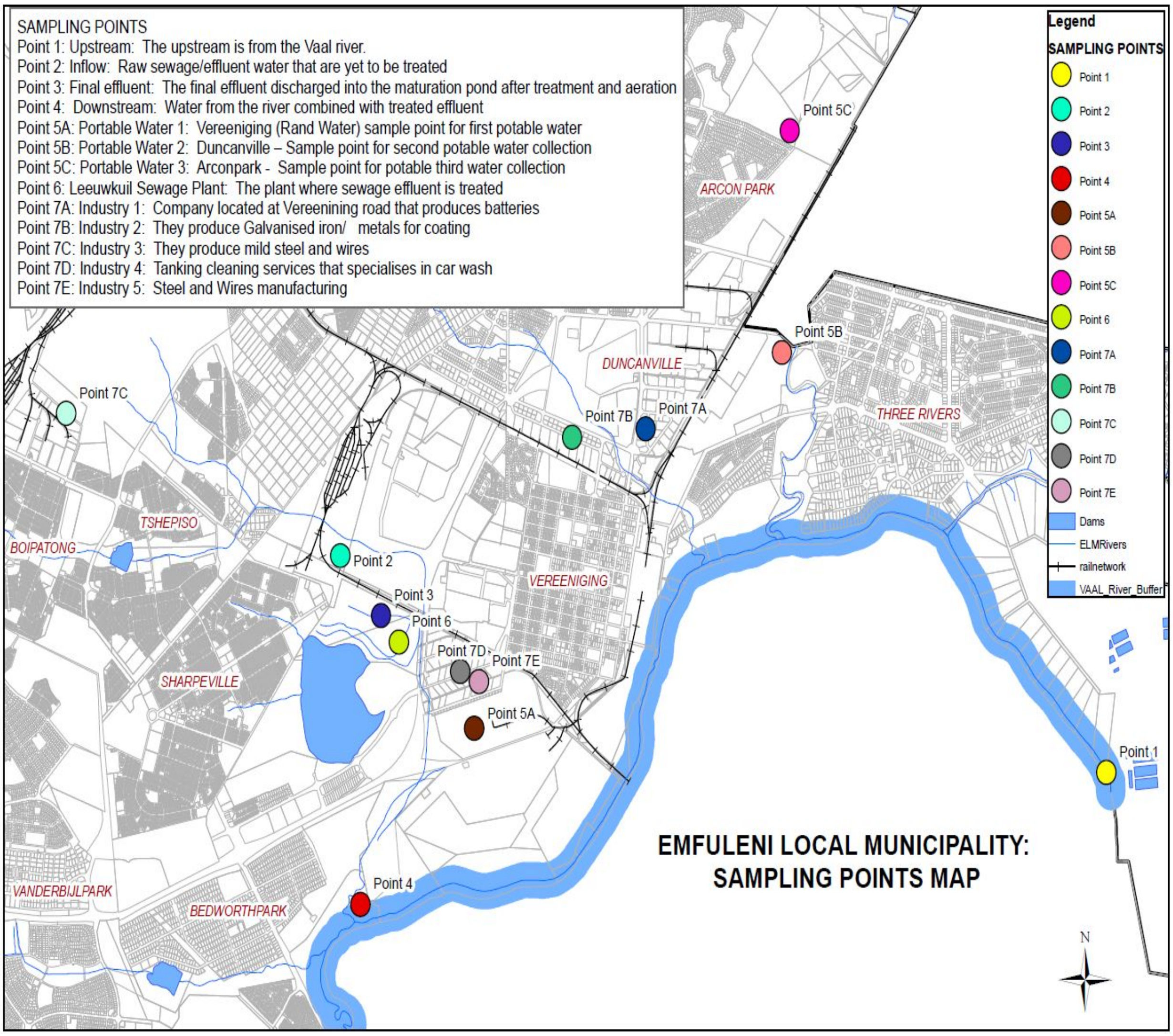


Figure 3.3: Maps Showing Sampling Points

3.4 Experimental Methods

The experimental work of the water analyses was conducted in two parts:

- Field work which included the infield (onsite) measurement of some physico-chemical parameters such as temperature (^oC), pH, conductivity (µS/cm) salinity (PSU), dissolved oxygen (mg/l), and total dissolved solids (mg/l)
- Laboratory analysis which include biological oxygen demand (BOD) (mg/l), Chemical oxygen demand (COD) (mg/l), heavy metal analysis (ICP-OES), microbial analysis(Isolation, purification, counting of colony, Gram staining, DNA Extraction and polymerase chain reaction PCR).

3.4.1 Determination of physico-chemical parameters

Onsite analysis of water samples included temperature, pH, electrical conductivity, salinity, total dissolved solids (TDS), and dissolved oxygen (DO) were measured using a multi-parameter ion specific meter (Hanna instruments, version HI9828, SN 08334776). The probe was initially rinsed with distilled water, and then followed by several rinses so as to optimize each water samples before actual reading was taken by immersing the probe into the water samples. All the measurements were taken in triplicates for the proper mean values

3.4.1.1 Heavy Metal Determination/Analysis

Elemental concentrations of aluminium (Al), Copper (Cu), Zinc (Zn), Manganese (Mn) and Lead (Pb) in the water samples and industrial effluent were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (PerkinElmer Optima 5300 DV).

Collected samples were digested using concentrated HNO_3. The essence of the digestion before analysis was to reduce organic matter interference and convert the metal to a form that can be analyzed by Inductively coupled argon plasma spectroscopy (ICP – OES) (Chinedu *et al*., 2011). All digested samples were filtered using 0.45 µm filter paper, prior to ICP-OES analysis. The target elements were analyzed by direct aspiration into the ICP-OES, data inclusion was based on correlation coefficient readings of >0.999 benchmarked against the standard curves for each of the metal standards at the respective absorbance wavelength, taking into consideration their respective method detection limits. A calibration blank and an independent calibration verification standard were analysed together with all samples to confirm the calibration status of the ICP-OES.

Table 3.3: Standard wavelength and deletion limits for heavy metals and plasma viewing position on the PerkinElmer optima 5300DV

Elements	Wavelength (nm)	Method detection limits (ppm) (Water)	Linear dynamic range (ppm)	Plasma viewing position
Al	396.153	0.008	1000	Radial
Cu	324.752	0.008	50	Axial
Mn	257.610	0.002	500	Radial
Pb	220.353	0.002	1000	Axial
Zn	202548	0.0009	100	Axial
Zn	.213.857	0.0017	100	Axial

Adapted From U.S. Environmental Protection Agency, 1996. Inductively Coupled Plasma-Atomic Emission Spectrophotometry, Method 6010B, Revision 2.0, SW-846 Manual, 3rd edition, Office of Solid Waste and Emergency Response

3.4.1.2 Chemical oxygen demand (COD)

USEPA (1995) stated that chemical oxygen demand (COD) is an important parameter used in assessing the degree of pollution and indices of self-purification of a river body. The COD was determined in the laboratory using standard Hanna instruments reactor HI839800 and HI 83099 COD and multi-parameter Photometer

The reactor was firstly preheated to 150 ˚C. Two syringes were supplied in the kit. One of the syringe was used to add 2ml of each eleven (11) sample to the vial in triplicate and the second syringe was used to add deionized water into another reagent vial in triplicate which was used as the blank (control). The vial was kept at an angle of 45^{o}. With the cap of the reagent tightly closed, the samples were mixed properly by inverting the vial a couple of times and then inserted immediately into the reactor and heated at 150 ˚C for 2 hours. At the end of the digestion period, the reactor switched off automatically and the vials containing the samples were allowed to cool down to about 120 ˚C for 20 minutes. Each vial was inverted severally while still warm and was then placed in the H1 740216 rack. The vials were left in the rack to cool to room temperature.

Colometric COD result was determined. First, the blank reading was read after setting the photometer machine to COD MR to confirm the zero reading after which the vials containing the eleven (11) samples in triplicates was now determined and recorded.

3.4.1.3. Biological oxygen demand measurement (BOD).

Biological Oxygen Demand (BOD) is an indicator of the concentration of biodegradable organic matter present in a water sample (Chinedu *et al.*, 2011). It can be used to gather the general quality of the water and its degree of pollution.

A sterilized 500ml plastic bottle in triplicate was used to collect BOD samples in all sampling points. Onsite analysis of water samples readings of dissolved oxygen was taken using a BOD/DO Hanna instrument version HI 98193, this reading is regarded as initial dissolved oxygen reading (DO_1). The BOD bottles were placed in a box, covered with a black plastic and brought back to UNISA Laboratory. The box containing BOD bottles were now sealed properly, put in the laboratory cupboard and was incubated for five (5) days at room temperature. After five days of incubation at room temperature, the dissolved oxygen level in the BOD bottles was measured using the same digital meter. The value of BOD as adapted by Mocuba (2010) and Chinedu *et al.* (2011) was determined by subtracting dissolved oxygen after incubation (DO_5) level from the DO_1 (measured in the field) level found five days previously:

BOD = DO (mg/l) (measured in the field) **-** DO (mg/l) (measured after incubation).

3.4.2 Microbiological analysis

Isolation of Bacteria (pure culture), Gram staining, extraction of DNA, PCR and tolerance test were carried out. The methods used are explained in detail as follows:

3.4.2.1 Isolation of bacteria (pure culture) from sample

The bacterial culturing of the water samples was examined using culture techniques. Approximately 1 ml of undiluted water samples from eleven sites was added to Nutrient agar (NA) media purchased from Sigma Aldrich, Pretoria RSA, using a sterile

Pasteur pipette (except for the inflow site that was diluted with sterile distilled water until a dilution of 10-5 was obtained and 1 ml of the diluted sample was now added to nutrient agar, and was spread using sterile plastic spreader and finally incubated at 37°C for 24 h. Distinct colonies that appeared on the media were directly streaked onto NA using sterile plastic loops and were incubated for 24hrs at 37°C. After incubation, microbial growth (based on the number of the colony) was observed. Distinct colonies were sub-cultured using streak method for purification (Abo-Amer *et al*., 2015). The pure cultures were identified based on gram staining and molecular techniques.

3.4.2.2 Gram Staining

Gram staining technique as described by Behera (2013) were adapted as a basis for the classification of isolated axenic cultures of bacteria and in the identification of gram positive (+ve) and gram negative bacteria (-ve).

3.4.2.3 Molecular techniques: extraction of DNA from bacteria isolates

DNA was extracted from each pure culture using a quick g-DNA extraction kit (Zymo Research, USA) according to the manufacturer's instructions. Approximately 1060 µl of storage buffer was added to each 20 mg tube of proteinase K and was maintained at -20°C. A loopful of cultured was mixed with 200 µl of saline water in the micro-centrifuge tube and vortexed at low intensity for 2 minutes. Approximately 200 µl of the sample together with 200 BioFluid and Cell Buffer and 20 µl Proteinase K was added to another micro-centrifuge tube. The tube was mixed thoroughly with the vortex machine and then incubated at 55°C for 10 minutes. After the incubation period, approximately 1 volume of Genomic Binding Buffer was added to the digested sample and then mixed thoroughly. The mixture was transferred to a Zymo-Spin 11C-XL column in a collection tube and centrifuged at 12000 rpm for 1 min. The collection tube together with the flow through the tube was then discarded.

Approximately 400 µl DNA pre-wash buffer was added to the column in a new collection tube and centrifuged for 1 min. The collection tube was later emptied. Afterward, approximately 700 µl/g – DNA wash buffer was added and centrifuged for 1 min and the collection tube was emptied also. Approximately 20 µl/g – DNA wash buffer was added again and centrifuged for 1 min and the collection tube with the flow through the tube was then discarded. The column was transferred to a clean centrifuge in order to elute the DNA. The supernatant (the amplicon) was collected and precipitated by adding approximately 70 µl DNA Elution, incubated for 5 minutes and then centrifuged for 1 mins. Immediately after the DNA extraction, polymerase chain reaction (PCR) was carried out using the universal bacterial 16S rDNA primers 27F (27F and 518R).

3.4.2.4. Amplification of 16S rDNA genes by polymerase chain reaction (PCR) and analysis of the PCR products.

The PCR amplification of the target DNA was carried out in a thermal cycler (MJ MiniTM Personal Thermal Cycler, Biorad SA) using 200 µl PCR tubes and a reaction mixture volume of 25 µl. The reaction mixture (working solution) was prepared, containing 12.5 µl × Dream Taq™ PCR master mix (10 × Dream Taq™ buffer, 2 µM dNTP mix and 1.25 Dream Taq™ polymerase), 1 µl of each PCR primer (27F and 518R) (10 µM) (synthesised by Inqaba Biotechnologies Industry, Pretoria, South Africa) and 2.5 µl of genomic DNA (25 ng/µl) was made up of 25 µl with ultra-pure nuclease-free water (8.5 µl).

Approximately 22.5 ml of the working solution was taken and added to the PCR tube that was properly labelled. Also, 1.5 ml of the extracted rDNA was added to the PCR tube and vortex to ensure homogeneity. The PCR tubes were then placed in the Thermal cycler under the following reaction conditions; Initial denaturation at 94°C for 5 min, 32 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were first analyzed by 1% agarose gel electrophoresis in 1X_ TBE buffer (Sigma SA) and were stained with 2% of 10 mg/ml ethidium bromide (Sigma SA) and visualize under short-wavelength UV light. The PCR products were done by Gel extraction Kit and sent to Inqaba, Pretoria, South Africa (SA) for sequencing.

3.4.2.5 Phylogenetic analysis

The 16s gene sequences obtained was first analyzed by BLAST (Basic Local Alignment Search Tool) algorithm. Based on the scoring index, the most similar sequences were aligned with the sequences of other representative bacterial 16S rDNA regions by using Clustal W software. The 16S rDNA sequences selected bacterial strains were then deposited in GenBank.

3.5 Heavy Metal Screening Test

To examine the ability of the 70 bacteria isolates to resist heavy metals, a screening test as described by Hookoom and Puchooa (2013) was adapted. A cut of range for concentration of all four (4) heavy metal used were determined as follows; lead (in lead nitrate - Pb) is 0.006 mg/l, Zinc (in Zinc nitrate -Zn) is 0.02 mg/l, Copper (in copper sulphate - Cu) is 0.005 mg/l and Aluminium (in Aluminium Nitrate - Al) is 0.2 mg/l. Overnight grown cultures of bacterial cells were inoculated on nutrient agar plates supplemented with different concentrations of heavy metals as indicated previously above. Zinc in Zinc nitrate, Lead to Lead nitrate, Aluminium in Aluminium nitrate and Copper in Copper sulphate were incubated at 37°C for 24 hours and cell growth observed.

The concentrations of each metal was increased by a common factor of 10 with concentrations as follows; (Pb =0.06mg/l, Zn= 0.2mg/l, Cu=0.05mg/l, Al=2mg/l). The numbers of bacteria isolates were reduced/screened to 39, based on only those that are resistance to the heavy metals being tested. In order to further reduce the number of bacteria isolates, the concentrations of each metal were further increased by a common factor of 20 bringing the concentrations as follows; (Pb =0.12 mg/l, Zn= 0.4 mg/l, Cu=0.01 mg/l, Al=4 mg/l). The numbers of bacteria isolates were further reduced to 22 based on only those that are resistance to the metals. It is this 22 isolates that molecular study was conducted on at Inqaba Biotec, Pretoria, South Africa for sequencing. Table showing the reaction of the isolates to different heavy metals and their progression in their reduction is shown in chapter 4.

3.6 Statistical Analysis

One-way analysis of variance (ANOVA) was done using SAS version 9.4 at a significance level of 0.05 to show the mean separation of the parameters measured across the sampling points. Also, Pearson correlation coefficient was also done using SAS version 9.4 at a significant of 0.05 to show the relationship between the parameters across the eleven (11) sampling points.

CHAPTER 4: RESULT AND DISCUSSION

4.1 Introduction

This chapter presents the research results, its interpretation and discussion of the findings. The study evaluated the water quality of sources within and around the Vaal areas, which is an urban area with industries, over a period of four seasons. The results presented in this chapter included data obtained on concentrations of heavy metals in industrial effluent water samples, Leeuwkuil Plant, Vaal River and potable water around Vaal, as well as their microbial characterisation and resistance to some heavy metals. The interpretations and discussions of the obtained results were based on the impact of heavy metals in industrial effluents on Leeuwkuil Plant and the environment in relation to compliance with the physico-chemical parameters stipulated standards by organisations such as Green Drop (2013), SAGES certification requirements (2013), SANS-241 (2015) and WHO (1984; 1989).

Table 4.1A: Elemental concentrations (ppm) in portable water, water from the Vaal River, Leeuwkuil Plant, and industries in the Vaal areas. Values (M±S.E.) followed by dissimilar letters in a column are significantly different at p≤0.05 and separated by different letters

	Elemental concentrations (mg/l)									
	Ag	**Al**	**B**	**Ca**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Na**
IND1	0.2±0.0b	10.6±3.6a	0.6±0.0c	186.3±16.5ab	0.2±0.0c	0.7±0.0a	14.0±1.3ab	8.5±0.8b	83.5±7.9a	213.0±18.7c
IND2	0.2±0.0b	0.2±0.1c	0.7±0.0c	37.8±3.9c	0.8±0.0a	0.2±0.0b	16.7±1.5a	8.6±0.9b	5.7±0.5b	432.1±78.6ab
IND3	0.2±0.0b	1.8±0.6b	5.4±0.8b	143.4±13.8ab	0.2±0.0c	0.2±0.0b	0.7±0.0d	63.2±5.6a	70.6±7.0a	49.1±4.8c
IND4	0.2±0.0b	14.0±4.7a	0.5±0.0c	472.3±45.8a	0.2±0.0c	0.3±0.0b	13.1±1.2ab	6.6±0.6bc	25.9±2.3b	97.4±9.0c
IND5	0.2±0.0b	1.8±0.6b	16.1±2.5a	293.7±21.3ab	0.5±0.0b	0.2±0.0b	6.4±0.6cd	9.7±0.9b	12.4±1.0b	866.4±85.0a
InF	0.4±0.2 b	0.2±0.0c	0.2±0.0c	35.2±2.1c	0.8±0.0a	0.2±0.0b	3.2±0.3d	8.5±0.5b	12.0±1.0b	52.8±5.0c
FinF	2.1±0.1a	0.2±0.0c	0.2±0.0c	33.9±7.5c	0.2±0.0c	0.2±0.0b	1.5±0.0d	8.6±0.9b	11.8±1.0b	59.4±6.0c
UpS	0.2±0.0b	0.2±0.0c	0.2±1.0c	67.1±5.5c	0.2±0.0c	0.2±0.0b	0.2±0.0d	8.2±0.2b	18.3±2.0b	53.6±5.0c
DoS	2.6±1.4a	0.2±0.0c	0.7±0.0c	58.8±4.2c	0.2±0.0c	0.3±0.0b	0.2±0.0d	8.3±0.3b	20.2±2.0b	51.2±5.0c
PO1	0.3±0.1b	0.2±1.0c	0.2±0.0c	20.2±0.5c	0.2±0.0c	0.2±0.0b	0.7±0.0d	2.0±0.0d	4.4±0.0b	5.5±0.0c
PO2	0.2±0.0b	0.2±0.0c	0.2±0.0c	27.7±2.8c	0.2±0.0c	0.2±0.0b	0.7±0.0d	3.7±0.8cd	6.5±0.0b	43.3±4.0c

*UpS= Upstream; *DoS= Downstream; *InF=Inflow; *FinE= Final Effluent; PO=Potable water, IND = Industry 1-5

Table 4.1B: Elemental concentrations (ppm) in portable water, waters from the Vaal River, Leeuwkuil plant, and industries in the Vaal areas. Values (M±S.E.) followed by dissimilar letters in a column are significantly different at p≤0.05 and separated by different letters

	Elemental concentrations (mg/l)									
	Ni	P	Pb	S	Sb	Se	Si	Sr	Te	Zn
IND1	0.3±0.0b	6.2±0.0a	4.8±0.0a	3502.8±105.3a	0.2±0.0a	0.2±0.0b	11.5±8.1a	1.8±0.0a	0.2±0.0a	7.40±0.40b
IND2	0.2±0.0b	1.1±0.0bc	0.2±0.0b	15.1±4.2b	0.2±0.0a	0.2±0.0b	6.4±0.0ab	1.8±0.0a	0.2±0.0a	88.79±8.43a
IND3	0.2±0.0b	0.8±0.0bc	0.2±0.0b	21.5±1.2b	0.2±0.0a	0.2±0.0b	0.2±0.0b	0.8±0.0b	0.2±0.0a	1.47±0.02c
IND4	0.4±0.0a	2.6±0.0abc	0.3±0.0b	21.0±2.1b	0.2±0.0a	0.2±0.0b	2.9±0.0ab	0.4±0.0b	0.2±0.0a	7.09±0.29b
IND5	0.2±0.0b	2.3±0.0abc	0.2±0.0b	7.9±1.9b	0.2±0.0a	0.2±0.0b	0.2±0.0b	0.2±0.0b	0.2±0.0a	0.30±0.07e
InF	0.2±0.0b	4.5±0.0ab	0.2±0.0b	166.8±37.8b	0.2±0.0a	3.3±0.0a	4.3±0.0ab	0.3±0.0b	0.2±0.0a	0.23±0.03e
FinE	0.2±0.0b	4.0±0.0abc	0.2±0.0b	20.4±0.9b	0.2±0.0a	1.4±0.0ab	1.8±0.0ab	0.2±0.0b	0.2±0.0a	0.28±0.45e
UpS	0.2±0.0b	0.8±0.0bc	0.2±0.0b	61.4±5.2b	0.2±0.0a	0.2±0.0b	2.7±0.0ab	1.7±0.0a	0.2±0.0a	0.20±0.00e
DoS	0.2±0.0b	1.3±0.0bc	0.2±0.0b	63.1±7.5b	0.2±0.0a	0.2±0.0b	4.7±0.0ab	0.2±0.0b	0.2±0.0a	0.20±0.00e
PO1	0.2±0.0b	0.4±0.0c	0.2±0.0b	8.5±0.8b	0.2±0.0a	1.6±0.0ab	2.5±0.0ab	0.2±0.0b	0.2±0.0a	0.21±0.01e
PO2	0.2±0.0b	1.6±0.0bc	0.2±0.0b	6.4±0.0b	0.2±0.0a	0.4±0.0b	1.0±0.0b	0.2±0.0b	0.2±0.0a	0.75±0.22d

*UpS= Upstream; *DoS= Downstream; *InF=Inflow; *FinE= Final Effluent; PO=Potable water, IND = Industry 1-5.

4.2 Heavy Metals and Physico-Chemical Parameters in Effluents from Industries and Leeuwkuil Wastewater Treatment Plant (Inflow and Final Effluent) Around Vaal Area

Heavy metal pollution is toxic to aquatic ecosystems and therefore a global concern, mainly due to the non-degradability abilities of some heavy metals and the difficulties faced in their remediation because they are recalcitrant (Patil *et al*., 2012; Mahlambi *et al*., 2015). Agricultural, mining, power generation, galvanising, paint and battery manufacturing activities have produced large quantities of heavy metals (Oven *et al*., 2016) that drains into water bodies. Heavy metals identified in this research are not an exception although they have their own beneficial properties in living organisms but in excess can pose serious threats to human wellbeing and environment (Oven *et al*., 2016; Tchnouwou *et al*., 2012).

4.2.1 Industrial effluents as a point source of heavy metals pollution in the Vaal Areas and Leeuwkuil Plant

Fergusson (1990) defined heavy metals as metallic elements with relative high density when compared to water. In recent years, environmental contamination by these heavy metals has created an increased ecological and global public health concern. Bradl (2002) further stated that an exponential increase of the use of heavy metals in several industrial, agricultural, domestic and technological applications has resulted to a dramatic rise in human exposure to these heavy metals. Industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources are the reported sources of heavy metals in the environment (He *et al*., 2005). Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations (Fergusson, 1990; Bradl, 2002; He *et al*., 2005).

In this study, samples collected from industrial effluents (five industries: IND1, IND2, IND3, IND4 & IND5), Leeuwkuil Wastewater Treatment Plant (Inflow and Final effluent), Vaal River (upstream and downstream) and potable waters (PO1&2), were analysed for the identification and determination of heavy metal concentrations. In total, 24 elements were detected in different concentrations (Table 4.1A & 4.1B), however the heavy metals namely Cd, Li, Mo and V were not shown on Table 4.1A and 4.1B due to their low concentrations without variance. The four identified heavy metals (Al, Cu, Pb, & Zn) were of interest in this study for two reasons. Firstly, they were tested on isolated bacteria from the study sites to assess their resistant to these heavy metals. Secondly, according to Jaishanker *et al*. (2014), these four heavy metals are among the few heavy metals commonly found in wastewater from most industries, and humans are also commonly exposed to them even though they are very persistent and toxic to the environment.

According to Table 4.1A and 4.1B below, a significant ($p<0.05$) higher amounts of metals were detected in industrial effluent water samples compared to those from other sampling points. For example, higher amounts of Al (10.6 mg/l) and Ca (472.3 mg/l) were detected in samples from industry 4 (IND4); Cr (0.8 mg/l), Fe (16.7 mg/l), & Zn (88.7 mg/l) in industry 2 (IND2) samples; K (63.2 mg/l) in industry 3 (IND3); Na (866.4 mg/l) in industry 5 samples and Cu (0.7 mg/l), Ni (0.3 mg/l), Mg (83.5 mg/l), Pb (4.8 mg/l), S (3502.8 mg/l), Si (11.5 mg/l) and Sr (1.8 mg/l) in industry 1 (IND1) samples compared to samples from the non-industrial sampling points (Table 4.1A & 4.1B). These results indicate industries as sources of metal pollution in the study site and could be attributed to what they produce and the reagents used. For instance, industry 1 (IND1) manufacture lead acid batteries, which requires organic reagents containing metals such as Cu, Ni, Mg, Pb, S & Si. Similarly, samples from industry 2 (IND2) (galvanizing industry) had the highest Cr & Fe concentration compared to other industries and that could be attributed to what they produce or the reagents used in galvanizing activities. Higher Al & Ca concentrations were detected in samples from Industry 4 (IND4), which could be attributed to compounds produced in tank cleaning service the industry carries out.

In this study, the higher concentrations of the heavy metals detected in industrial effluents, which are toxic in excess amounts to humans and the environment, are due mainly to the nature of products and reagents used by these industries in the Vaal area. For example, chromium is widely employed in numerous industrial processes and as a result, is a contaminant of many environmental systems (Cohen *et al*., 1993; Norseth, 1981; Wang *et al*., 2006). In this study, chromium concentration varied across the sampling points and although, chromium is needed in trace amounts for glucose metabolism, over dose can cause liver necrosis, nephrites, gastrointestinal irritation, ulcers (coetaneous, nasal and mucus membrane) in humans and wildlife (Katole *et al*., 2013). Chromium concentration of samples from industry 2 (IND2) (a galvanized industry) and inflow are the highest (0.8mg/mL) when compared to the other industries

(Table 4.1A).This may be attributed to what they produce or the reagents used in galvanizing activities. It is important to highlight that majority of the heavy metals concentrations in the Leeuwkuil Wastewater Treatment Plant were drastically reduced compared to those in samples from industrial effluents (Table 4.1A and 4.1B). According to Table 4.1A, the highest concentration of Fe (16.7 mg/l) was detected in samples from industry 2 (IND2) followed by industry 1 (IND1) (14.0 mg/ml) & industry 4 (IND4) (13.1 mg/ml). It was further observed that Fe in inflow (3.2 mg/ml) was higher than the concentration in final effluent (1.5 mg/ml) indicating the role of the treatment plant in the reduction of the metal. Furthermore, the concentration of Fe in inflow was observably lower than the ones from the industries which implied that there could be serial dilutions of the sample before the entrance into the treatment plant. The highest Al concentration (14.0 mg/l) was detected in samples from Industry 4 (IND4) (tank cleaning service industry) compared to other industries and samples from the Leeuwkuil Wastewater Treatment Plant (Table 4.1A). Furthermore, a relatively high amount of Ni was detected in effluents from Industry 4 (IND4) (Table 4.1B), both high values of Al and Ni can be linked to the type of chemicals present such as aluminium and nickel complexes in the washed tank. The concentration of zinc in samples revealed significantly high concentration (88.79 mg/l) in industry 2, which can be attributed to waste product released in the industry during their galvanizing activities. The highest concentration of Pb (4.8 mg/l) was detected in samples from Industry 1(IND1) when compared to other industries, inflow and final effluent. The highest concentration of Cu (0.7 mg/l) was detected in samples from industry 1(IND1) (Table 4.1A).

Copper is an essential micronutrient in human health but can cause health problems when exposed to an extreme concentration above recommended limits of <2 (SANS-241, 2015 and WHO, 984; 1989). High dosage of copper can cause development of anaemia (Madsen *et a*l., 1990; Bent and Bohm, 1995) and neurological complications, hypertension, liver and kidney dysfunctions in humans and other animals (Rao *et al*., 2001, Krishna and Govil, 2004). There is a very narrow range of concentrations between beneficial and toxic effects of copper (Tchounwou *et al*., 2012). It is important to note that heavy metals such as Pb, Cd, Hg and Cr are ranked among the priority metals that are considered to be persistent and pose public health of significance, as they are known to induce organ damage at lower levels of exposure (Tchnouwou *et al.,* 2012; Manyatshe *et al*., 2016; Jaishankar *et al*., 2014).

In this study, it was clearly shown that effluent discharge and land use are the major pollution sources of the Vaal River. This observation was supported with higher amount of Zn, Cu and Pb from the final effluent of the Leeuwkuil Treatment Plant that is discharged into the Vaal River (Table 4.1B). Clearly, according to findings in this study, industrial effluents are point sources of the heavy metals pollution. In agreement with our findings, industrial activities were reported to influence the concentrations of heavy metals such as Pb, Cu, Mn, Zn, Fe and Cd in Ogun River in South West Nigeria (Jaji *et al*., 2007). Furthermore the work of many researchers (Bailey *et al*., 1999; Khraisheh *et al*., 2004; Sekhar *et al*., 2004; Calamari and Naeve, 1994; Helmer and Hespanhol, 1997; Kamika and Momba, 2013; Vodela *et al*., 1997; Igwilo *et al*., 2006; Jaji *et al*., 2007) also complied with the findings of this study regarding industries as a point source of heavy metal pollution. In India, heavy metal concentrations in industrial effluents were reported to be above permissible limits for International organisations like WHO, USEPA, EUC, and EPA (Mohod and Dhote, 2013). These results are consistent with the findings of this research work where Cu, Pb and Zn were found to be above SAGES limits of 0.01, 0.01 and 0.1 respectively in both industrial and final effluent from Leeuwkuil Treatment Plant (Table 4.2). There is no stipulated Green Drop certification requirements for these metals, hence it becomes difficult to conclude that Leeukuil Treatment Plant is effective in reducing the concentration of Al, Cu, Pb and Zn from industrial effluent (Table 4.2). Clearly, using SAGES (2013) standard, it is an indication that the Leeuwkuil Treatment Plant needs to be reviewed.

Seasonally, heavy metal such as Al, Zn, Cu and Pb showed variation across sampling points. This could be attributed to the quantity of wastewater released from both industrial and domestic activities that may impact negatively on the Wastewater Treatment Plant efficiency in each of the seasons. For instance, the concentration of Zn was highest in summer (81.12 mg/ml) compared to the lowest in spring (0.2 mg/ml) for samples collected from industry 2, which had the highest Zn among the effluent samples (Table 4.2). The work done by Jaji *et al*. (2007) was in agreement with this finding, where they observed seasonal influence on the concentrations of Cu, Pb, Mn and Cd associated with industrial wastewater sources. Heavy metal concentrations above permissible limits in industrial effluents can reach rivers and be accumulated in tissues and organs of living organs such as catfish (Osman and Kloas, 2010). Therefore, there is need for industries to have effective and efficient treatment systems and continuous monitoring of the quality of effluent being released into the main treatment plant in order to reduce treatment costs.

The excessive concentration of metals such as Pb, Zn, Cu, and other heavy metals from the final effluent identified in this study are of concern as they can affect the water quality causing it to be considered unsuitable for drinking, irrigation and other water uses (Jaishankar *et al*., 2014). Furthermore, such alterations in water quality of the river due to these heavy metals can affect other parameters such as BOD, COD, TDS, total suspended solids (TSS) and faecal coli forms (Patil *et al*., 2012). For example, death of aquatic organisms due to excessive Pb can cause decomposed organisms in the water to influence parameters such as BOD, COD, TDS and TSS. The detection of high concentrations of Pb in the final effluent of this study is of great concern because it exceeded the SAGES (2013) standard.

4.2.2 Physico-chemical characteristics of effluents from industries and Leeuwkuil Wastewater Treatment Plant

A comparative study was conducted to analyse the influence of abiotic factors responsible for heavy metals concentrations in the collected water samples. Temperature, pH, biological oxygen demand (BOD), dissolved oxygen (DO), chemical oxygen demand (COD), total dissolved solutes (TDS), salinity, and electrical conductivity (EC) were determined and compared with the Green Drop (2013) certification requirements and SAGES (2013) standard (Table 4.2).

Temperature is a critical water quality parameter, since it directly influences the amount of dissolved oxygen that is available to aquatic organisms. All the organisms have a range of temperatures at which they carry out essential activities such as reproduction, optimal growth and general fitness (Dallas and Day, 2004). Change in temperature affects the metabolic rate, respiration, the distribution and survival of aquatic organisms by altering physiological processes and enzyme activities leading to death in aquatic organisms (McKee and Wolf 1963; Eaton, 2005). If the temperature changes are not regularly monitored properly, it can affect the ability of aquatic organisms to grow, reproduce, escape predators, and compete for habitat (Chapman and WHO, 1996). According to results obtained in this study, seasonal variation significantly ($p<0.05$) affected the temperature of samples from all seven sampling points. For instance, the highest sample temperatures were recorded during summer for all industrial effluent samples compared to the lowest values during winter (Table 4.2).

Similarly, among the sampling sites, significant differences ($p<0.05$) in sample temperatures were recorded during each season. In summer, the highest sample temperature (28.2^{o}C) was recorded for Industry 3 compared to Inflow, which had the lowest temperature (26.3^{o}C). A similar pattern was also noted during each of the other seasons (Table 4.2). These temperatures varied significantly across the seven sampling sites and ranged between 26.3^{o}C and 28.2^{o}C during summer, 22.7^{o}C to 26.9^{o}C during autumn, 15.7^{o}C to 16.7^{o}C during winter and 20.33^{o}C to 21.18^{o}C during spring. Such observed changes in the temperature were affected by seasonal variations. According to Eckenfelder and Wesley (2000), a rise in the inflow temperature above 35^{o}C has the tendency to cause negative changes in the biological activity during the treatment process and can cause the reduction of the efficiency of nutrient removal while high temperature in the effluent can lead to the disruption of aquatic organism's activities in the receiving water bodies. However, this was not the case in this study as temperature values were in compliance with the green drop standard for effluent.

The pH measurement is among one of the most important and commonly used tests in water chemistry (APHA, 1995). In this study, seasonal variation significantly ($p<0.05$) affected the pH of samples from all seven sampling points. In general, most sites recorded significantly higher pH values during winter compared to spring (Table 4.2). It was also noted that the pH values varied significantly across the sampling points in each season. For example, among the seven sampling points, five sites (Industries 1, 2, 4 & 5 and Final effluent) had the highest pH values in winter compared to other sites (Industry 3 and Inflow). Zamxaka *et al*. (2004) reported variations in water sample pH values with the Gogogo sites (Sites 1 to 8) having relatively lower pH values compared to the sites in Nkonkobe (Sites 9 to 17). They further confirmed the effect of season by reporting that the overall pH values were relatively higher in winter compared to summer (Zamxaka *et al*., 2004), which is in support of our findings as shown in Table 4.2. In general, similar differences in the pH values were noted in our study, when sampling sites were compared during each season.

The effluent pH across all seven sampling sites ranged from 6.8 to 12.5 with the lowest values in autumn (Industry 1) and the highest values recorded in winter in (Industry 4) (Table 4.2). These results indicated that all the industries produced alkaline wastewater (>pH 7) except for Industry 1 (summer and autumn) and Industry 2 (in spring), which were slightly acidic (<pH 7). The acidity in Industry 1 samples could be traced to the waste from battery production, whereas, Industry 2 are involved in coating steel and wires (galvanization). Industries and wastewater treatment plants can release acidic (organic and inorganic) compounds and other products in their effluents that can influence pH of receiving water bodies (Bosch, 1999). However, it is important to note that the most values obtained in this study from the seven sampling points (Table 4.2) are within the stipulated Green Drop certification requirements (5.5 – 9.5) and posed no threat to the receiving water bodies (Jaji *et al*., 2007; Igbinosa and Okoh, 2009).

Table 4.2 shows the BOD results of samples from the industries, Inflow and Final effluents. Similar to temperature and pH, seasonal variation significantly ($p<0.05$) affected the BOD values of all sampled sites (industries and the Leeuwkuil plant). For example, samples from Industry 1 had the highest BOD value obtained in winter (6.83 mg/l) compared to the lowest BOD (5.60 mg/l) in spring (Table 4.2). Similar to our finding, Vaishali and Punita (2013) reported significant differences in BOD values with post winter having the highest value compared to post monsoon season. In our study however, during each season, significant differences were recorded among sampling sites. Although there is no stipulated Green Drop certification requirement for BOD, the decrement in the value in the final effluent implied Leeuwkuil Treatment Plant is effective in the removal of organic matter. This decrement in BOD regimes in the inflow and final effluent samples could also be traced to the serial dilution of the industrial effluent by the domestic effluent (Akbar *et al*., 2010).

In addition, seasonal variation significantly ($p<0.05$) affected the DO values of all sampled sites (industries and the Leeuwkuil Plant). The values of DO in the industries, inflow and final effluent were observed to be low and varied significantly across the sampling points in each of the seasons. Our results are in agreement with findings reported by Sangeeta and Neha (2015) that seasonal variation affected the DO values in eight sampling points in the Nalasopara region with the monsoon season having the highest mean value (3.073 mg/l) compared to summer (1.711 mg/l). In another study, DO values varied significantly and ranged from 3.9–6.6 mg/l when the physico-chemical qualities of the final effluents of an urban wastewater treatment plant in South Africa were assessed between August 2007 and July 2008 (Odjadjare and Okoh, 2010). However, there is no stipulated SAGES (2013) standard recorded for DO for the effluents hence, it becomes difficult to evaluate the effectiveness of Leeuwkuil Wastewater Treatment Plant. However, it is notable that there is an increase in the concentration of DO in the final effluent (which was lower in the inflow), one can conclude that Leeuwkuil Treatment Plant is effective in removing organic pollutant that were present in the inflow hence the reason for high DO in the final effluent.

The measurement of the total quantity of oxygen required for oxidising all organic material into carbon dioxide and water was also taken into consideration in the current study. Seasonal averages of COD from industrial effluent and Leeuwkuil Treatment Plant (inflow and final effluent) showed significant ($p<0.05$) variations in all four seasons. For example, the highest COD was in autumn (320 mg/l) compared to the lowest in winter (184 mg/l) for samples from industry 1 (Table 4.2). In our study, the COD values ranged from 3 to 909 mg/l during summer, 3 to 1172 mg/l during autumn, 136 to 1209 mg/l in winter and 0 to 1493 mg/l during spring. Seasonal variation was also reported to influence the COD of final effluents of an urban wastewater treatment plant in South Africa (Odjadjare and Okoh, 2010). Although in our study, COD values from industries did not meet the green drop standard of effluent indicating high level of pollution, these were noticeably reduced at the final effluent in summer, autumn and spring when compared to the Green Drop certification. Implying that the Leeuwkuil Wastewater Treatment Plant was effective in their treatment during the three seasons.

Table 4.2: Characterisation of industrial and Leeuwkuil treatment plant effluents during the seasons and their compliance with physico-chemical parameters standards

Parameters	**Seasons**	**Sampling sites**							**Pf values**	**Standards**	
		Industry 1	**Industry 2**	**Industry 3**	**Industry 4**	**Industry 5**	**Inflow**	**Final effluent**		**Green Drop 2013**	**SAGES 2013**
Temperature (ºC)	Summer	27.40±0.7dA	26.55±0.7fA	28.22±0.7Aa	27.73±0.7cA	27.93±0.8bA	26.31±0.8gA	26.60±0.7eA	<.0001	30	-
	Autumn	24.15±1.0cB	23.80±1.1eB	24.29±1.1Bb	24.38±1.1aB	24.14±1.0dB	22.73±1.1fB	26.97±1.0aA	<.0001	30	-
	Winter	16.41±0.8cD	15.72±0.8gD	16.20±0.8dD	15.83±0.7fC	15.95±0.9eD	16.61±0.7bD	16.69±0.8aC	<.0001	30	-
	Spring	20.78±1.0cC	20.92±1.1bC	20.33±1.1gC	20.71±1.0fD	21.18±1.0aC	20.75±1.1dC	20.7±1.0eB	<.0001	30	-
pH	Summer	6.94±1.1gC	8.67±1.1eA	9.18±1.06cB	10.76±1.01aB	10.53±1.0bC	9.02±1.0dA	7.68±1.1fB	<.0001	5.5 - 9.5	5.5 - 9.5
	Autumn	6.78±0.9gC	8.18±0.96dA	10.20±0.97bA	7.74±0.99Fc-	10.27±1.0aC	7.87±0.9eC	8.21±0.9cA	<.0001	5.5 - 9.5	5.5 - 9.5
	Winter	8.23±1.5gA	8.74±1.5dA	9.99±1.5cB	12.49±1.5aA	12.17±1.5bA	8.51±1.5eB	8.35±1.4fA	<.0001	5.5 - 9.5	5.5 - 9.5
	Spring	7.02±1.8fB	6.37±1.8gB	9.35±1.8cB	12.48±1.7aA	11.54±1.9bB	7.76±1.7dC	7.74±1.8eB	<.0001	5.5 - 9.5	5.5 - 9.5
BOD (mg/l)	Summer	5.80±1.8bB	5.57±1.8cB	4.20±1.8fC	5.40±1.6dB	6.51± 1.83aA	4.88±1.8eA	3.26±1.8gB	<.0001	-	-
	Autumn	5.65±2.3dB	6.96±2.3aA	6.62±2.24cA	5.12±2.2eB	6.65±2.25bA	3.65±2.2fB	3.05±2.2gB	<.0001	-	-
	Winter	6.83±1.8aA	5.43±1.8cB	5.30±1.8dB	6.25±8.12bA	5.17±1.82eB	2.61±1.8gC	4.44±1.8fA	<.0001	-	-
	Spring	5.60±1.8cB	5.90±1.8bB	5.26±1.8eB	6.93±1.83aA	5.33±1.8dB	2.13±1.8gC	4.22±1.8fA	<.0001	-	-
DO (mg/l)	Summer	1.43±1.16fC	2.00±1.2bA	1.72±1.2eA	1.81±1.2cB	0.99±1.2gB	1.73±1.2dA	2.96±1.2aA	<.0001	-	-
	Autumn	1.69±1.3cB	0.67±1.3gB	0.98±1.3eB	1.77±1.3bB	0.78±1.3fB	1.63±1.3dA	2.970±1.3aA	<.0001	-	-
	Winter	1.21±1.59fC	2.10±1.6dA	2.17±1.7aA	2.12±1.6cA	1.14±1.6gB	1.32±1.6eA	2.15±1.7bA	<.0001	-	-
	Spring	1.92±1.2dA	2.10±1.2bA	1.63±1.2fA	2.08±1.2cA	1.85±1.2eA	1.00±1.0fA	2.78±1.2aA	<.0001	-	-

COD (mg/l)	Summer	227.7±189.4fB	277.3±205.6dA	909±589.2aB	620.8±423.2bB	275±2.1eB	455.7±368.3cA	3±2.3gB	<.0001	75	75
	Autumn	320±279.5fA	1172±785.1aB	872±534.7cB	1128±782.6bA	670±456.9dA	360±28.9eB	3±2.7gB	<.0001	75	75
	Winter	184±120.3eC	174±145.3fC	878± 786.3bB	1209±934.2aA	200±15.7dB	468±32.4cA	136±98.5gA	<.0001	75	75
	Spring	292±256.9dB	218±187.4eC	1493±936.3aA	714±634.5bB	95±6.9fC	452±34.6cA	0±0gB	<.0001	75	75
TDS (mg/l)	Summer	1892±1145.2dB	2122±1256.4cA	22±15.7gA	2452±1632.3bB	3901±1987.2aA	328±303.8eA	265±204.4fA	<.0001	25	25
	Autumn	2434±1435.6cA	1826±1189.4dB	19±14.9gA	3117±1825.9aA	2468±1356.8bB	354±323.3eA	253±199.6fA	<.0001	25	25
	Winter	1845±1168.3cB	2223±1163.8bA	14±12.3gB	1417±1105.7dC	3636±1968.5aA	337±302.6eA	281±224.9fA	<.0001	25	25
	Spring	1301±1126.9cC	1138±987.8dB	12±8.12gB	2335±1235.6bB	4611±2864.9aA	321±298.8eA	232±200.4fA	<.0001	25	25
EC (µS/cm)	Summer	3603±2673.4bA	3782±2562.9aA	45±32.8gA	1954±1346.9cB	1425±1256.2dB	669±54.9eA	627±567.8fA	<.0001	150	70 - 150
	Autumn	2743±1893.7bB	3594±2362.8aA	39±26.9gA	1797±1538.7cB	1109±985.7dB	710±67.8eA	505±475.4fA	<.0001	150	70 - 150
	Winter	1845±1534.7cC	2223±1452.8bB	14±8.9gC	1417±1209.2dB	3636±2986.7aB	337±29.7eB	281±169.3fC	<.0001	150	70 - 150
	Spring	2600±1894.6cB	2276±1468.3dB	25±18.9gB	4673±3672.8bA	9224±7869.2aA	642±53.7eA	464±369.5fB	<.0001	150	70 - 150
Salinity (psu)	Summer	2.05±1.42dB	2.14±1.6cA	24.77±18.92aA	1.50±0.96eB	6.20±5.2bA	0.33±0.24fA	0.26±0.20gA	<.0001	-	-
	Autumn	6.79±5.4bA	1.87±0.97dA	23.80±19.47aA	0.89±0.56eC	5.82±3.45cA	0.35±0.24fA	0.26±0.19gA	<.0001	-	-
	Winter	1.96±0.86dB	2.39±1.67cA	17.90±13.89aB	0.88±0.56eC	6.02±4.79bA	0.34±0.23hA	0.28±0.20iA	<.0001	-	-
	Spring	1.36±0.68dB	1.21±0.97eB	14.38±11.24aB	2.52±1.56cA	5.18±4.2bB	0.33±0.24fA	0.24±0.22gA	<.0001	-	-
Aluminium (mg/l)	Summer	10.25±3.2aC	0.20±0.05eA	3.00±0.82cA	7.85±2.01bC	2.95±0.54dB	0.20±0.05eA	0.20±0.05eA	<.0001	-	-
	Autumn	11.47±4.4bB	0.36±0.07eA	4.41±2.04dA	16.30±6.80aA	6.92±3.06cA	0.20±0.05fA	0.20±0.05fA	<.0001	-	-
	Winter	10.40±2.56bC	0.37±0.07cA	0.20±0.05dB	13.30±4.2aB	0.20±0.05dC	0.20±0.05dA	0.20±0.05dA	<.0001	-	-
	Spring	15.20±4.36bA	0.20±0.05cA	0.20±0.05cB	18.70±7.82aA	0.20±0.05cC	0.20±0.05cA	0.20±0.05cA	<.0001	-	-
Zinc (mg/l)	Summer	0.48±0.08cB	81.12±15.8aA	0.20±0.05eB	4.49±1.4bA	0.20±0.05eA	0.20±0.05eA	0.22±0.06dA	<.0001	-	0.1
	Autumn	28.54±11.7bA	77.37±20.3aA	2.97±0.98dA	3.55±1.2cA	0.41±0.31fA	0.20±0.05gA	0.51±0.34eA	<.0001	-	0.1

	Winter	0.32±0.04cB	68.18±18.85aA	0.20±0.05eB	3.91±1.2bA	0.21±0.06dA	0.20±0.05eA	0.20±0.05eA	<.0001	-	0.1
	Spring	0.29±0.06cB	0.20±0.05dB	2.5±18.9aB	4.32±1.4bA	0.20±0.05dA	0.20±0.05dA	0.20±0.05dA	<.0001	-	0.1
Copper (mg/l)	Summer	0.70±0.2aA	0.20±0.05cA	0.20±0.05cA	0.31±0.08bA	0.20±0.05cA	0.20±0.05cA	0.20±0.05cA	<.0001	-	0.01
	Autumn	0.76±0.4aA	0.35±0.1cA	0.20±0.05dA	0.54±0.15bA	0.20±0.05dA	0.20±0.05dA	0.20±0.05dA	<.0001	-	0.01
	Winter	0.62±0.21aA	0.20±0.05cA	0.20±0.05cA	0.21±0.08bA	0.20±0.05cA	0.20±0.05cA	0.20±0.05cA	<.0001	-	0.01
	Spring	0.81±0.23aA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	<.0001	-	0.01
Lead (mg/l)	Summer	5.03±1.46aA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	<.0001	-	0.01
	Autumn	4.83±1.3aB	0.20±0.05cA	0.20±0.05cA	0.42±0.2bA	0.20±0.05cA	0.20±0.05cA	0.20±0.05cA	<.0001	-	0.01
	Winter	4.3±1.36aB	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	<.0001	-	0.01
	Spring	4.4±1.5aB	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	0.20±0.05bA	<.0001	-	0.01

Values are means of triplicates ± Standard deviations (SD); Means with similar small letters (a-g) across a row are not significantly different ($P < 0.05$). Means with similar capital letters (A-C) within a column are not significantly different ($P < 0.05$). BOD= Biological oxygen demand, DO= Dissolved oxygen, COD= Chemical oxygen demand, TDS= Total dissolved solutes, EC= Electrical conductivity. Green drop standard is South African effluent standard. SAGES = South African General Effluent Standard, 2013.

Seasonal averages of TDS profile of the industrial, inflow and treated effluents samples vary significantly ($p<0.05$) in all the seasons and ranged from 22 to 3901 mg/l during summer season; 19 to 3117 mg/l during autumn season; 14 mg/l to 3636 mg/l during winter season and 12 mg/l to 4611 mg/l during the spring season (Table 4.2). Higher levels of TDS were observed in all the industrial effluents except in industry 3, inflow and final effluent. High TDS in industries 1, 2, 4 and 5 could be attributed to high dissolvable ions in the effluents especially in industries 4 and 5. Similarly, the TDS values of final effluents of an urban wastewater treatment plant varied significantly with season and sampling points (Odjadjare and Okoh, 2010). In our study, although industry 3 produced lower TDS, the final effluent however, did not comply with Green Drop certification of 25 mg/l indicating that the Leeuwkuil Treatment Plant was not effective in reducing the total dissolved solutes from the industrial effluents. The issue with high TDS is that it can be toxic to freshwater animals by causing osmotic stress and affecting the osmoregulatory capability of the organisms (McCulloch *et al*., 1993).

Electrical conductivity (EC) is a measure of water's ability to conduct electric current and it is related to the amount of dissolved minerals in water. However, it does not indicate the elements present (Nazir *et al*., 2015). Conductivity of the samples (industries, inflow and treated effluents) varied significantly ($p<0.05$) in all the seasons and among all sampling points and ranged from 39 to 3594 μS/m in autumn season; 45 to 3782 μS/m during summer season; 14 to 3636 during μS/m winter season and 25 to 9224 μS/m during spring season (Table 4.2). This study showed that the values of EC in the final effluent were lower in all the seasons but it did not meet the Green Drop certification requirements (150 μS/cm). This high value of EC may be attributed to the presence of contaminants such as sodium, potassium, chloride, and sulphate (Nazir *et al*., 2015). This implied that the Leeuwkuil Wastewater Treatment Plant was not effective in maintaining the values of EC within the recommended standard.

The term salinity refers to saltiness and is defined with reference to the electrical conductivity of seawater (Dallas and Day, 2004). Since the quantity of dissolved organic matter in seawater is very small relative to the amount of inorganic matter, salinity and TDS are virtually identical in seawater. According to Table 4.2, the salinity of samples (industries, inflow and treated effluents) varied significantly ($p<0.05$) in all the seasons and among all sampling points and ranged from 0.35 to 23.80 psu during autumn; 0.33 to 24.77 psu during summer; 0.34 to 17.90 psu during winter and 0.33

to 14.38 psu during spring (Table 4.2). Similarly, the salinity of final effluent of an urban wastewater treatment plant varied significantly with season and sampling points (Odjadjare·and Okoh, 2010). In our study, higher levels of salinity were observed in samples from industry 3 (wire industry) indicating high dissolvable salt in their effluent water. The high salinity in industry 3 can be traced to oxidation of cations and anions used in wire manufacturing. According to the results, salinity in inflow samples is lower in all the seasons compared to the industrial samples due to serial dilution of industrial effluent by domestic effluent. Furthermore, salinity in inflow was found to be higher than in the final effluent in all the seasons. However, there are no set standard for salinity level for effluent discharged into the aquatic ecosystems in South Africa. The water quality criteria for South African coastal zones put the acceptable limit of salinity in marine ecosystem for all biological activity at 33-36 psu (SANCOR, 1984), indicating effectiveness by the treatment plant in removing dissolved salts present in waste water.

In summary, the industries were observed to have high values for the heavy metals and the water quality parameters (BOD, DO, COD, TDS, EC, and salinity) measured in this study. This indicated them as point source of pollution in Vaal area of South Africa. The Leeuwkuil Wastewater Treatment Plant was also assessed on its ability to reduce the contaminants to the required and acceptable limit using Green Drop and SAGES certification requirements. There were no stipulated Green Drop standard for the four heavy metals (Al, Cu, Zn, Pb), BOD, salinity, and DO. Amongst the four heavy metals of interest in this study, Cu, Zn, and Pb were observed to be above the SAGES certification requirements whereas Al has no SAGES certification requirements for comparison. Furthermore, water parameters such as TDS (25 mg/l) and EC (150 μS/cm) did not meet the Green Drop standard. An overall assessment of the Leeuwkuil Wastewater Treatment Plant showed the treatment plant needs to be reviewed as it was found to be ineffective in maintaining TDS and EC but was only effective in maintaining temperature, pH, and COD within the Green Drop standard and SAGES standards.

4.2.3 Correlation coefficient analysis of the heavy metals and physico-chemical parameters of effluents from industries and the Leeuwkuil Wastewater Treatment Plant

Analysis of the data obtained in this study showed strong significant ($p<0.05$) correlations between physico-chemical variables (Tables 4.3-4.6); and in the case of regression, a significantly high dependence of one variable on the order as shown Figures 4.1-4.4 for samples from the industries, inflow and final effluent for all the seasons. The analysis of the interrelationship between physical parameters and heavy metals gave an insight on the influence of the industrial effluents on the wastewater treatment plants. Verandani and Vardhan (2012) stated that the study of correlation coefficients of water quality parameters helps to evaluate the concentration of the various pollutants, which will aid in assessing the overall quality of water. Strong correlation between variables is within the correlation coefficients range of 0.8 to 1 and -0.8 to -1, moderate correlation in the range of 0.5 to 0.8 and -0.5 to -0.8, and weak correlation in the range of 0.0 to 0.5 and -0.0 to -0.5.

Table 4.3: Pearson's correlation coefficients of physico-chemical parameters for the effluents samples during summer

	pH	BOD	DO	COD	TDS	EC	Salinity	Al	Zn	Cu	Pb
Temperature	0,446515	0,285862	-0,54819	0,577608	0,335755	-0,17517	0,682544	0,53353	-0,39425	0,151501	0,088029
pH		0,371355	-0,41169	0,488847	0,478116	-0,28314	0,170358	-0,05347	-0,06756	-0,52585	-0,64446
BOD			-0,85386	-0,01479	0,8727	0,594333	-0,18862	0,417871	0,205302	0,321719	0,289776
DO				-0,33668	-0,61582	-0,20652	-0,22732	-0,42567	0,142392	-0,27703	-0,27424
COD					-0,2195	-0,36776	0,741774	0,159102	-0,16014	-0,17785	-0,24925
TDS						0,520927	-0,28386	0,349175	0,186824	0,161635	0,099676
EC							-0,45805	0,4201	0,624391	0,582467	0,560374
Salinity								-0,02458	-0,17153	-0,2086	-0,16387
Al									-0,33816	0,849981	0,735257
Zn										-0,20136	-0,17349
Cu											0,975572

r-Values≥0.492 or =−0.492 are significant at $p < 0.05$.

Table 4.4: Pearson's correlation coefficients of physico-chemical parameters for the effluents samples during winter

	pH	BOD	DO	COD	TDS	EC	Salinity	Al	Zn	Cu	Pb
Temperature	-0,62665	-0,5061	-0,21799	-0,29731	-0,61774	-0,61774	-0,1601	-0,21394	-0,58123	0,230549	0,239868
pH		0,294096	0,014836	0,575691	0,453104	0,453104	0,201925	0,30797	-0,2172	-0,35865	-0,3728
BOD			0,114118	0,159207	0,407374	0,407374	0,130844	0,113135	0,113135	0,556414	0,545843
DO				0,424983	-0,49109	-0,49109	0,219185	-0,02771	0,339929	-0,47393	-0,48022
COD					-0,34968	-0,34968	0,320958	0,472718	-0,26364	-0,27551	-0,29304
TDS						1	-0,18126	0,112987	0,283994	0,153692	0,152934
EC							-0,18126	0,112987	0,283994	0,153692	0,152934
Salinity								-0,31186	-0,14396	-0,16588	-0,15967
Al									-0,20475	0,546822	0,526943
Zn										-0,17878	-0,17542
Cu											0,999722

r-Values≥0.492 or =−0.492 are significant at $p < 0.05$.

Table 4.5: Pearson's correlation coefficients of physico-chemical parameters for effluents samples during autumn

	pH	BOD	DO	COD	TDS	EC	Salinity	Al	Zn	Cu	Pb
Temperature	0,028449	-0,38604	0,696853	-0,42779	-0,18928	-0,25745	-0,07645	-0,07381	-0,21444	-0,10898	-0,06922
pH		0,445901	-0,47494	0,238806	-0,26996	-0,54094	0,593374	-0,2291	-0,29819	-0,71359	-0,58717
BOD			-0,89414	0,718913	0,396425	0,416418	0,515112	0,217522	0,492615	0,143697	0,072607
DO				-0,71024	-0,26052	-0,31841	-0,40268	-0,02063	-0,42897	0,071457	0,114528
COD					0,438634	0,40218	0,199515	0,316789	0,438361	0,102218	-0,30622
TDS						0,64127	-0,34125	0,775549	0,243503	0,670334	0,356476
EC							-0,38427	0,21337	0,870216	0,650704	0,434234
Salinity								0,037429	-0,15838	-0,14146	0,046401
Al									-0,19979	0,722113	0,442847
Zn										0,30728	0,180551
Cu											0,840165

r-Values≥0.492 or =−0.492 are significant at $p < 0.05$.

Table 4.6: Pearson's correlation coefficients of physico-chemical parameters for effluents samples during spring

	pH	BOD	DO	COD	TDS	EC	Salinity	Al	Zn	Cu	Pb
Temperature	0,098693	0,061147	0,127998	-0,8115	0,771055	0,770896	-0,54106	-0,07613	-0,78899	0,017278	0,017278
pH		0,407588	-0,15825	0,263728	0,646876	0,647159	0,326824	0,332007	0,200657	-0,35474	-0,35474
BOD			0,483471	0,15002	0,420076	0,420149	0,211721	0,570929	0,1532	0,15853	0,15853
DO				-0,44884	0,051771	0,051767	-0,22887	0,125914	-0,20828	0,00938	0,00938
COD					-0,35176	-0,35153	0,829566	0,078051	0,935799	-0,15058	-0,15058
TDS						1	-0,05307	0,188759	-0,3479	-0,03281	-0,03281
EC							-0,0529	0,188751	-0,3477	-0,03315	-0,03315
Salinity								-0,21541	0,939742	-0,19612	-0,19612
Al									-0,13424	0,546918	0,546918
Zn										-0,19357	-0,19357
Cu											1

r-Values≥0.492 or =−0.492 are significant at $p < 0.05$.

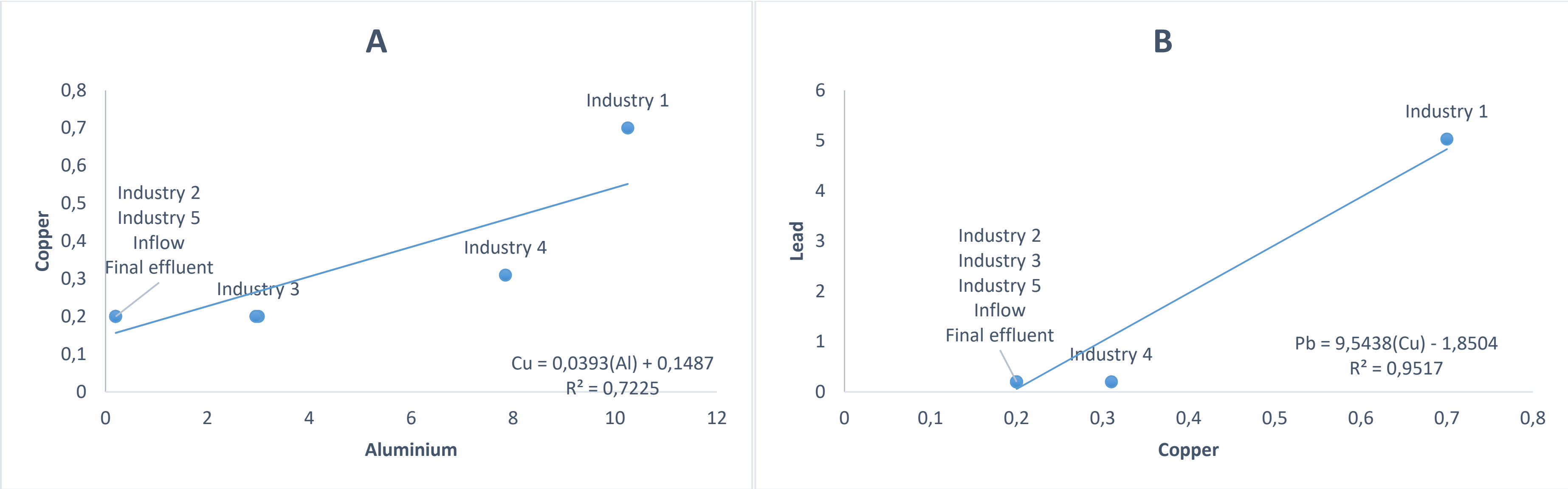


Figure 4.1: Linear regression graph to predict the physico-chemical parameters in the seven effluent samples. Pearson's correlation between copper and aluminium (A), Lead and copper (B) during summer for the effluent samples are strong

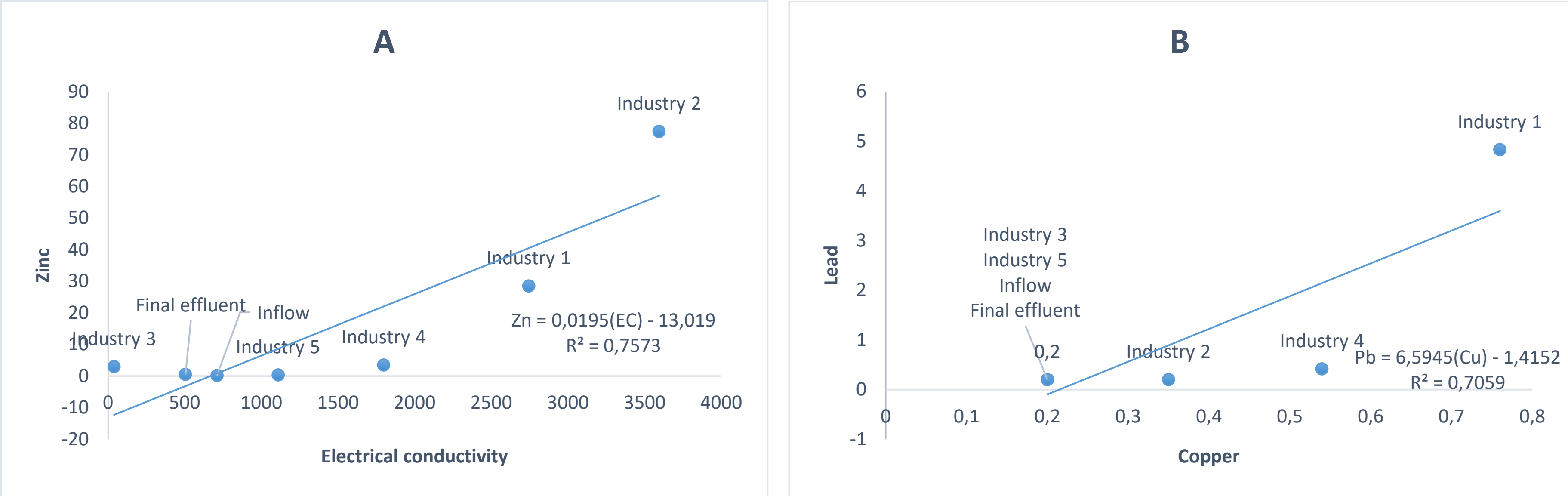


Figure 4.2: Linear regression graph to predict the physico-chemical parameters in the seven effluent samples. Pearson's correlation between zinc and electrical conductivity (A), Lead and copper (B) during autumn for the effluent samples are strong (r-value is between 0.8 to 1) and are represented with linear regression equation

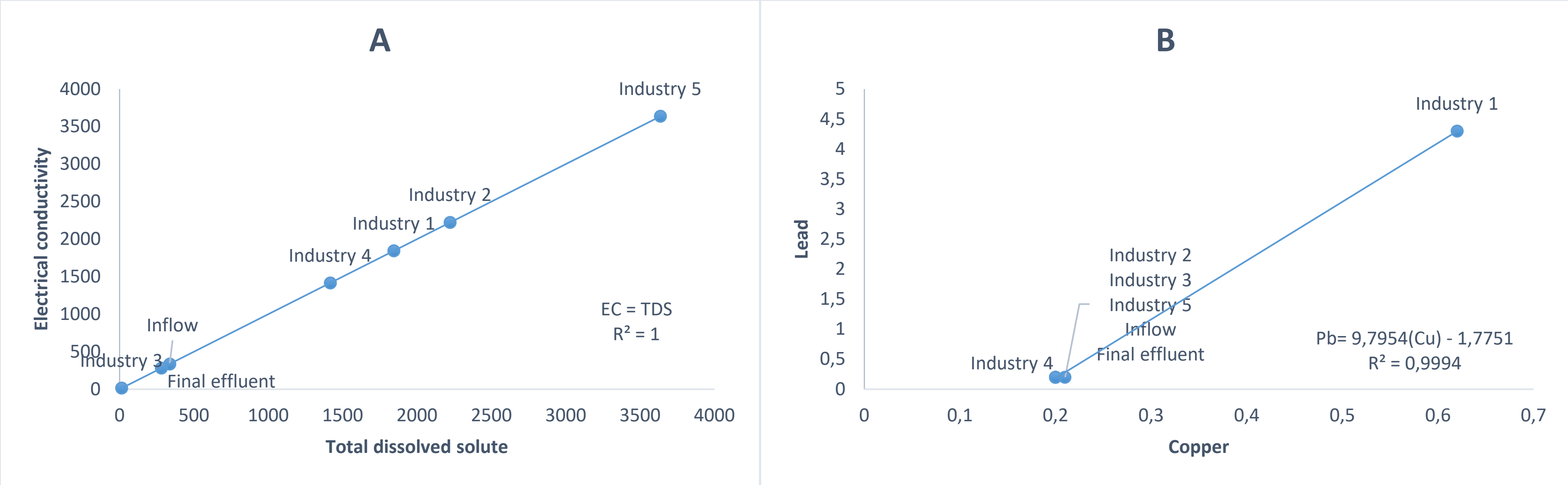


Figure 4.3: Linear regression graph to predict the physico-chemical parameters in the seven effluent samples. Pearson's correlation between electrical conductivity and total dissolved solids (A), Lead and copper (B) during winter for the effluent samples are strong (r-value is between 0.8 to 1) and are represented with linear regression equation

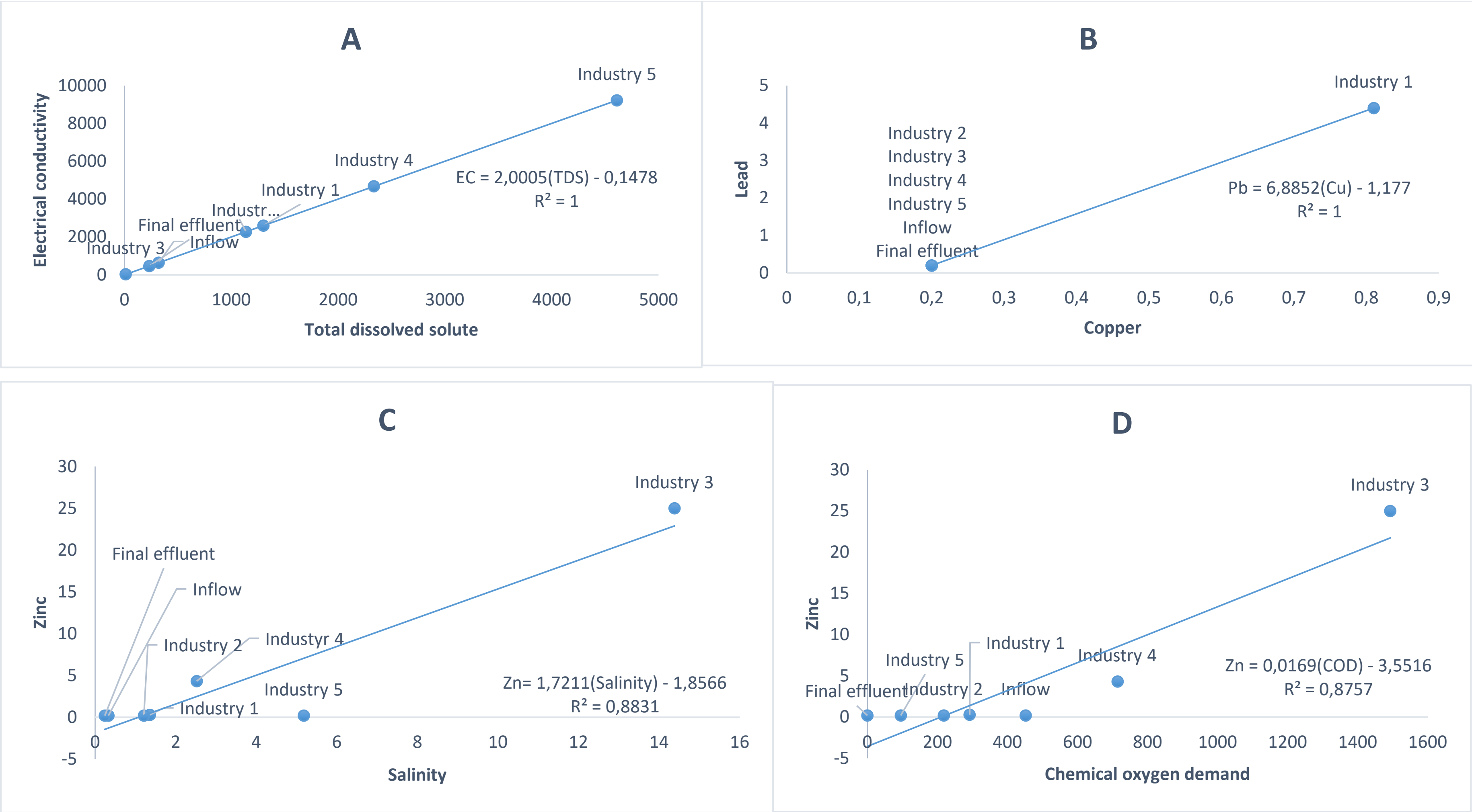


Figure 4.4: Linear regression graph to predict the physico-chemical parameters in the seven effluent samples. Pearson's correlation between electrical conductivity and total dissolve solids (A), Lead and copper (B), zinc and salinity (C) zinc and chemical oxygen demand (D) during spring for the effluent samples are strong and are represented with linear regression equation

According to Table 4.3 and Figure 4.1, at a significant level of $p<0.05$, during the summer season, a strong and positive correlation was obtained between the following parameters; temperature and COD ($r^2=0.5776$), temperature and salinity ($r^2=0.6825$, $p<0.05$), temperature and Al ($r^2=0.5335$), BOD and EC ($r^2=0.5943$), BOD and TDS ($r^2=0.8727$), TDS and EC ($r^2=0.5209$), EC and Zn ($r^2=0.6243$), EC and Cu ($r^2=0.5824$), EC and Pb ($r^2=0.5603$), Al and Cu ($r^2=0.8499$), Al and Pb ($r^2=0.7352$) and Cu and Pb (($r^2=0.9755$) at $p<0.05$). Similarly, during winter and at significant level of $p< 0.05$ (Table 4.4 and Figure 4.3), positive correlation also existed between pH and COD (($r^2=0.5756$), BOD and Pb ($r^2=0.5458$), Al and Cu (r^2 (=0.5468), Al and Pb (($r^2=0.5269$), and Cu and Pb ($r^2=0.9997$). In a similar study in the Nalasopra region, strong positive correlations were noted between some physico-chemical parameters (Sangeeta and Neha, 2015). Similar strong positive correlations between various pairs of metals including manganese, copper, cadmium, nickel, cobalt and chromium in the soil water system have been reported (Akbar *et al*., 2010).

In addition to the noted correlations between pairs of physico-chemical parameters during summer and winter, similar strong correlations were observed with Cu and pH, Al and TDS and Zn and EC during autumn at a significant level of $p< 0.05$ (Table 4.5 and Figure 4.2). For example, a significant positive correlation existed between temperature and dissolved oxygen ($r=0.6968$), pH and salinity ($r=0.5933$), BOD and salinity ($r^2=0.5151$), BOD and Zn ($r^2=0.4926$), TDS and EC ($r^2=0.6412$), TDS and Al ($r^2=0.7755$), TDS and Cu ($r^2=0.6703$), EC and Zn ($r^2=0.8702$), EC and Cu ($r^2=0.6507$), Al and Cu ($r^2=0.7221$), and Cu and Pb ($r^2=0.8401$) (Table 4.5).

A similar correlation pattern was observed between BOD and the different variables across the four seasons indicating high dependency of BOD on the concentration levels of the variables across the seasons. This also implied that seasonal variation had a significant influence on the wastewater released by the industries. The wastewater released by the industries also impacted the quality of the effluent from the Leeuwkuil Wastewater Treatment Plant.

Spring also recorded positive correlations between certain physico-chemical parameters as shown in Table 4.6 and Figure 4.4. For example, a strong positive relationship at significant level of $p< 0.05$ existed between temperature and DO ($r^2=0.7710$), temperature and EC ($r^2=0.7708$), pH and DO ($r^2=0.6468$), pH and EC

(r^2=0.6471), BOD and Al (r^2=0.5709), COD and salinity (r^2=0.8295), COD and Zn (r^2=0.9357), salinity and Zn (r^2=0.9397), Al and Cu (r^2=0.5469), Al and Pb (r^2=0.5469), and Cu and Pb (r^2=1). In general, these results demonstrated strong correlations between pairs of heavy metals in all seasons. This is a clear indication of common origins (industries) of these contaminants and their influence on the Leeuwkuil wastewater treatment plant in Vaal area.

4.3 Heavy Metals and Physico-Chemical Parameters in Upstream and Downstream (Vaal River) and Potable Water around the Vaal Area

In this study, samples collected from the Vaal River (upstream and downstream) and potable waters (PO1 & PO2), were analysed for identification and determination of heavy metal concentrations. The same number of heavy metals was identified in upstream, downstream, potable water 1 and 2 (Table 4.1A & 4.1B). Notably from this study, the concentrations of most heavy metals identified (Al, Cr, Cu, Fe, Ni, Pb, and Zn) in potable water were observed to be lower than the concentrations of the ones in upstream and downstream. This result implied that the purification plants were effective in reducing the concentrations of these heavy metals.

4.3.1 Heavy metal analysis of samples from Vaal River and Potable water

Previous studies on water quality of river showed that leachate, runoff from domestic activities and industrial effluent contributes to the pollution of rivers and the reduction of their quality for domestic, aesthetic, industrial and other uses (Fadiran and Mamba, 2005; Mtetwa, 1996). Lead (Pb) concentrations in upstream and downstream (Vaal River) were observed to be high (Table 4.1 and 4.7). This result was consistent with the findings of Osman and Kloas (2010), where they observed Pb concentrations to be higher in Nile River as a result of urban effluent draining into the river. In Bangladesh, a similar report showed high level of heavy metal pollution as a result of industrial effluents, urban and agricultural wastes being discharged into rivers and other forms of water bodies (Alam *et al*., 2007). According to the Department of Environment (2001), Rupsha River is the most polluted river with heavy metals and this is attributed to the increased number of industries located around the area. This was also observed in this study in Vereeninging area of South Africa where 24 heavy metals were identified in industrial and wastewater treatment plant effluents, and the Vaal River. The presence of these heavy metals in the Vaal River implied that there is a tendency for fishes in the river to be contaminated with heavy metals through bioaccumulation. Samad *et al.* (2015) discovered Pb, Zn, Fe and Mn in Bangladesh River (Rupsha River), and Fe, Cu, Zn, Pb, Cr, Mn and Ni in the fish and crayfish muscles of the same river. This is an indication that the fish diffused or ingested these metals and over time, it bioaccumulated in their muscles (Manyatshe *et al*., 2016). This can create a health hazards for human being especially if the bioaccumulation factor is beyond the acceptable limits. Furthermore, the concentration of Fe in upstream and downstream is low (0.2 mg/l) as compared to final effluent (1.5 mg/l). We can conclude that there could be absorption of this particular metal below the sediments in the Vaal River (Varol and Sen, 2012).

The presence of high Pb concentration in the Leeuwkuil Plant impacted on the quality of wastewater discharged into Vaal River by the plant. This trend was also observed in potable waters where Pb concentrations were above the recommended limit. Lead is non-biodegradable and can therefore persist and build up to toxic levels in living organisms (Bent and Bohm, 1995). Their accumulation in aquatic organisms can reach humans through the food chain and potable water, and can pose health related complications in humans. For instance, Pb is carcinogenic and persistence (Patil *et al*., 2012; Mahlambi *et al*., 2015; Manyatshe *et al*., 2016) and its accumulation in excessive concentrations through the food chain can cause neurological and behavioural disorders especially in children, anaemia, impaired kidney and testicular function in humans (Barzilay *et al*., 1999).

In the Vaal area, the Vaal River is exposed to threat of heavy metals pollution arising from anthropogenic activities such as land use, urbanisation and industrialisation. The main concern arises as this river serves as a backbone of the country's economy as it provides water services to the economic hub (Gauteng province) of the country (Tempelhoff, 2006). Furthermore, the Vaal River contributes 25% to the Gross Domestic Product (GDP) of the country's economy and has over 12 million people who directly depend on it for water (Tshwane University of Technology, 2009). Therefore, a proper evaluation to assess any presence of toxic constituents is beyond environmental health but also for society's wellbeing. As a result, the current study further evaluated samples from Vaal River and potable waters for their quality and concentrations of heavy metals in relation to seasonal variations together with their potential associated effects on humans and the environment.

Table 4.7: Characterisation of receiving water and potable water during the seasons and their compliance with standards

Parameters	Seasons	Sampling sites				Pf values	Standards	
		Upstream	Downstream	Potable water 1	Potable water 2		SANS-241, 2015	WHO, 1984; 1989
Temperature (ºC)	Summer	27.78±0.73Ba	27.25±0.74cA	26.70±0.76dA	28.48±0.71aA	<.0001	25	35
	Autumn	23.95±1.05bB	24.58±1.05aB	23.13±1.08dB	23.67±1.06cB	<.0001	25	35
	Winter	16.45±0.78cD	16.60±0.81bC	18.76±0.79aD	16.17±0.86dD	<.0001	25	35
	Spring	21.03±1.10C	24.47±1.07aB	20.57±1.03cC	20.57±1.03cC	<.0001	25	35
pH	Summer	8.84±1.04dA	9.15±1.0cA	9.20±1.05bA	9.24±1.0Aa	<.0001	5 - 9.7	7 - 8.5
	Autumn	8.58±0.99bA	8.52±0.98cA	8.99±0.98aA	8.99±0.98aA	<.0001	5 - 9.7	7 - 8.5
	Winter	8.51±1.5dA	8.65±1.52bA	8.54±1.45cA	8.82±1.48aA	<.0001	5 - 9.7	7 - 8.5
	Spring	7.79±1.79cB	7.77±1.78dB	8.45±1.81bA	8.66±1.79aA	<.0001	5 - 9.7	7 - 8.5
BOD (mg/l)	Summer	4.060±1.82bA	4.240±1.84aA	0.870±1.8cB	0.690±1.87dB	<.0001	-	<3
	Autumn	4.890±2.11aA	4.210±2.12bA	0.920±2.11cB	0.640±2.12dB	<.0001	-	<3
	Winter	4.510±1.8aA	4.090±1.81bA	1.190±1.82cA	1.180±1.8dA	<.0001	-	<3
	Spring	4.530±1.8bA	4.680±1.82aA	1.040±1.8dA	1.830±1.8cA	<.0001	-	<3
DO (mg/l)	Summer	3.050±1.18dA	3.270±1.19cA	4.980±1.19aB	4.080±1.18bB	<.0001	-	6
	Autumn	3.390±1.35cA	2.190±1.34eB	4.520±1.36bB	4.550±1.37aB	<.0001	-	6
	Winter	3.870±1.67cA	3.560±1.66dA	5.810±1.7bA	5.980±1.7aA	<.0001	-	6
	Spring	3.880±1.22cA	3.790±1.22dA	4.480±1.4bB	4.750±1.4aB	<.0001	-	6
COD (mg/l)	Summer	42.5±41.2aBC	41.0±38.2bB	0±0cC	0±0cC	<.0001	-	10
	Autumn	39±23.4bC	48±34.5aB	13±12.2cB	2.0±1.3dC	<.0001	-	10
	Winter	53±48.5cB	55±51.7bA	38±31.4dA	71±60.6aA	<.0001	-	10

	Spring	87±68.5bA	3.0±1.98aC	0±0dC	52±48.3cB	<.0001	-	10
TDS (mg/l)	Summer	311±278.2aAB	300±268.5bA	130±103.7cA	127±105.3dA	<.0001	≤ 1 200	<600
	Autumn	285±243.8bB	315±302.8aA	89±78.9cB	78±67.5dB	<.0001	≤ 1 200	<600
	Winter	377±325.7aA	358±321.7bA	77±64.2cB	75±63.7dB	<.0001	≤ 1 200	<600
	Spring	381±365.2aA	312±298.6bA	61±58.3cB	75±63.7dB	<.0001	≤ 1 200	<600
EC (µS/cm)	Summer	620±530aB	598±489.4bB	261±158.4dA	262±186.7cA	<.0001	≤ 170	250
	Autumn	552±463.3bB	630±578.3aA	181±109cB	157±96.8dB	<.0001	≤ 170	250
	Winter	377±205.6aC	358±245.6bC	77±67.8cC	75±67.8dC	<.0001	≤ 170	250
	Spring	763±643.8aA	625±503.4bA	121±89.6cB	120±98.6dB	<.0001	≤ 170	250
Salinity (psu)	Summer	0.30±0.22aA	0.29±0.22bA	0.13±0.09cA	0.12±0.07dA	<.0001	-	-
	Autumn	0.35±0.25aA	0.30±0.22cA	0.08±0.02dB	0.34±0.22bA	<.0001	-	-
	Winter	0.37±0.28aA	0.36±0.26bA	0.08±0.02cB	0.08±0.02cB	<.0001	-	-
	Spring	0.37±0.28aA	0.31±0.22bA	0.07±0.02cB	0.07±0.05cB	<.0001	-	-
Aluminium (mg/l)	Summer	0.20±0.05bA	0.20±0.05bA	0.2±0.09aA	0.20±0.05bA	<.0001	≤ 0.3	0.2
	Autumn	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 0.3	0.2
	Winter	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 0.3	0.2
	Spring	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 0.3	0.2
Zinc (mg/l)	Summer	0.22±0.06cA	0.35±0.07aB	0.3±0.04bA	0.20±0.05dA	<.0001	≤ 5	3
	Autumn	0.34±0.26cA	0.77±0.75aA	0.23±0.05dA	0.64±0.43bA	<.0001	≤ 5	3
	Winter	0.20±0.05aA	0.20±0.05aB	0.20±0.05aA	0.20±0.05aA	<.1	≤ 5	3
	Spring	0.20±0.05aA	0.20±0.05aB	0.20±0.05aA	0.20±0.05aA	<.1	≤ 5	3
Copper (mg/l)	Summer	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 2	2

	Autumn	0.20±0.05bA	0.28±0.08aA	0.20±0.05bA	0.20±0.05bA	<.0001	≤ 2	2
	Winter	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 2	2
	Spring	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 2	2
Lead (mg/l)	Summer	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 0.02	0.01
	Autumn	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 0.02	0.01
	Winter	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 0.02	0.01
	Spring	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	0.20±0.05aA	<.1	≤ 0.02	0.01

Values are means of triplicates ± Standard deviations (SD); Means with the same letter (a-g) across the row are not significantly different ($P < 0.05$). Means with similar capital letters (A-C) within a column are not significantly different ($P < 0.05$). BOD= Biological oxygen demand, DO= Dissolved oxygen, COD= Chemical oxygen demand, TDS= Total dissolved solutes, EC= Electrical conductivity. SANS is South Africa National Standard for drinking water (SANS - 241:2015). WHO 1984; 1989.

4.3.2 Physico-chemical characteristics of samples from Vaal River and Potable water

A comparative study was also conducted for samples from upstream, downstream, potable water 1and 2 to analyse the influencing abiotic factors responsible for heavy metals concentrations. Temperature, pH, biological oxygen demand (BOD), dissolved oxygen (DO), chemical oxygen demand (COD), total dissolved solids (TDS), salinity, and electrical conductivity (EC) were determined and compared to the SAN-241 (2015) and WHO (1984; 1989) standards for drinking water (Table 4.7).

Similarly, temperatures for upstream, downstream, potable water 1 and 2, samples varied significantly ($p<0.05$) across the seasons and ranged from 26.7 to 28.5^{o}C during summer, 23.1 to 24.6^{o}C during autumn, 16.2 to 18.8^{o}C during winter and 20.6 to 24.5^{o}C in spring as illustrated in Table 4.7. Our results are similar to findings reported by Sangeeta and Neha (2015) that seasonal variation affected the physico-chemical water parameters of the Nalasopara Region in India. The highest water sample temperature was in summer compared to winter and monsoon seasons (Sangeeta and Neha, 2015). In another related study, Odjadjare and Okoh (2010) assessed the physico-chemical quality of an urban municipal wastewater effluent and reported on similar influence of seasons with respect to variations in temperature of water samples from study sites in the Eastern Cape, South Africa. However, the measured temperatures for the upstream, downstream, potable water 1 and 2 in this study were found to be below the set values based on South African National Standard (SANS-241, 2015) (except in summer) and World Health Organization Standard (WHO, 1984; 1989), 25^{o}C and 35^{o}C, respectively. The fluctuations in average temperature values could be attributed to changes in the seasons.

Similar to temperature, seasonal variation significantly ($p<0.05$) partly affected pH values of the Vaal River (upstream and downstream) and that of potable waters (PO1& PO2) as shown in Table 4.7. In the Vaal River, the lowest pH values were recorded during spring compared to other seasons (Table 4.7). In summer, significant differences in pH values were noted among sampling sites, for example, the pH value of potable water 2 (pH 9.2) was higher than that for upstream (pH 8.8) and similarity in differences among sites were also shown during other seasons (Table 4.7). It is important to note that upstream, downstream, potable water 1 and 2 were within alkaline values. The values ranged from 8.8 to 9.2 during summer, 8.5 to 8.9 during autumn, 8.5 to 8.8 during winter and 7.8 to 8.7 during spring (Table 4.7). This also corresponded with the work done by Karen and Cornelius, (2012) where the pH values of the Vaal River catchment areas sampled were found to be in the alkaline range (pH 7-8). The pH values for upstream and downstream, were within permissible limits of SANS (2015) and WHO (1984; 1989) standard but potable water 1 and 2 exceeded those limit. This could be due to the chemicals that were used during water purification process which increased the pH value of potable water. This implied that the purification process needs to be reviewed (Table 4.7).

The BOD values obtained for upstream, downstream, potable water samples were found to vary significantly ($p<0.05$) across the- sampling points in each of the seasons. The BOD values ranged from 0.69 to 4.24 mg/l during summer, from 0.64 to 4.89 mg/l during autumn, 1.18 to 4.51 mg/l in winter and from 1.04 to 4.68 mg/l during spring (Table 4.7). Overall, the BOD values range recorded in our study for upstream, and downstream (4.060-4.680 mg/l) and there was no significant difference across the seasons. This result was not in agreement with the work done by Sibanda *et al*. (2014) where they observed the range of BOD values of the Tyume River to be between 0.78 - 2.76 mg/l and there were significant differences across seasons. This observation could be attributed to more wastewater discharge, external pollution and land use due to high industrialized activities taking place in the studied areas than the areas around the Tyume River (Sibanda *et al*., 2014). The wide BOD range noted for the Vaal River in this study could be attributed to increased urbanisation, population and industrialised activities taking place in the Vaal areas compared to few industries and less population in the rural settings along the Tyume River in the Eastern Cape (Sibanda *et al*., 2014). Notably, the BOD values in our study are higher than the World Health Organization (WHO, 1984; 1989) standard of <3 mg/l, which is an indication of pollution in the Vaal River (both in upstream and downstream). However, the BOD values of the potable waters were found to be lower than the WHO standard, which is an indication that potable water distributed to the populace through the purification plant posed no threat to human beings, although there is no SANS-241 (2015) standard for BOD. Based on WHO (1984; 1989) standard, the purification plant is effective in maintaining the BOD levels within the acceptable limits.

The DO values of upstream showed no significant ($p<0.05$) difference across the seasons unlike the DO values for downstream. The DO values for upstream and downstream were observed to be lower than the two potable waters sampled in this study and so did not meet SANS-241(2015) and WHO (1984; 1989) standards (Table 4.7) indicating higher pollution than the potable water channels. Notably, the lower values observed in DO of upstream indicating that there was an external source of pollution probably due to human activities such as farming, fishing, and animal excreta. Another plausible reason could be because of serial dilution as the river flows from upstream to downstream in all seasons. The purification plant on the other hand is not effective in maintaining DO using WHO (1984; 1989) standards.

Seasonal averages of COD varied significantly across the sampling points in each of the seasons for upstream, downstream, potable waters. In summer, COD values ranged from 0 to 42.5 mg/l, in autumn (2 to 48 mg/l), during winter (38 to 71 mg/l) and in spring (0 to 87 mg/l) (Table 4.7). WHO (1984; 1989) standard revealed that upstream in all seasons and downstream in all the seasons except in spring, potable water (in winter and autumn) did not meet this international standard. Clearly, it was evident that the Vaal River was contaminated with chemical organic matters but the purification plant could reduce those toxic constituents in most seasons.

The TDS values for upstream, potable waters vary significantly across the seasons. However, the TDS values for downstream showed no significant difference across seasons. This was not in agreement with the work done by Sibanda *et al*. (2014) were they observed that TDS varied significantly across seasons for all the samples from a typical rural based river. However, their work was in agreement with the seasonal variations obtained in this study for upstream. Vaishali and Punita (2013) assessed the effect of seasonal variation on water quality of the River Mini and reported significant variation in the TDS. This result was consistent with findings in this study for the Vaal River. The highest TDS were recorded in winter season indicating an increase in organic matter in the River Mini water during the season, which they attributed to the increase in anthropogenic interferences of the surrounding areas (Vaishali and Punita, 2013). This was consistent with the results obtained for downstream in this study. An evaluation of the physico-chemical characteristics of the Buffalo River in the Eastern Cape Province of South Africa, revealed similar effect of season on the TDS were the values ranged from 20.3 – 23.350 mg/l across sites

(Chigor *et al*. 2013). TDS for upstream and downstream were below the limits for SANS (2015) (1200 mg/l) and WHO (1984; 1989) limit of 600 mg/l. Furthermore, the potable waters were also found to meet both standards indicating the effectiveness of the purification plant.

The EC values for downstream, upstream and potable waters (1 and 2) ranged from 261 to 620 µS/cm in summer, 157 to 630 µS/cm in autumn, 75 to 377 µS/cm in winter and 120 to 763 µS/cm in springs, respectively as illustrated in Table 4.7. These values are higher compared to those reported by Jordaan and Bezuidenhout (2013) for the Vaal River in 2012 (17.91 to 78 µS/cm). This implied that the rate of pollution has increased over the years as a result of technological advancement due to the release of pollutants capable of increasing the electrical conductivity of waterbodies. Furthermore, the EC of the Buffalo River in the Eastern Cape Province of South Africa showed higher EC values compared to our study (42.3–46,693 µS/cm) (Chigor *et al*., 2013). This implied that the location activities influence the level of EC and possibly other physico-chemical parameters as a result of pollution. Hence, it is important to monitor the quality of water sources on a regular basis as the values obtained in this study did not meet the SANS (2015) limit of ≤ 170 µS/cm and WHO (1984; 1989) limit of 250 µS/cm during the sampling period indicating pollution of the Vaal River.

The salinity of downstream, upstream and potable waters (1 and 2) were equally determined as shown in Table 4.7. Seasonal variation did not significantly affect the salinity of samples from the Vaal River; however, it affected the salinity of potable waters. For instance, the highest salinity in potable water (0.34 psu) was recorded in autumn compared to the lowest in spring (0.07 psu). The impacts of excess salinisation on water resources include decrease crop yield, increased formation of scale in aquatic organisms and increased requirements for pre-treatment of water for selected industrial use such as boiler feed water (DEAT, 2000). There was no stipulated standard for salinity and as a result, it is difficult to ascertain the effectiveness of the purification plant.

In summary, most of the water quality parameters of upstream and downstream did not meet the SANS (2015) and WHO (1984; 1989) standards indicating partly the influence of the final effluents discharged by the Leeuwkuil Wastewater Treatment Plant and possibly, other external source is responsible for the pollution. However, the purification plant was found to be effective in maintaining the values of Cu, Zn, Al, temperature, BOD, COD, and TDS although more review needs to be done in the plant to ensure that the plant is capable of maintaining all the water qualities within the stipulated limits.

4.3.3 Correlation coefficient of heavy metals and physico-chemical parameters of samples from Vaal River and Potable water

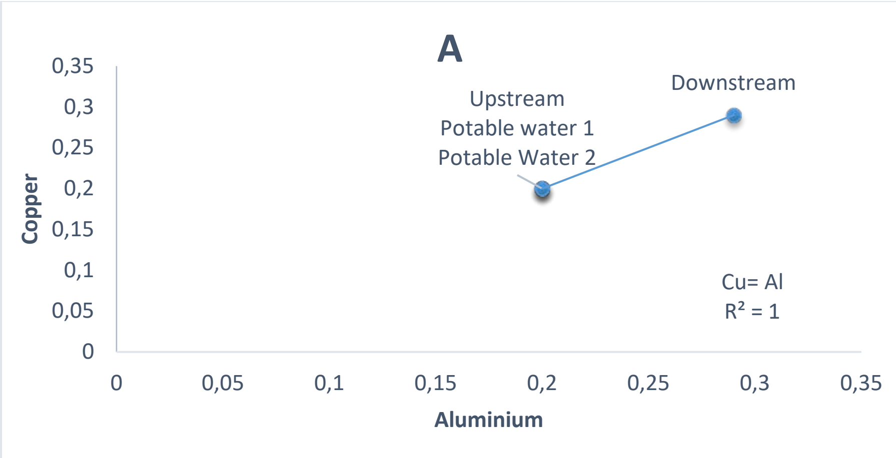
A correlation analysis was used to evaluate the degree of interrelation and association between two or more variables of the Vaal River and potable water treatment process. Strong positive correlations (0.8-1) at significant level of $p < 0.05$ were observed between BOD, COD, TDS, EC and salinity during the summer season. For example, it was evident that there were stronger correlations between some heavy metals (Al and Cu, Al and Pb, Cu and Pb) with a correlation coefficient of 1 (Table 4.8 and Figure 4.5). Regression analysis demonstrated strong associations between some parameters that will help predict water quality indices.

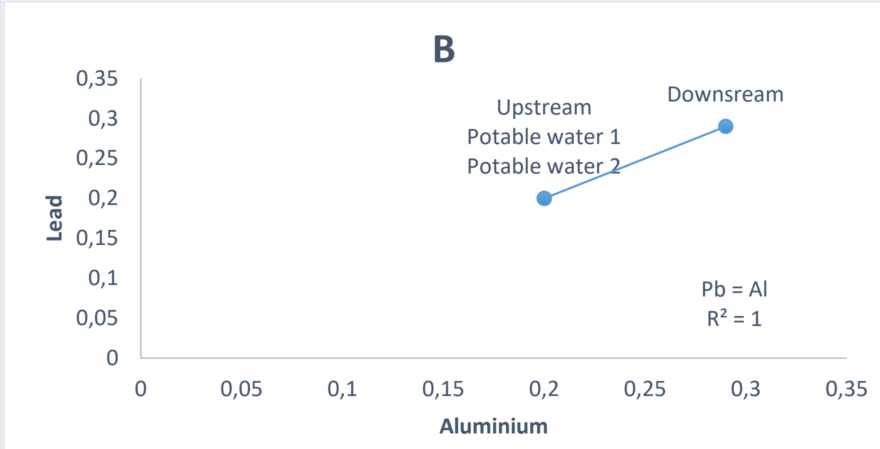
Table 4.8: Pearson Correlation of physico-chemical parameters of samples from Upstream, Downstream and Potable Water 1 & 2 during summer

	PH	BOD	DO	COD	TDS	EC	Salinity	Al	Zn	Cu	Pb
Temperature	-0,071	-0,103	-0,37874	-0,04976	-0,05591	-0,04227	-0,08482	-0,265544	-0,94898	-0,26554	-0,265544
PH		-0,689	0,838845	-0,73081	-0,74423	-0,74357	-0,74474	0,1555970	0,283293	0,15559	0,1555970
BOD			-0,86168	0,997297	0,996338	0,995812	0,996853	0,6073222	0,110186	0,60732	0,6073222
DO				-0,8848	-0,87808	-0,88592	-0,86069	3,754E-16	0,729918	3,75E-16	3,75E-16
COD					0,999757	0,999811	0,999007	0,556427	0,043273	0,556427	0,5564276
TDS						0,999902	0,99956	0,540908	0,042431	0,540908	0,5409089
EC							0,999046	0,540256	0,030508	0,540256	0,5402564
Salinity								0,5424508	0,06792	0,542450	0,5424508
Al									0,500835	1	1
Zn										0,500835	0.500832
Cu											1

r-Values ≥0.492 or =−0.492 are significant at $p < 0.05$.

.





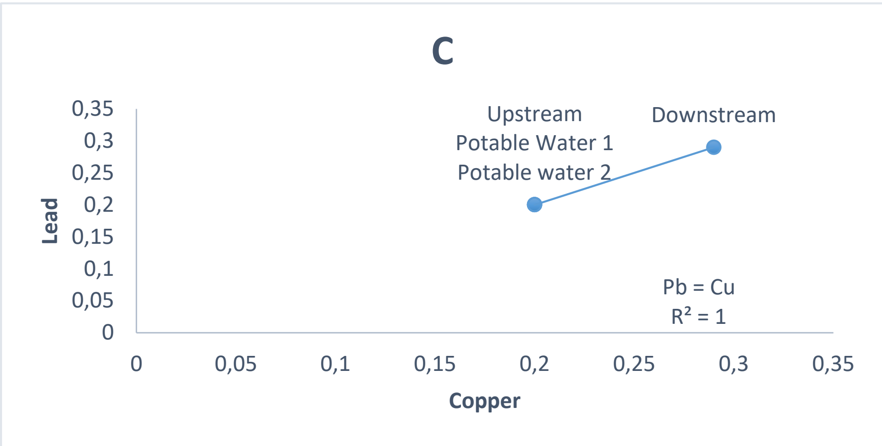


Figure 4.5: Linear regression graph to predict the physico-chemical parameters (copper, aluminium, lead) in upstream, downstream and potable water samples. Pearson's correlation between copper and aluminium (A), lead and aluminium (B), lead and copper (C) during summer for water samples are strong (with value 1) and are represented with linear regression equation.

Table 4.9: Linear-regression equations to predict water quality of samples from upstream, downstream and potable water during summer

R^2 Value	Regression equations
0.9946	COD= 12.342 (BOD)-9.5471
0.9995	TDS= 4.2415 (COD)+128.46
0.9916	BOD=0.0097 (EC)- 1.7401
0.9996	EC= 83274 (COD) +261.41
0.9998	EC= 1.963 (TDS)+9.275
0.9937	Salinity= 0.0503 (BOD)+ 0.086
0.9991	Salinity= 0.001 (COD) +0.0015
0.9991	Salinity= 0.001 (TDS) +0.0015
0.9981	Salinity= 0.0005 (EC) +0.0029

Table 4.10: Pearson Correlation of physico-chemical parameters of samples from upstream, downstream and potable water 1 & 2 during autumn

	PH	BOD	DO	COD	TDS	EC	Salinity	Al	Zn	Cu	Pb
Temperature	-0,07152	-0,0702	0,186317	-0,30255	-0,11867	-0,13151	0,816656	0,954687	0,420984	-0,26554	-0,74835
PH		-0,7991	0,338591	-0,58744	-0,64501	-0,62038	-0,41019	-0,00478	0,347234	0,155597	0,338652
BOD			-0,83615	0,943454	0,974816	0,967199	0,495294	-0,28918	0,149628	0,468775	-0,52946
DO				-0,94287	-0,93741	-0,94803	-0,39116	0,466915	-0,54403	-0,87622	0,510262
COD					0,982	0,984632	0,302599	-0,54048	0,244939	0,69511	-0,38617
TDS						0,999471	0,474995	-0,37191	0,317274	0,653802	-0,54506
EC							0,464401	-0,38793	0,335793	0,677757	-0,53949
Salinity								0,624387	0,586989	0,170801	-0,98539
Al									0,186322	-0,52223	-0,52223
Zn										0,726774	-0,70035
Cu											-0,33333

r-Values≥0.492 or =−0.492 are significant at $p < 0.05$.

Table 4.11: Linear-regression equations to predict water quality of samples from upstream, downstream and potable water during autumn

R^2 Value	Regression equations
0.8901	COD= 9.2658 (BOD)+0.8065
0.9503	TDS= 55.757 (BOD) +43.158
0.9643	TDS= 5.719 (COD) 45.914
0.9355	EC= 108.25 (BOD)+ 91.521
0.9695	EC=11.22 (COD)+ 93.878
0.9989	EC=1.9557 (TDS)+5.0004
0.9114	AL=0.012 (Temp)- 0.1241

Table 4.12: Pearson Correlation of physico-chemical parameters of samples from upstream, downstream and potable water 1 & 2 during winter

	PH	BOD	DO	COD	TDS	EC	Salinity	Al	Zn	Cu	Pb
Temperature	-0,5154	-0,4568	0,39924	-0,8737	-0,4536	-0,4536	-0,4571	-0,4621	-0,3053	-0,4621	-0,2212
PH		-0,45036	0,412953	0,846885	-0,43397	-0,43397	-0,42168	0,903227	-0,57046	0,903227	0,095077
BOD			-0,97964	-0,02928	0,998797	0,998797	0,997531	-0,57659	0,652236	-0,57659	0,497249
DO				0,069872	-0,98809	-0,98809	-0,99071	0,61832	-0,49203	0,61832	0.052346
COD					-0,02898	-0,02898	-0,02288	0,82716	-0,06173	0,82716	0,037037
TDS						1	0,999762	-0,58069	0,614323	-0,58069	0,53914
EC							0,999762	-0,58069	0,614323	-0,58069	0,53914
Salinity								-0,57717	0,597424	-0,57717	0,556921
Al									-0,33333	1	-0,33333
Zn										-0,33333	-0,33333
Cu											-0,33333

R-Values≥0.492 or =−0.492 are significant at $p < 0.05$.

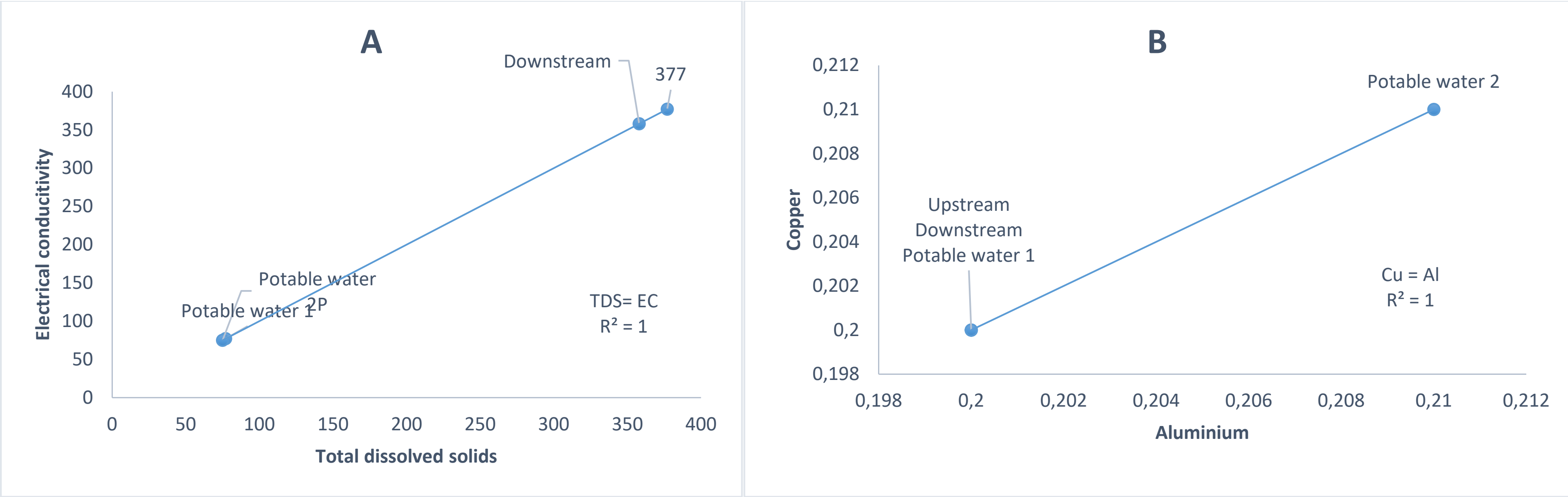


Figure 4.6: Linear regression graph to predict the physico-chemical parameters (electrical conductivity, total dissolved solids, copper, aluminium,) in upstream, downstream and potable water samples. Pearson's correlation between electrical conductivity and total dissolved solids (A), and copper and aluminium (B), lead and aluminium (B), during winter for water samples are strong (with value 1) and are represented with linear regression equation.

Table 4.13: Linear-regression equations to predict water quality variable of samples from upstream, downstream and potable water during winter

R^2 Value	Regression equations
0.9976	TDS= 93.145 (BOD) -33.699
0.9976	EC= 93.145 (BOD) -33.699
0.9951	Salinity= 0.0909 (BOD) -0.0267
0.9995	Salinity= 0.001 (TDS) + 0.0059
0.9995	Salinity= 0.001 (EC) + 0.0059
0.8158	Pb= 0.0322 (pH) – 0.0754

‘

Table 4.14: Pearson Correlation of physico-chemical parameters of samples from upstream, downstream and potable water 1 & 2 during spring

	PH	BOD	DO	COD	TDS	EC	Salinity	Al	Zn	Cu	Pb
Temperature	-0,66872	0,68144	-0,70508	0,80451	0,52856	0,533036	0,543718	-0,38533	-0,22271	-0,38585	0,9933670
PH		-0,93451	0,996847	-0,64533	-0,95698	-0,96529	-0,96729	0,413321	-0,55231	-0,38585	-0,581575
BOD			-0,91457	0,913746	0,969322	0,96461	0,967248	-0,7099	0,541391	-0,71057	0,5951713
DO				-0,5866	-0,93126	-0,94161	-0,94429	0,365528	-0,49454	0,366411	-0,623547
COD					0,826901	0,803713	0,804514	-0,91662	0,804514	-0,91697	6,626E-17
TDS						0,999332	0,999233	-0,59691	0,709151	-0,59766	0,4275312
EC							0,999919	-0,56818	0,706128	-0,56894	0,4322116
Salinity								-0,57035	0,697097	-0,57112	0,4436069
Al									-0,33333	-0,33333	-0,333333
Zn										-0,33378	-0,333333
Cu											-0,333782

r-Values≥0.492 or =−0.492 are significant at $p < 0.05$.

As shown in Table 4.9, it was evident that during the summer season, the association between the COD and BOD (r^2= 0.9946); TDS and COD (r= 0.9995); EC and TDS (r^2= 0.9998); Salinity and BOD; Salinity and COD (r^2 = 0.9991), were very strong (Table 4.9). Similarly, in autumn, strong correlations were revealed between certain phyico-chemical parameters at significant level of $p< 0.05$. For example, the concentration of BOD correlated strongly and positively (with correlation coefficients >0.9) with COD, TDS and EC (Table 4.10). Similarly, COD correlated strongly with TDS and EC, however, the strongest correlation among heavy metals was between Zn and Cu (0.7) (Table 4.10). Linear regression analysis revealed an r^2 value of 0.9989; 0.9695, 0.9114, 0.9643, 1.0, 1.0 and 1.0 for the associations between EC and TDS; EC and COD; Al and Temp; TDS and COD, Pb and Al, Cu and Pb, Cu and Al respectively (Table 4.11). In our study, the higher correlation coefficients noted between heavy metals would imply same origin of the metals identified (Verandani and Vardhan, 2012).

Similar to autumn, strong positive correlations existed between certain phyico-chemical parameters during winter at a significant level of $p< 0.05$. For example, BOD correlated strongly with TDS (0.99), EC (0.99) and salinity (0.99) and between EC and TDS (1), TDS and salinity (0.99), EC and salinity (0.99). Similarly, pH correlated strongly with COD (0.85), and Al correlated well with Cu with a coefficient value of 1 (Table 4.12 and Figure 4.6). Nevertheless, interrelation and association between variables during winter season revealed that Pb, Zn and Cu had a moderate correlation with the other water parameters when compared to Al that had strong correlation with Cu (Table 4.12). Furthermore, linear regression analysis revealed the following r^2 values for the following parameters; salinity and BOD (r^2= 0.9951); salinity and TDS (r^2= 0.9995); salinity and EC (r^2= 0.9995) (Table 4.13).

Taken together, the interrelationship and associations between some physico-chemical parameters of the effluents from wastewater treatment, Vaal River and potable treatment process showed correlation coefficient values and linear regression equations similar to findings reported by other authors (Akbar *et al*., 2010; Vaishali and Punita, 2013; Sangeeta and Neha, 2015). This could imply that the industrial effluents influenced the Leeuwkuil Wastewater Treatment Plant which in turn influenced Vaal River and the purification plant.

4.4. Identification of Microbial Presence and their Resistance to Heavy Metals

4.4.1 Microbiological characterisation

The physico-chemical parameters of the water identified can reveal particular conditions for the ecology of aquatic organisms and possibly suggest suitable management strategies for maintaining the quality of water (Abdel-Raouf *et al*., 2012; Mbalassa *et al*., 2014). The municipal treatment plants is aimed at removing or reducing organic wastes in order to avoid a decrease in dissolved oxygen in the receiving watershed, eliminating contaminants in order to avoid excessive richness of nutrients, and protect human health by deactivating microorganisms capable of causing disease (Dixit *et al*., 2015; Akpor and Muchie, 2010). Based on these, it became necessary to assess the microbial compositions of waste obtained from the industries, Leeuwkuil Wastewater Treatment Plant (inflow and final effluent) and purification plant (potable water 1 and 2). Identification of harmful microorganisms in potable water 1 and 2 in this study could pose a serious health concern to the people living around Vaal area.

4.4.1.1 Culture morphology

In this study, a total of seventy pure strains of bacteria were isolated from the sampling points. Observations of the pure cultures in petri plates revealed morphological variations in terms of the colony form, texture, opacity, elevation and colour. Colonies with similar morphological characteristics were grouped together and selected for the screening process.

4.4.1.2 Screening for resistant microbial isolates towards high heavy metal concentration

In this study, heavy metal screening tests were carried out for all 70 isolates obtained by exposing the isolates to increasing concentrations of four metals (Al, Cu, Pb and Zn). The results showed that 22 isolates were resistant (+) to a factor of 10 increased concentrations of Al, Cu, Pb, and Zn (Table 4.15). Among the 22 isolates, 90% (20) showed resistance to Cu and Pb, whilst 86% (19) were resistant to Al and 82% (18) to Zn (Table 4.15).

Table 4.15: Heavy metal resistance for pre-screening test of bacterial isolates

STRAIN CODE	ORIGIN	SEASON COLLECTED	RESPONSE TO ELEMENTAL CONCENTRATIONS (mg/l)			
			Al 4	Cu 0.01	Pb 0.12	Zn 0.4
25	inflow	Spring	+	+	+	+
26	inflow	Spring	+	+	+	+
27	inflow	Spring	+	+	+	+
68	inflow	Spring	-	+	+	+
35	upstream	Autumn	+	+	+	+
31	upstream	Spring	+	-	-	-
41	final effluent	Autumn	+	+	+	+
47	final effluent	Winter	+	+	+	+
16	final effluent	Winter	+	+	+	+
67	final effluent	Spring	-	+	+	+
39	downstream	Autumn	+	+	+	+
8	Downstream	Winter	+	+	+	+
57	Downstream	Winter	+	+	+	+
21	Downstream	Spring	+	+	+	+
64	downstream	Spring	+	+	+	-
66	Downstream	Spring	+	+	+	-
13	potable water 1	Winter	-	+	+	+
4	potable water 2	Summer	+	+	+	+
32	potable water 2	Spring	+	+	-	+
70	industry 2	Spring	+	-	+	+
44	industry 4	Autumn	+	+	+	+
17	industry 4	Winter	+	+	+	+

+ represent bacterial isolates resistant to heavy metals, - represent bacterial isolates non-resistant to heavy metals

Notably, all the bacteria in samples from industry 4 during autumn and winter showed resistance to all selected heavy metal. This trend was also observed in potable water

2 during summer, downstream (autumn and winter), final effluent (autumn and winter) and inflow in autumn. Therefore, it was evident that heavy metal resistant bacteria were common among the isolates collected from the study sites. These 22 resistant bacterial isolates could have adapted to the high concentrations of the heavy metals derived from industries within and around the study sites. It is interesting to note that heavy metal resistance bacteria have been reported to enhance bioremediation technologies (Tchounwou *et al*., 2012).

Microbes have developed mechanisms to survive high concentrations of heavy metals. The survival mechanisms include active transport of the metals away from the cell, enzymatic detoxification of the metals to less toxic forms and reduction in metal sensitivity of cellular targets (Bruins *et al*., 2000; Nies and Silver, 1995; Silver, 1996). The detoxification mechanisms maybe for one metal or group of chemically related metals and can possibly vary depending on the microbe involved (Nies and Silver, 1995). The identified heavy metal resistant bacteria in this study can be used in the future for the bioremediation of the environment polluted with heavy metals, hence the purpose of their identification. These identified heavy metal bacteria also play an important role in the wastewater treatment plants in Vaal in the decomposition of wastewater and probably in reducing the concentrations of heavy metals that came from the industrial effluent. Therefore, there was a need to further characterise these isolated heavy metal bacterial colonies using gram staining and molecular process.

4.4.1.3 Gram staining of bacterial isolates

To help with the preliminary identification of the 22 heavy metal resistant isolates, a gram staining technique was employed in this study. According to this staining method, Figure 4.7 below shows examples of the two common bacterial colonies identified gram negative rod (A) and gram positive rod (B). It was evident that there was high presence of both gram negative and gram positive bacterial isolates in all sampling points (Figure 4.7). Furthermore, the metals selected were known to be resistant by a broader spectrum of bacterial isolates hence the need for a molecular studies.

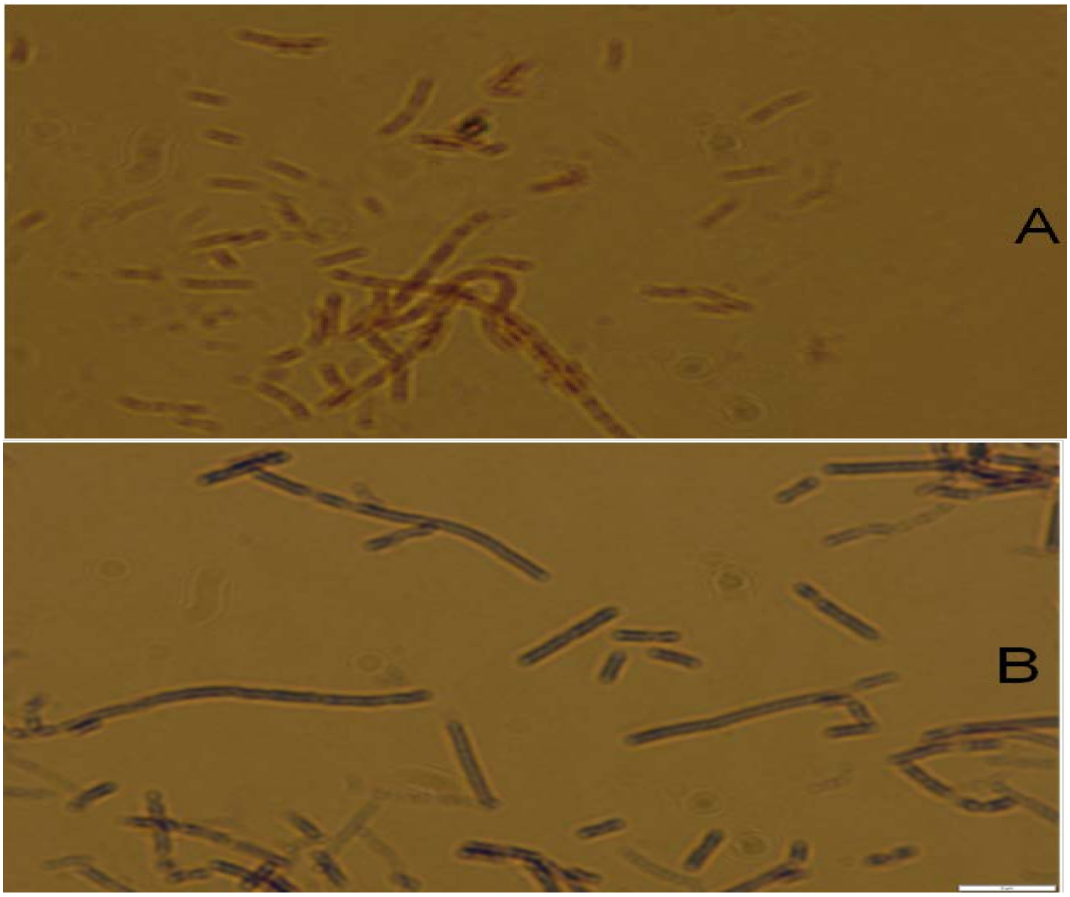


Figure 4.7: Two samples of gram stained bacterial isolates from the sampling points. Gram negative rod (A) (bacteria take up safranin pigment and turn reddish pink) and gram positive rod (B) (bacteria take up the crystal violet and turn purple).

4.4.2 Molecular characterisation

Molecular characterisation of bacteria was achieved using DNA extraction which was amplified further with PCR techniques. According to Table 4.16 and Figure 4.8, four major clusters of bacteria (out of the 22 heavy metal resistant bacteria) were revealed with majority showing 100 percent similarity to *Bacillus* strains (Cluster 1), those highly similar to *Sphingomonas* sp., *Pseudomonas* sp., *Alcanivorax* sp. (Cluster 2). Cluster 3 included those found to show high similarity to species of *Serratia marcescens, Enterobacter* and *Klebsiella* and a group of bacterial were completely isolated (Cluster 4).

Specifically, the deletion of gaps allowed for a range of identification of close relatives with similarities as close as 99% to 100%. For example, isolate E60 was identified as *Sphingomonas* sp. belonging to the genus of Enterobacter and sharing common ancestry with *Bacillus* sp., therefore having descending phylogenetic relationship with *Bacillus toyonensis* strain (E17), *Bacillus thuringiensis* strain (E29) and *Bacillus* sp. strain (E31). Similarly, the descending relationship included the cluster of the other ten organisms which are *Staphylococcus pasteuri* (E13), *Bacillus tequilensis* (E37) and *Bacillus* sp. strain (E68), however; the cluster in similarity is highest with organisms *Alcanivorax* sp. (E54), *Pseudomonas aeruginosa* strain (E66), *Serratia marcescens* strain (E21), *Serratia marcescens* strain (E40), *Klebsiella* sp (E55), *Enterobacteriaceae bacterium* (E33), and *Enterobacter aerogenes* (E67) respectively (Table 4.16 and Figure 4.8).

These bacteria identified are said to be resistant to the four heavy metals (Al, Cu, Pb and Zn) used in this study. The more the presence of these heavy metals and other heavy metals that are identified in both industrial and Leeuwkuil treatment plant, the more the persistent of this bacteria since the metals may serve as nutrient to many of them. Therefore, there is a need for the removal of this heavy metals to prevent bioaccumulation in living organisms (which can be toxic if ingested in large quantity) of which the pathogenic bacteria can be harmful to other living organisms and can cause disease if not properly destroyed. The diversity of bacterial species isolated from the study sites evaluated in this study are in agreement with results reported by Jordaan and Bezuidenhout (2013) when they assessed the impact of physico-chemical water quality parameters on bacterial diversity in the Vaal River. In this study, *Serratia marcescens*, *Bacillus tequilensis*, *Pseudomonas aeruginosa*, and

Sphingomonas spp were found in the upstream and downstream of Vaal River which is similar to the bacteria species found in Vaal River by Jordaan and Bezuidenhout (2013). *Serratia marcescens* is pathogenic because of its involvement in opportunistic infection in human especially in the urinary tract, respiratory tract, wound and the eye (Auwaerter, 2007). *Pseudomonas aeruginosa* is also an opportunistic bacterium in humans because it is capable of causing disease in an immune-compromised person (Hall *et al*., 2004). The presence of these pathogenic bacteria in the Vaal River poses threat to the life of people living around the area and making use of the water from the river for their day to day activities. *Sphingomonas* spp is also found useful despites the role it plays in human diseases (Ryan and Adley, 2010). They have degradable ability and can be applied in bioremediation of environmental contaminants (Yabuuchi and Kosako, 2015). *Staphylococcus pasteuri* is a coagulase-negative, Gram positive organism which is emerging as an agent of nosocomial infections and a blood derivatives contaminant, though its role in causing human disease mostly remains controversial (Savini *et al*., 2009). This organism was discovered to be resistant to several classes of antibiotics such as methicillin/oxacillin, macrolides, lincosamides, streptogramins, tetracyclines, chloramphenicol, streptomycin, fosfomycin, as well as quaternary ammonium compounds. In this study, the organism was found in the potable water. This showed the inability of the purification plant to eradicate these pathogenic bacteria and as a result, could cause a lot of health hazard to the people in Vaal River.

Serratia marcescens, *Bacillus thuringiensis*, and *Alcanivorax* spp are found in the final effluent. The presence of *Serratia marcescens* in final effluent and upstream and downstream indicated the movement of the bacterium from the final effluent into the Vaal River. This implied that Leeuwkuil Wastewater Treatment Plant was not effective in controlling the microbial composition of the waste. *Bacillus thuringiensis* is known to be a biological pesticide and in this study, it was found to be resistant to heavy metals. This implied that the bacterium could have the tendency to bioremediate environmental contaminants. *Alcanivorax* spp play some roles in biotechnology where they are used to breakdown oil especially, the hydrocarbons (Yakimov *et al*., 1998). In this study, the bacterium was found to be resistant to heavy metals. This implies that the organism could be used in bioremediation.

Table 4.16: Characterisation of bacterial isolates collected from study sites

Sequence length (nt)	Strain No	Origin	Closest similarity	Percentage similarity	Accession No
402	13	Potable water 1	*Staphylococcus pasteuri* partial 16S rRNA gene, strain mammoth-5	99	KY670723
455	17	Industry 4	*Bacillus toyonensis* strain Se8 16S ribosomal RNA gene, partial sequence	100	KY670724
420	21	Downstream	*Serratia marcescens* strain U10 16S ribosomal RNA gene, partial sequence	100	KY670725
454	41	Final effluent	*Bacillus thuringiensis* strain NBBT7 16S ribosomal RNA gene, partial sequence	99	KY670726
446	35	Upstream	*Bacillus* sp. strain M 16S ribosomal RNA gene, partial sequence	99	KY670727
433	44	Industry 4	Enterobacteriaceae bacterium EF44 16S ribosomal RNA gene, partial sequence	99	KY670728
452	57	Downstream	*Bacillus tequilensis* strain SLI23 16S ribosomal RNA gene, partial sequence	99	KY670729
429	47	Final effluent	*Serratia marcescens* strain U10 16S ribosomal RNA gene, partial sequence	99	KY670730
436	67	Final effluent	*Alcanivorax* sp. BI06 16S ribosomal RNA gene, partial sequence	99	KY670731
468	68	Inflow	*Klebsiella* sp. SS1-29 16S ribosomal RNA gene, partial sequence	96	KY670732
409	31	Upstream	*Sphingomonas* sp. D31C2 16S ribosomal RNA gene, partial sequence	99	KY670733
464	26	Inflow	*Enterobacter aerogenes* KCTC 2190 16S ribosomal RNA gene, partial sequence	99	KY670734
447	32	Potable water 2	*Bacillus* sp. strain 90.2.7 16S ribosomal RNA gene, partial sequence	100	KY670735
440	64	Downstream	*Pseudomonas aeruginosa* strain PUFSTf05 16S ribosomal RNA gene, partial sequence	100	KY670736

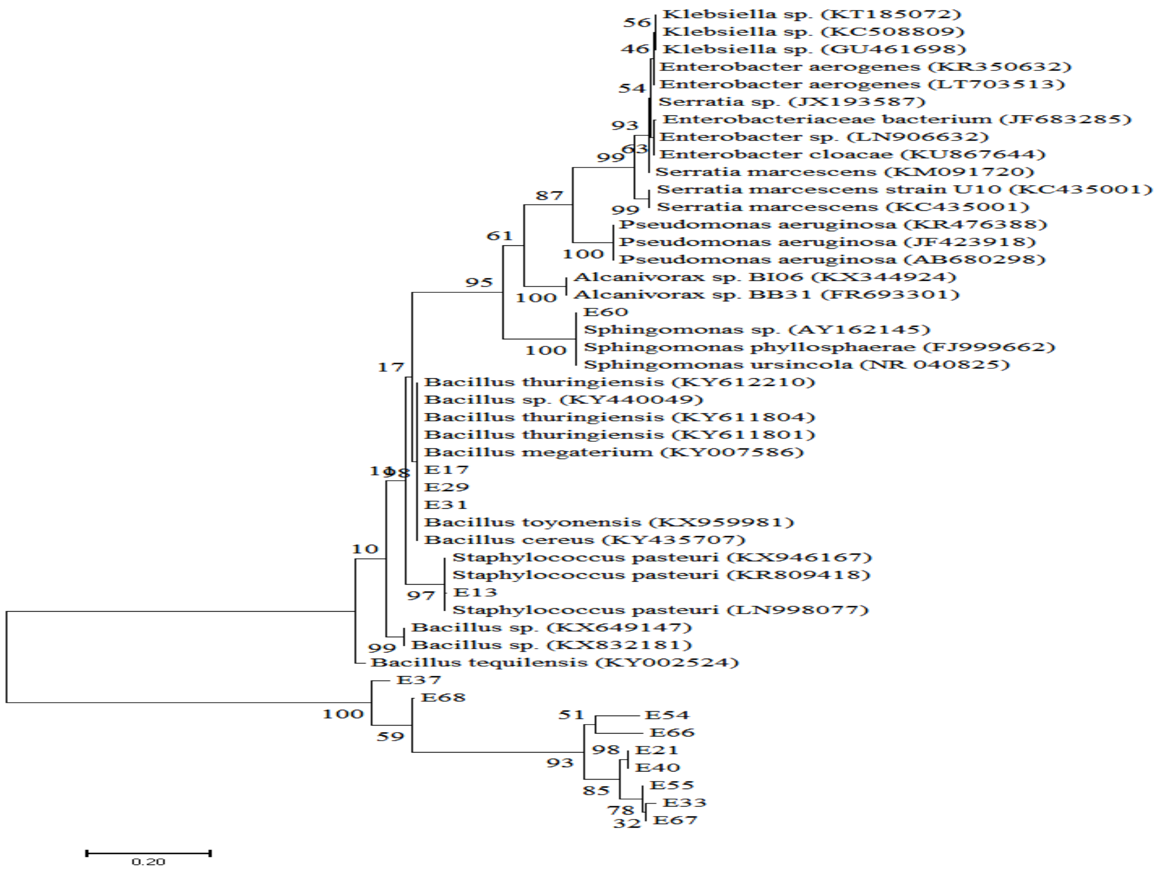


Figure 4.8: Phylogenetic tree showing the relationship among the screened bacterial species with similarities based on 16S rDNA gene sequences (ML, 1000 rounds bootstrap replications)

4.5. Evaluation of the Effectiveness of the Leewkuil Treatment Plant

Pollution of Vaal River could have an adverse effect on the purification plant. The effect could include high cost of purification of water from the downstream of the river. The correlation coefficients of physico-chemical parameters of water samples from upstream, downstream, potable water 1 and 2 during summer, autumn winter and spring showed significant positive correlation were found between pH and dissolved oxygen during summer (r^2=0.8388), pH and chemical oxygen demand (r^2=0.8468), aluminium (r^2=0.9032), copper (r^2=0.9032) during winter, pH and dissolved oxygen (r^2=0.9968) during spring. Biological oxygen demand also correlated positively with COD, TDS, EC, salinity, Al, Cu, and Pb in summer; COD, TDS, EC, salinity, and Cu in autumn; TDS, EC, salinity, Zn and Pb in winter; and COD, TDS, EC, salinity, Zn and Pb in spring. The correlation of one parameter with other parameters in the different seasons showed the influence of the seasonal variation on the effluents from the Leeuwkuil Wastewater Treatment Plants discharged into the Vaal River and the purification plant. The more polluted the river is the higher the pressure on the purification plant to produce quality water for the populace.

4.6 Conclusions

The contamination of heavy metals in water and other aquatic environments have gained greater momentum and attention due to its abundance, persistence and ecotoxicity. In this study, a total of 24 heavy metals (Al, Cd, Cr, Cu, Fe, Mg, Ni, Pb, Sr, & Zn) were identified from all the sampling points. The water quality test indicated that wastewater from the industries is polluted with heavy metals and the correlation study done showed their influence on the Leeuwkuil treatment plant and purification plant. The water quality test done also signalled the presence of microorganisms in the samples. Microbial characterisation and heavy metal sensitivity test done showed the presence of bacterial with 22 of them resistant to the selected heavy metals in this study. Furthermore, the presence of the pathogenic bacteria in potable water showed that more attention is required on the purification plant.

CHAPTER 5: SUMMARY, RECOMMENDATIONS AND CONCLUSION

5.1 Summary

The objectives of this study were to identify and quantify heavy metals and physico-chemical parameters in effluent water from industries and Leeuwkuil plant, and water samples from Vaal River and potable water as well as comparing the selected heavy metals and physico-chemical parameters to current national and international standards [SAGES, Green Drop certification requirement, South African National standards (SANS) and World Health Organization (WHO)]. Further objective of this study were to identify and characterise bacteria that are resistant to the selected heavy metals in all the sampling points and to determine the correlation between the quality of potable water and the effectiveness of the treatment plants. The findings of this study are summarised below:

5.1.1 Heavy metals and physico-chemical parameters of the effluents and water samples

Twenty four heavy metals (Ag, Al, B, Ca, Cr, Cu, Fe, K, Mg, Na, Ni, P, S, Pb, Sb, Se, Sr, Si, Te, Cd, Li, Mo, V & Zn) were identified in effluent water from industries and Leeuwkuil plant, and water samples from Vaal River and potable water. The industries recorded high values for heavy metals and water quality parameters (BOD, DO, COD, TDS, EC, and salinity) indicating them as point source of pollution in Vaal area of South Africa. The Leeuwkuil Wastewater Treatment Plant was also assessed and was found to be ineffective in maintaining Cu, Zn, Pb, (using SAGES standard), TDS and EC but was only effective in maintaining temperature, pH, and COD within the green drop and SAGES standards. This indicated that Leeuwkuil Wastewater Treatment Plant needs to be reviewed.

The assessment of heavy metals and physico-chemical parameters from Vaal River (upstream and downstream) and potable water showed that most of the water quality parameters of samples from Vaal River did not meet the SANS-241(2015) and WHO (1984; 1989) standards indicating partly the influence of the final effluents discharged by the Leeuwkuil wastewater treatment plant and possibly, other external source that is responsible for the pollution. However, the purification plant was found to be effective in maintaining the values of Cu, Zn, Al, temperature, BOD, COD, and TDS although more review is needed to be done in the plant.

5.1.2 Identification and characterisation of heavy metal resistant bacteria

Seventy bacteria isolates obtained in this study were identified and exposed to increasing concentrations of four metals (Al, Cu, Pb and Zn). The results showed that 22 isolates were resistant (+) to increased concentrations of Al, Cu, Pb, and Zn. Among the 22 isolates, 90% (20) showed resistance to Cu and Pb, whilst 86% (19) were resistant to Al and 82% (18) to Zn. The heavy metal resistant bacteria such as *Sphingomonas* sp., *Bacillus toyonensis* strain (E17), *Bacillus thuringiensis* (E29), *Bacillus* sp. strain (E31), *Staphylococcus pasteuri* (E13), *Bacillus tequilensis* (E37) and *Bacillus* sp. strain (E68), *Alcanivorax* sp. (E54), *Pseudomonas aeruginosa* (E66), *Serratia marcescens* (E21), *Serratia marcescens* strain (E40), *Klebsiella* sp (E55), *Enterobacteriaceae bacterium* (E33), and *Enterobacter aerogenes* (E67) were identified based on their similarities in the NCBI database. It is believed that some of these resistant bacteria like *Sphingomonas spp* and *Alcanivorex* (although causing diseases in human) were helpful in the bioremediation of the heavy metals contaminations. However, some of them like *Serratia marcescens*, *Pseudomonas aeruginosa*, *Staphylococcus pasteuri* and *Sphingomonas* spp are said to be pathogenic and must be removed so it does not affect human health.

5.1.3 Correlation between the quality of potable water and the effectiveness of treatment plants in Vaal area

The correlation coefficients of heavy metals and physico-chemical parameters of water samples from Vaal River and potable water during summer, autumn winter and spring showed significant positive correlation between pH and dissolved oxygen during summer, pH and chemical oxygen demand, aluminium, copper during winter, pH and dissolved oxygen during spring. Biological Oxygen Demand also correlated positively with COD, TDS, EC, salinity, Al, Cu, and Pb in summer; COD, TDS, EC, salinity, and Cu in autumn; TDS, EC, salinity, Zn and Pb in winter; and COD, TDS, EC, salinity, Zn and Pb in spring. The correlations between these parameters in the different seasons showed the influence of the seasonal variation on the effluents from the Leeuwkuil Wastewater Treatment Plants discharged into the Vaal River and the purification plant. This implied that increase in the pollution of Vaal River will result in a corresponding increase in the pressure on the purification plant to produce quality water for the people around the area. This may possibly influence the effectiveness of Leeuwkuil Wastewater Treatment Plants and the purification plant unless they are constantly reviewed to ensure consistency in their treatments.

5.2 Recommendations

The following recommendations are made from this study

- Effluent discharge need to be closely monitored by the municipal officials to ensure compliances by the industries to South Africa standards.
- Industries need to partake in environmental conferences or meetings so as to update themselves with current and latest publications and legal notices. This is very important to help them improve in their operations and partake in corporate social investment, including sponsoring community campaigns on pollution.
- Constant upgrading of Leeuwkuil Wastewater Treatment Plant should be monitored to reduce overload especially in some seasons. Leeuwkuil is already operating above design capacity especially in summer/spring, which could be one of the reasons for not maintaining TDS and EC within green drop limit.
- The final effluent discharged into Vaal River partly influenced BOD, DO, COD and EC concentrations of Vaal river as they were above the SANS-241 (2015) and WHO (1984; 1989) Standards. This pollution could have resulted also due to external activities around the river or possibly due to contamination from land use around Vaal, bearing in mind that Vaal is an industrialised area. Concerted effort can be made by the municipal to divert all storm water to drain pipes so that the storm water can be effectively treated.
- It is imperative for the government (or higher body) to monitor effluent and waste water before disposal into the river by the sewage plant to avoid pollution of water bodies.
- The publics who come to the river to perform their ritual activities hence polluting the river should be educated about the negative impact of the activities to water bodies.
- Monthly inspections need to be conducted by the municipality as well as industries to ensure that effluent does not exceed the stipulated limits. The municipality should also ensure that all industries are certified by ISO 14001 standard. Heavy fines should be imposed on industries that fails to meet requirements.

5.3 Recommendations for further research

It is believed that some of these resistant bacteria were helpful in the bioremediation of the heavy metals contaminations. However, in this study, detail work on the identification of their role in the bioremediation was not done. Hence, the bioremediation study of these heavy metals resistant bacteria is required for future study. Therefore, more work needs to be done in identifying the mechanisms of operation of the bacteria in degrading the heavy metals.

The present study ascertained the effectiveness of Leeuwkuil Treatment Plant through the measurement of physico-chemical parameters and the heavy metals. This provided a baseline information on the treatment plants but more work needs to be done in verifying the treatment capacity and the availability of the workers and equipment needed for the effective treatment of the waste by the treatment plants.

The study provided a baseline information on the impact of the five production industries on the treatment plants in Vaal, Vereeninging, South Africa. It is recommended that future studies will comprise more industries that were not studied in this research and are within the area.

High pH observed in potable water 1 and 2 could have resulted from contamination in the chemical used by purification plant in purifying water. This can be considered for future study.

5.4 Conclusion

The findings in this study showed industries as a point source of heavy metal pollutions in Vaal area. This posed serious challenge to the treatment plants in this area of South Africa. The correlation study done showed the influence of industrial effluents on the Leeuwkuil treatment plant and purification plant. The water quality test done also signalled the presence of microorganisms in the samples and were confirmed through microbial characterisation with the identification of heavy metal resistant bacteria. Furthermore, pathogenic bacteria were identified in potable water suggesting more review on the purification plant.

REFERENCE

Abbasi U, Jin W, Pervez A, Bhatti ZA, Tariq M, Shaheen S, Iqbal A, Mahmood Q (2016). Anaerobic microbial fuel cell treating combined industrial wastewater: Correlation of electricity generation with pollutants. Bioresource technology 200: 1 - 7.

Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM (2012). Microalgae and wastewater treatment. Saudi journal of biological sciences 19(3): 257 - 275.

Abo-Amer AE, El-Shanshoury AERR, Alzahrani OM (2015). Isolation and molecular characterization of heavy metal-resistant Alcaligenes faecalis from sewage wastewater and synthesis of silver nanoparticles. Geomicrobiology Journal 32(9): 836 - 845.

Abo-Amer, A.E., El-Shanshoury, A.E.R.R. and Alzahrani, O.M., 2015. Isolation and molecular characterization of heavy metal-resistant Alcaligenes faecalis from sewage wastewater and synthesis of silver nanoparticles. Geomicrobiology Journal *32*(9): 836-845.

Abraham JV, Butler RD, Sigee DC (1997). Ciliate populations and metals in an activated-sludge plant. Water Research 31(5):1103 - 1111.

Adebisi SA, Fayemiwo KA (2010). Pollution of Ibadan soil by industrial effluents. New York Journal 3(10): 37 - 41.

Adepoju-Bello AA, Alabi OM (2005). Heavy metals: A review. The Nigerian Journal of Pharmacy 37: 41 - 45.

Adepoju-Bello AA, Ojomolade OO, Ayoola GA, Coker HAB (2009). Quantitative analysis of some toxic metals in domestic water obtained from Lagos metropolis. The Nigerian Journal of Pharmacy 42(1): 57 - 60.

Adewumi JR, Ilemobade AA, Van Zyl JE (2010). Treated wastewater reuse in South Africa: Overview, potential and challenges. Resources, Conservation and Recycling 55(2): 221 - 231.

Africa Development Bank Group (2015). Annual group report. Rural Water Supply and Sanitation Initiative (RWSSI) and Rural Water Supply and Sanitation Initiative Trust Fund (RWSSI-TF). Water and Sanitation Department.

Ahmad K, Bhatti LA, Muneer M, Iqbal M, Iqbal Z (2012). Removal of heavy metals (Zn, Cr, Pb, Cd, Cu and Fe) in aqueous media by calcium carbonate as an adsorbent. International Journal of Chemical and Biochemical Sciences 2:48 - 53.

Aina EOA, Adedipe NO (1996). Water quality monitoring and environmental status in Nigeria. FEPA Monograph 6, FEPA, Abuja, Nigeria, 239.

Ajao AT, Oke MA, Ajijolakewu AK, Odebisi MB (2012). Physicochemical and Ecotoxicological Evaluation of Raw and Biologically Treated Textile Effluent. Journal of Scientific Theory and Methods 2012: 270 - 281.

Akaninwor JO, Wegwu MO, Nwaichi EO (2007). Levels of some heavy metals and physico-chemical properties of effluents from a beverage company in Rivers State, Nigeria. Journal of Applied Sciences and Environmental Management 11(1).

Akbar Jana F, Ishaqa M, Ihsanullahb I, Asimc SM (2010). Journal of Hazardous Materials 176: 609 - 616.

Akpor OB, Muchie M (2010). Remediation of heavy metals in drinking water and wastewater treatment systems: Processes and applications. International Journal of Physical Sciences 5(12): 1807 - 1817.

Akpor OB, Ohiobor GO, Olaolu TD (2014). Heavy metal pollutants in wastewater effluents: Sources effects and remediation. Advances in Bioscience and Bioengineering 2(4): 37 - 43.

Alam S (2010). Globalization, Poverty and Environmental Degradation: Sustainable Development in Pakistan. Journal of Sustainable Development Published by Canadian Centre of Education 103.Science 3(3).

Alam MJ, Islam MR, Muyen Z, Mamun M, Islam S (2007). Water quality parameters along rivers. International Journal of Environmental Science and Technology 4(1): 159 - 167.

Alinnor IJ, Obiji IA (2010). Assessment of trace metal composition in fish samples from Nworie River. Pakistan Journal of Nutrition, 9(1): 81 -85.

Alo BI, Olanipekun A (2006). Heavy metal pollution in different environmental media in Africa: Problems and prospects (with case studies from Nigeria). FMEnv/University of Lagos Centre for Environmental Human Resources Development Department of Chemistry, University of Lagos, Akoka, Lagos, Nigeria and Pollution Control Department, Federal Ministry of Environment, Abuja, Nigeria.

Amoo IA, Ajayi OO, Ipinmoroti KO, Amuda OS (2004). Performance of some coagulants in the treatment of effluent from soap/detergent industry. Proceeding 27th of International Conference of Chemical Society of Nigeria, Benin 415 - 420.

Angelakis AN, Snyder SA (2015). Wastewater treatment and reuse: Past, present, and future. Water (7): 4887 - 4895.

APHA (1995). Standard methods for the examination of water and waste water, 19th. Ed, American Public Health Association, American Water Works Association & Water Environment Federation, Washington, DC.

Appenroth KJ (2010). Definition of “heavy metals” and their role in biological systems. In Soil heavy metals . Springer Berlin Heidelberg19 - 29.

Asante KA, Agusa T, Subramanian A, Ansa-Asare OD, Biney CA, Tanabe S (2007). Contamination status of arsenic and other trace elements in drinking water and residents from Tarkwa, a historic mining township in Ghana. Chemosphere 66:1513 - 1522.

Auwaerter P (2007). "Serratia species". Point-of-Care Information Technology ABX Guide. Johns Hopkins University.

Ayres RU (1992). Toxic heavy metals: materials cycle optimization. Proceedings of the National Academy of Sciences 89(3): 815 - 820.

Bahri A, Drechsel P, Brissaud F (2008). Water reuse in Africa: challenges and opportunities. First African water week, “Accelerating Water Security for Socio-Economic Development of Africa*”, Tunis, Tunisia. Google Scholar*.

Bailey SE, Olin TJ, Bricka RM, Adrian DD (1999). A review of potentially low-cost sorbents for heavy metals. Water research 33(11): 2469 - 2479.

Baloyi C, Gumbo JR, Muzerengi C (2014). Pollutants in sewage effluent and sludge and their impact on downstream water quality: a case study of Malamulele sewage plant, South Africa. Water Pollution XII 182: 15.

Banza CLN, Nawrot TS, Haufroid V, Decrée S, De Putter T, Smolders E, Kabyla BI, Luboya ON, Ilunga AN, Mutombo AM, Nemery B (2009). High human exposure to cobalt and other metals in Katanga, a mining area of the Democratic Republic of Congo. Environmental research 109(6): 745 - 752.

Barzilay JMD, Winkler G, Weinberg MD, William Eley J (1999). The water we drink. Water quality and its effects on health: New Brunswick, New Jersey, Rutgers University Press152.

Battarbee R, Anderson N, Appleby P, Flower RJ, Fritz S, Haworth E, Higgit S, Jones V, Kreiser A, Munro MA, Natkanski J, Richardson N, Rippey B, Stevenson AC (1988). Lake Acidification in the United Kingdom, ENSIS, London.

Behera S (2013). Physiochemical and Microbial Analysis of Soil and Water of Dhobiajharan Village (Proposed Coal Mine Site-Tubed) (Doctoral dissertation).

Bent S, Böhm K (1995). Copper-induced liver cirrhosis in a 13-month old boy. Gesundheitswesen (Bundesverband der Arzte des Offentlichen Gesundheitsdienstes (Germany) 57(10): 667 - 669.

Bhagirath B, Ratna R (2002). Environmental and accountability impact of industrial pollution on rural communities. Economic and political weekly 19.

Bielská L (2013). Chemical methods for prediction of bioavailability of persistent organic pollutants in solid environmental matrices (Doctoral dissertation, Masarykova univerzita, Přírodovědecká fakulta).

Binning K, Baird, D (2001). Survey of heavy metals in the sediments of the Swartkops River Estuary, Port Elizabeth South Africa. Water SA *27*(4): 461 - 466.

Bosch S (1999). Environmental scoping report for the conveyance, treatment and disposal of wastewater for the town of Keiskammahoek, in the Eastern Cape. CEN Integrated Environmental Management Unit, Port Elizabeth.

Bradl H (2002). Adsorption of heavy metal ions on clays. Encyclopedia of surface and colloid science 373 - 384.

Bruins MR., Kapil S, Oehme FW (2000). Microbial resistance to metals in the environment. Ecotoxicology and environmental safety 45(3): 198-207.

Buamah R, Petrusevski B, Schippers JC (2008). Presence of arsenic, iron and manganese in groundwater within the gold-belt zone of Ghana. Journal of Water Supply: Research and Technology 57: 519 - 529.

Burger J, Gaines KF, Boring CS, Stephens WL, Snodgrass J, Dixon C, McMahon M, Shukla S, Shukla T, Gochfeld M (2002). Metal levels in fish from the Savannah River: potential hazards to fish and other receptors. Environmental research 89(1): 85 - 97.

Calamari D, Naeve H. (1994). Review of pollution in the African aquatic environment. Committee for Inland Fisheries of Africa (CIFA) Technical Paper No. 25, FAO, Rome, 118

Chapman DV, World Health Organisation (1996). Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring / edited by Deborah Chapman, 2nd ed. London: E and FN Spon. http://www.who.int/iris/handle/10665/41850.

Chigor VN, Sibanda T, Okoh AI (2013). Variations in the physicochemical characteristics of the Buffalo River in the Eastern Cape Province of South Africa. Environmental monitoring and assessment 185(10): 8733 - 8747.

Chikoto T (2009). Informal Settlements in South Africa. A Research Report Submitted in fulfilment of part of the requirements for the Degree of BSc (Hons) (Quantity Surveying). In the faculty of Engineering, Built environment of Information Technology, University of Pretoria.

Chindah A, Braide A, Sibuendu O (2004). Distribution of hydrocarbons and heavy metals in sediment and a Crustacean (*Shrimps-Penaeus notialis*) from the bonny/new Calabar River estuary, Niger Delta. African Journal of Environmental Assessment and Management 9:1 - 14.

Chinedu SN, Nwinyi OC, Oluwadamisi AY, Eze VN (2011). Assessment of water quality in Canaanland, Ota, Southwest Nigeria. Agriculture and Biology Journal of North America 2(4):577 - 583.

Chirisa I, Bandauko E, Matamanda A, Mandisvika G (2017). Decentralized domestic wastewater systems in developing countries: the case study of Harare (Zimbabwe). Applied Water Science *7*(3): 1069 - 1078.

Cohen MD, Kargacin B, Klein CB, Costa M (1993). Mechanisms of chromium carcinogenicity and toxicity. Critical reviews in toxicology *23*(3): 255-281.

Corcoran E, Nellemann C, Baker E, Bos R, Osborn D, Savelli H (2010). Sick Water? The central role of wastewater management in sustainable development: A Rapid Response Assessment. United Nations Environment Programme (UNEP), Un-Habitat, Grid-Arendal.

Cornish GA, Kielen NC (2004). Wastewater irrigation–hazard or lifeline? Empirical results from Nairobi, Kenya and Kumasi, Ghana. Wastewater use in irrigated agriculture 69 - 79.

Dallas HF, Day JA (2004). The effect of water quality variables on aquatic ecosystems: a review. Pretoria: Reported to Water Research Commission by Freshwater Research Unit, University of Cape Town. WRC Report No. TT 224/04

Dan'azumi S, Bichi MH (2010). Industrial pollution and implication on source of water supply in Kano, Nigeria. International Journal of Engineering and Technology 10(1): 101 - 109.

De Villiers S, Thiart C (2007). The nutrient status of South African rivers: concentrations, trends and fluxes from the 1970's to 2005. South African Journal of Science 103: 343 - 349.

DEAT (2000).White paper on integrated pollution and waste management for South Africa: A policy on pollution prevention, Waste minimization, impact management and remediation, Department of Environmental Affairs and Tourism, 80.

Department of Environment (2001). Water Quality Data of Rivers Buriganga, Meghna, Balu, Shitalakhya and Jamuna. Department of Environment, Dhaka, Bangladesh.

Department of Water Affairs (1988). Evaporation map for Namibia. Report No: 11/1/8/1/H1, October 1988, p.11.

Department of water and environmental affairs (DWEA) (2009).Gauteng water for growth and department. Draft 1.1. March.

Department of water and forestry (1996). South African water quality guidelines. Domestic uses. 2nd edition. Department of water affairs and forestry, Pretoria. Volume 1.

Dhokpande SR, Kaware JP (2013). Biological methods for heavy metal removal–A review. International Journal of Engineering Science and Innovative Technology. 2(5): 304-309

Dixit R, Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D (2015). Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7(2): 2189 - 2212.

Du Plessis S, Smit B (2005). Economic Growth in South Africa since 1994. Stellenbosch Economic Working Papers: 1 / 2006. Bureau of Economic Research University of Stellenbosch.

Duruibe JO, Ogwuegbu MOC, Egwurugwu JN (2007). Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences 2(5): 112 - 118.

Eaton AD (2005). Standard Methods for the Examination of Water and Waste Water. 21 st Edn. American Public Health Association, Washington, ISBN. 0875530478. pp. 343 - 453.

Eberhard R, Robinson P (2003). Guidelines for the Development of National Water Policies and Strategies to support IWRM. Draft. SADC Water Sector Co-ordination Unit, Gaborone, Botswana.

Eckenfelder W, Wesley J (2000). Literature Review of Temperature Effects in Biological Wastewater Treatment, Boston.

Emfuleni Local Municipality Wastewater Risk Abatement Plan (2013). Emfuleni Local Municipal. https://en-wikipedia.org/wiki/Emfuleni_Local_Municipality Arial view of Study Area.

Ensink JH, Simmons RW, van der Hoek W (2004). Wastewater use in Pakistan: The cases of Haroonabad and Faisalabad. Wastewater use in irrigated agriculture: Confronting the livelihood and environmental realities 91 - 99.

Ethan JN, Richard WM, Michael GK (2003). The effect of an industrial effluent on an urban stream benthic community: water quality vs. habitat quality. Environmental Pollution 123 (1):1 - 13.

Fadiran AO, Mamba SM (2005). Analysis of nitrates and nitrites in some water and factory effluent samples from some cities in Swaziland. Bulletin of the Chemical Society of Ethiopia 19(1): 35 - 44.

Fakayode S (2005). Impact assessment of industrial effluent on water quality of the receiving Alaro River in Ibadan, Nigeria. African Journal of Environmental Assessment and Management 10:1 - 3.

FAO (2005). Irrigation in Africa in figures. AQUASTAT Survey – 2005. Water Report 29.FAO, Rome.

Faruqui N, Niang S, Redwood M (2004). Untreated wastewater reuse in market gardens: a case-study of Dakar, Senegal. In Wastewater Use in Irrigated Agriculture. Confronting the Livelihood and Environmental Realities, Scott CA, Faruqi NI, Raschid-Sally L (eds). CABI Publishing: Wallingford, UK 113 - 126.

Fazli MM, Soleimani N, Mehrasbi M, Darabian S, Mohammadi J, Ramazani A (2015). Highly cadmium tolerant fungi: their tolerance and removal potential. Journal of Environmental Health Science and Engineering 13(1): 19

Fergusson JE (1990). Heavy elements, chemistry. Environmental impact and health effect, Pergamon.

Fernández-Luqueño F, López-Valdez F, Gamero-Melo P, Luna-Suárez S, Aguilera-González EN, Martínez AI, García-Guillermo MDS, Hernández-Martínez G, Herrera-Mendoza R, Álvarez-Garza MA, Pérez-Velázquez IR (2013). Heavy metal pollution in drinking water-a global risk for human health: A review. African Journal of Environmental Science and Technology 7(7): 567 - 584.

Fonseca M, De Oliveira M, Arakaki L (2006). Removal of cadmium, zinc, manganese and chromium cations from aqueous solution by clay mineral. Journal of Hazardous Materials Science 137: 288 - 292.

Gbadebo AM, Taiwo AM, Eghele U (2010). Environmental impacts of drilling mud and cutting wastes from the Igbokoda onshore oil wells, Southwestern Nigeria. Indian Journal of Science and Technology 3(5): 504 - 510.

Ghazy SE, El-Asmy AA, EL-Nokrashy AM (2008) Separation of chromium (III) and chromium (VI) from environmental water samples using eggshell sorbent. Indian Journal of Science and Technology 1 (6): 1 - 7.Gray CA (1997). Field assessment of numerical impacts of coastal sewage disposalon fish larvae relative to natural variability. Environmental Biology of Fishes 50: 415 - 434.

Haferburg G, Kothe E (2010). Metallomics: lessons for metalliferous soil remediation. Applied microbiology and biotechnology 87(4): 1271 - 1280.

Haji HT (2011). Impact of Climate Change on Surface Water Availability in the Upper Vaal River Basin.(Doctorate Dissertation, Tshwane University of Technology).

Halder JN, Islam N (2015). Water Pollution and its Impact on the Human Health. Journal of Environment and Human 2(1): 2373 - 8332.

Hall J, Hodgson G, Kerr KG (2004). Provision of safe potable water for immune-compromised patients in hospital. Journal of Hospital Infections 58: 155 - 158.

Hammer, M.J., and M.J. Hammer, Jr., 2004. Water Quality. In: Water and Waste Water Technology. 5th Edn. New Jersey: Prentice-Hall 139 - 159.

Hanif HN, Bhatti Ali MA (2010). Heavy metal tolerance and biosorption potential of white rot fungi. Asian Journal of Chemistry 22: 335-345.

He ZL, Yang XE, Stoffella PJ (2005). Trace elements in agroecosystems and impacts on the environment. Journal of Trace elements in Medicine and Biology 19(2-3): 125 - 140.

Helmer R, Hespanhol I (1997). Wastewater as a resource. Chapter 4: Water Pollution Control: A Guide to the Use of Water Quality Management. United Nations Environment Program, the Water supply and Sanitation Collective Council, and the World Health Organization.

Hookoom M, Puchooa D (2013). Isolation and identification of heavy metals tolerant bacteria from industrial and agricultural areas in Mauritius. Current research in microbiology and biotechnology 1(3): 119 - 123.

Horan NJ (1990). Biological Wastewater Treatment Systems: Theory and Operation John Wiley & Sons. New York. 310p.

Hurse TJ, Connor MA (1999). Nitrogen removal from wastewater treatment lagoons. Water science and technology 39(6): 191 - 198.

Hutton M, Symon C (1986). The Quantities of Cadmium, Lead, Mercury and Arsenic Entering the U.K. Environment from Human Activities. Science of the Total Environment. 57: 129 - 150.

Ibeto CN, Okoye COB (2010). High levels of Heavy metals in Blood of Urban population in Nigeria. Research Journal of Environmental Sciences 4(4): 371 - 382.

Ibrahim S, Tayel I (2005). “Effect of Heavy Metals on Gills of Tilapia Ziilli Inhabiting the River Nile Water (Damietta Branch and El-Rahawey Drain),” Egypt Journal of Aquatic Biology & Fish (9)2:111- 128.

Igbinosa EO, Okoh AI (2009). Impact of discharge wastewater effluents on the physico-chemical qualities of a receiving watershed in a typical rural community. International Journal of Environmental Science and Technology 6(2): 175 - 182.

Igwilo IO, Afonne OJ, Maduabuchi UJM, Orisakwe OE (2006). Toxicological study of the Anam River in Otuocha, Anambra state, Nigeria. Archives of environmental and occupational health 61(5): 205 - 208.

Ilhan S, Nourbakhsh MN, Kiliçarslan S, Ozdag H (2004). Removal of chromium, lead and copper ions from industrial waste waters by Staphylococcus saprophyticus. Turkish Electronic Journal of Biotechnology 2(2): 50 - 57.

Jaafar R, Omar I, Jidon AJ, Wan-Khamizar BW, Siti-Aishah BM, Sharifah-Noor-Akmal SH (1993). Skin cancer caused by chronic arsenical poisoning: A report of three cases. Medical Journal of Malaysia 48: 86 - 92.

Jaishankar M, Mathew BB, Shah MS, Murthy KTP, Gowda KRS (2014). Biosorption of few heavy metal ions using agricultural wastes. Journal of Environment Pollution and Human Health 2(1): 1 - 6.

Jaji MO, Bamgbose O, Odukoya OO, Arowolo TA (2007). Water quality assessment of Ogun River, south west Nigeria. Environmental monitoring and assessment 133(1-3): 473 - 482.

Jakimska A, Konieczka P, Skóra K, Namieśnik J (2011). Bioaccumulation of Metals in Tissues of Marine Animals, Part I: the Role and Impact of Heavy Metals on Organisms. Polish Journal of Environmental Studies 20(5).

Jamatia A, Chakraborty S, Das D, Jamatia SK, Ray S, Das MK (2014). Evaluation of Physiochemical characteristics of disposed Rubber industry effluent: a case study of Bodhjungnagar industrial growth centre. IOSR Journal of Engineering. 4: 44 - 50.

Jenkins D, Richard M, Daigger G (2003). Manual on the Causes and Control of Activated Sludge Bulking and Foaming. 2nd edition. Lewis publishers, Boca.

Jimena MG, Roxana O, Catiana Z, Margarita H, Susana M, Ines-Isla M (2008). Industrial effluents and surface waters genotoxicity and mutagenicity evaluation of a river of Tucuman, Argentina. J. of hazardous Material 155(3): 403 - 406.

JMP (2012): Progress on Drinking Water and Sanitation – (2012) update. 60 p. http://www.wssinfo.org/fileadmin/user_upload/resources/

Jordaan K, Bezuidenhout CC (2013). The impact of physico-chemical water quality parameters on bacterial diversity in the Vaal River, South Africa. Water SA 39(3): 385 - 396.

Kamika I, Momba MN (2013). Assessing the resistance and bioremediation ability of selected bacterial and protozoan species to heavy metals in metal-rich industrial wastewater. BMC microbiology 13(1): 28.

Katole SB, Kumar P, Patil RD (2013). Environmental pollutants and livestock health: a review. Veterinary Research International 1(1): 1 - 13.

Keller S (2012). Water Pollution. http://www.sswm.info/content/water-pollution.

Keraita B, Drechsel P, Huibers FP, Raschid-Sally L (2002). Wastewater use in informal irrigation in urban and periurban areas of Kumasi, Ghana. Urban Agriculture Magazine 8: 11 - 13.

Khanijo I (2002). Nutrient removal from waste water by wetland system. Wetlands 10.

Khraisheh MA, Al-degs YS, Mcminn WA (2004). Remediation of wastewater containing heavy metals using raw and modified diatomite. Chemical Engineering Journal 99(2): 177 - 184.

Kott Y, Roze N, Sperber S, Betzer N (1974). Bacteriophages as viral pollution indicators. Water research 8(3): 165 - 171.

Kraemer A, Choudhury K, Kampa E (2001). Protecting Water Resources: Pollution Prevention. (Thematic Background Paper). International Conference on Freshwater in Bonn.

Krishna AK, Govil PK (2004). Heavy metal contamination of soil around Pali industrial area, Rajasthan, India. Environmental Geology 47(1): 38 - 44.

Krishnani K, Meng X, Christodoulatos C, Boddu V (2008). Biosorption mechanism of nine different heavy metals onto biomatrix from rice husk. Journal of Hazardous Materials Science 153:1222 - 1234.

Kumar PR, Pinto LB, Somashekar RK (2010). Assessment of the efficiency of sewage treatment plants: a comparative study between nagasandra and mailasandra sewage treatment plants. Kathmandu university journal of science, engineering and technology 6(2): 115 - 125.

Lahnsteiner J, Lempert G (2007). Water management in windhoek, namibia. Water Science and Technology *55*(1 - 2): 441 - 448.

Lakherwa D (2014) Adsorption of heavy metals: a review. International Journal of Environmental Research 4(1): 2249 - 3131

Larsdotter K (2006). Microalgae for phosphorus removal from wastewater in a Nordic climate (Doctoral dissertation from School of Biolotechnology, Royal Institute of Technology, Stockholm, Sweden).

Lenntech Water Treatment and Air Purification (2004). Water Treatment, Published by Lenntech, Rotterdamseweg, Netherlands.

Lenntech WT (2009). Chemical Properties, Health and Environmental Effects of Copper. Lenntech Water Treatment and Purification Holding B.V. www.lenntech.com/periodic/elements/cu .htm.

Lim SS, Lee DS, Lee SI, Park HJ, Kwak JH, Choi WJ (2010). Reduction of ammonia volatilization and nitrogen loss by addition of zeolite and phospho-gypsum to cattle manure mixed with rise hull and sawdust as bedding materials. In *The 9th International* Conference of the East and Southeast Asia Federation of Soil Science Societies 434 - 435.

Lofrano G, Brown J (2010). Wastewater management through the ages: A history of mankind. Science of the Total Environment 408(22): 5254 - 5264.

Longe EO, Omole DO (2008). Analysis of pollution status of river Illo, Ota, Nigeria. The Environmentalist 28(4): 451 - 457.

Madsen H, Poultsen L, Grandjean P (1990). Risk of high copper content in drinking water. Ugeskr. Laeger. 152 (25): 1806-1809.

Madyiwa S (2006). Modelling the lead and cadmium uptake by star grass under irrigation with treated wastewater. PhD Thesis. University of Pretoria, Pretoria, South Africa.

Mahlaka R (2015). Vaal River City, a new riverfront development pushing boundaries: A mixed-income and high-development emerges. Density. http:// www. moneyweb.co.za/investing/property/vaal-river-city-new-riverfront-development-pushing-bound.

Mahlambi MM, Ngila CJ, Mamba BB (2015). Recent developments in environmental photocatalytic degradation of organic pollutants: the case of titanium dioxide nanoparticles—a review. Journal of Nanomaterials 2015: 5 - 24.

Maila MP, Cloete TE (2005). The use of biological activities to monitor the removal of fuel contaminants—perspective for monitoring hydrocarbon contamination: a review. International Biodeterioration and Biodegradation 55(1):1 - 8.

Mamabolo MF (2012). Effects of cooperative governance in the sewage treatment works in the upper Vaal River (Doctoral dissertation, North-West University).

Mann US, Dhingra A, Singh J (2014). Water Pollution: Causes, Effects And Remedies. International Journal of Advanced Technology in Engineering and Science*:* 02(08): 70 - 74.

Manyatshe AI, Fosso-Kankeu E, van der Berg D, Lemmer N, Waanders F, Hlanganani T (2016). Assessment of seasonal variations in surface water quality of the Mooi and Vaal rivers network, South Africa. International Conference on Advances in Science, engineering, Technology and Natural Resources (ICASETNR-16): Nov 24-25.

Marcovecchio JE, Botté SE, Freije RH (2007). Heavy metals, major metals, trace elements. Handbook of water analysis 2: 275 - 311.

Markandya A (2004). Water quality issues in developing countries. World Bank and University of Bath 1 - 33.

Martin S, Griswold W (2009). Human health effects of heavy metals. Environmental Science and Technology briefs for citizens 15:1 - 6.

Mateo-Sagasta J, Raschid-Sally L, Thebo A (2015). Global wastewater and sludge production, treatment and use. In Wastewater 15 - 38. Springer Netherlands.

Matowanyika W (2011) Impact of Alexandra Township on the water quality of the Jukskei River. A research report submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science.

Mbalassa M, Bagalwa JJM, Nshombo M, Kateyo ME (2014). Assessment of physicochemical parameters in relation with fish ecology in Ishasha River and Lake Edward, Albertine Rift Valley, East Africa. International Journal of Current Microbiology and Applied Sciences: 3(6): 230 - 244.

McCulloch WL, Goodfellow Jr WL, Black JA (1993). Characterization, identification and confirmation of total dissolved solids as effluent toxicants. Environmental. Toxicology. Risk Assessment, 2: 213 - 227.

McKee JE, Wolf HW (1963). Water Quality Criteria, State Water Quality Control Board, Sacramento, Calif. Publication, No. 3-A, 93.

Mejáre M, Bülow L (2001). Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. TRENDS in Biotechnology 19(2): 67 - 73.

Mema V (2010). Impact of poorly maintained waste water and sewage treatment plants: Lessons from South Africa. ReSource 12: 60 - 65.

Metcalf L, Eddy HP (1972). Wastewater engineering collection treatment disposal. McGraw-Hill Interamericana.

Mills-Knapp S, Traore K, Ericson B, Keith J, Hanrahan D, Caravanos J (2012). The World's Worst Pollution Problems: Assessing the Health Risks at Hazardous Waste Sites. Blacksmith Institute, New York.

Milner MJ, Kochian LV (2008). Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system. Annals of botany 102(1): 3 - 13.

Mocuba JJ (2010). Dissolved Oxygen and Biochemical Oxygen Demand in the waters close to the Quelimane sewage discharge (Master's thesis, The University of Bergen).

Mohod CV, Dhote J (2013). Review of heavy metals in drinking water and their effect on human health. International Journal of Innovative Research in Science, Engineering and Technology 2(7): 2992 - 2996.

Momba MN, Sibewu M, Mandeya A (2009). Survival of somatic and F-RNA coliphages in treated wastewater effluents and their impact on viral quality of the receiving water bodies in the Eastern Cape Province-South Africa. Journal of Biological Sciences 9(7): 648 - 654.

Morrison G, Fatoki OS, Persson L, Ekberg A (2001). Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment Plant on the Keiskamma River-pH, electrical conductivity, oxygen-demanding substance (COD) and nutrients. Water SA: 27(4):475 - 480.

Morrison GL, Anderson T, Behnia M (2004). Seasonal performance rating of heat pump water heaters. Solar Energy *76*(1-3):147 - 152.

Mostert E (2009). International co-operation on Rhine water quality 1945–2008: An example to follow? Physics and Chemistry of the Earth, Parts A/B/C, 34(3): 142 - 149.

Movahedian H, Bina B, Asghari GH (2005). Toxicity evaluation of wastewater treatment plant effluents using Daphnia magna. Journal of Environmental Health Science and Engineering 2(2): 1 - 4.

Mtetwa S (1996). Effluent and wastewater standards in Zimbabwe. Lake Chivero. A Polluted Lake. University of Zimbabwe Publications, Harare, Zimbabwe 124 - 133.

Mukherjee S, Nelliyat P (2006). Ground Water Pollution and Emerging Environmental Challenges of Industrial Effluent Irrigation: A Case Study of Mettupalayam Taluk, Tamilnadu. Available at SSRN: https://ssrn.com/abstract=1021153 or http://dx.doi.org/10.2139/ssrn.1021153

Muneer M, Bhatti IA, Adeel S (2010). Removal of Zn, Pb and Cr in textile wastewater using rice husk as a biosorbent. Asian Journal of Chemistry 22: 7453 - 7459.

Murray K, Du Preez M, Van Ginkel C (2000). National Eutrophication Monitoring Programme. Implementation Manual Draft. Water Research Commission, Pretoria.

Naidoo S, Olaniran AO (2014). Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources. International Journal of Environmental Research and Public Health 11: 249 - 270.

Nasrullah RN, Bibi H, Iqbal M, Durrani MI (2006). Pollution load in industrial effluent and ground water of Gadoon Amazai Induatrial Estate (GAIE) Swabi, NWFP. Journal of agricultural and biological science 1(3): 18 - 24.

Nazir R, Khan M, Masab M, Rehman HU, Rauf NU, Shahab S, Ameer N, Sajed M, Ullah M, Rafeeq M, Shaheen Z (2015). Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. Journal of Pharmaceutical Sciences and Research 7(3): 89.

Nhapi I, Gijzen H (2002). Wastewater Management in Zimbabwe. Proceedings of the 28th WEDC Conference on sustainable environmental sanitation and water services. Kolkata, India, 2002.

Nies DH (2003). Efflux-mediated heavy metal resistance in prokaryotes. FEMS microbiology reviews 27(2-3): 313 - 339.

Nies DH, Silver S (1995). Ion efflux systems involved in bacterial metal resistance. Journal of industrial microbiology. 14(2): 186-199.

Njoku KL, Akinola MO, Oboh BO (2009). Phytoremediation of crude oil contaminated soil: the effect of growth of Glycine max on the physico-chemistry and crude oil contents of soil. Nature and Science 7(10): 79 - 87.

Norseth T (1981). The carcinogenicity of chromium. Environmental health perspectives 40: 121.

Nriagu JO (1989). A global Assessment of Natural Sources of Atmospheric Trace Metals. Nature 338: 47 - 49.

Ntuli F (2012). Management and Control of Industrial Effluents Discharged to Public Sewers: A Case Study. World Academy of Science, Engineering and Technology, 6(8): 121 - 126.

Oarga A (2009). Life in extreme environments. Revista de Biologia e ciencias da Terra 9(1):1 - 10.

Obodo GA (2004). The bioaccumulation of heavy metals in fish from Anambra River. Journal of Chemical Society of Nigeria 29(1): 60 - 62.

Odjadjare EE, Okoh AI (2010). Physicochemical quality of an urban municipal wastewater effluent and its impact on the receiving environment. Environmental monitoring and assessment 170(1-4): 383 - 394.

Odjegba VJ, Bamgbose NM (2012). Toxicity assessment of treated effluents from a textile industry in Lagos, Nigeria. African Journal of Environmental Science and Technology 6(11): 438 - 445.

Odjegba VJ, Bamgbose NM (2012). Toxicity assessment of treated effluents from a textile industry in Lagos, Nigeria. African Journal of Environmental Science and Technology *6*(11): 438 - 445.

Odoemelam SA (2005). Bioaccumulation of trace elements in fish from Oguta Lake. Journal of the Chemical Society Nigeria 30: 18 - 20

Ogunfowokan AO, Okoh EK, Adenuga AA, Asubiojo OI (2005). An assessment of the impact of point source pollution from a university sewage treatment oxidation pond on a receiving stream – a preliminary study. Journal of Applied Sciences 5(1):36 - 43.

Ogunleye IO, Izuagie AA (2013). Determination of heavy metal contents in some industrial effluents from Ondo State, Nigeria. Journal of Environmental Chemistry and Ecotoxicology 5(8): 216 - 219.

Okoh AI, Odjadjare EE, Igbinosa EO, Osode AN (2007). Wastewater treatment plants as a source of microbial pathogens in receiving watersheds. African Journal of Biotechnology 6(25).

Okonkwo JO (2010). Sanitation, Potable Water Supply and Environmental Protection. Benefits, Progress and Issues for Sustainability in Developing Countries. Aisa Policy brief Number 23.

Olafisoye OB, Adefioye T, Osibote OA (2013). Heavy metals contamination of water, soil, and plants around an electronic waste dumpsite. Polish Journal of Environmental Studies 22(5): 1431 - 1439.

Olaniyi I, Odoh R, Onyebuchi NJ (2012). Effect of industrial effluent on the surrounding environment. Archives of Applied Science Research 4(1): 406 - 413.

Olowu RA, Ayejuyo OO, Adewuyi GO, Adejoro IA, Denloye AAB, Babatunde AO, Ogundajo AL (2010). Determination of heavy metals in fish tissues, water and sediment from Epe and Badagry Lagoons, Lagos, Nigeria. Journal of Chemistry 7(1): 215 - 221.

Opelo RJ, Little CJP (2004). Strategic development for the wastewater and sanitation sector of Botswana.

Organisation for Economic Co-operation and Development (OECD) (2008).OECD Environmental data: Compendium 2006 - 2008.

Osman AG, Kloas W (2010). Water quality and heavy metal monitoring in water, sediments, and tissues of the African catfish Clarias gariepinus (Burchell, 1822) from the river Nile, Egypt. Journal of Environmental Protection 1(4): 389 - 400.

Oven M, Saghir KM, Huda QA, Nadeen FM, Almeelbi T (2016). Heavy metals: Biological importance and detoxification strategies. Journal of Bioremediation & Biodegradation 7: 1 - 15.

Owa FW (2014). Water pollution: sources, effects, control and management. International Letters of Natural Sciences 3.

Pacific Institute (2010): World Water Quality Facts and Statistics. Oakland: Pacific Institute.

Pandey S, Rai R, Rai LC (2012). Proteomics combines morphological, physiological and biochemical attributes to unravel the survival strategy of *Anabaena* sp. PCC 7120 under arsenic stress. Journal of proteomics 75(3): 921 - 937.

Paranychianakis NV, Salgot M, Snyder SA, Angelakis AN (2015). Water reuse in EU states: necessity for uniform criteria to mitigate human and environmental risks. Critical Reviews in Environmental Science and Technology 45(13): 1409 - 1468.

Parkman M, Patchett J, Odongo O (2008). Kisumu Water Supply and Sanitation Project, Long Term Action Plan: Water Design Report, Lake Victoria South Water Services Board, Kisumu, Kenya.

Patil PN, Sawant DV, Deshmukh RN (2012). Physico-chemical parameters for testing of water-A review. International Journal of Environmental Sciences, 3(3): 1194 - 1207.

Peplow D (1999). Environmental impacts of mining in Eastern Washington. University of Washington Water Center.

Piddock LJ (2006). Multidrug-resistance efflux pumps? Not just for resistance. Nature Reviews Microbiology 4(8): 629 - 636.

projections, and key challenges for sustainability. Technology in Society 28(1-2): 63–80.

Prüss A, Kay D, Fewtrell L, Bartram J (2002). Estimating the burden of disease from water, sanitation, and hygiene at a global level. Environmental health perspectives 110(5): 537.

Qadir M, Sharma BR, Bruggeman A, Choukr-Allah R, Karajeh F (2007). Non-conventional water resources and opportunities for water augmentation to achieve food security in water scarce countries. Agricultural water management 87(1): 2 - 22.

Qasem A (2011). Performance Assessment Model for Wastewater Treatment Plants (Doctoral dissertation, Concordia University).

Raja R, Hemaiswarya S, Kumar NA, Sridhar S, Rengasamy R (2008). A perspective on the biotechnological potential of microalgae. Critical reviews in microbiology 34(2): 77 - 88.

Rajaram T, Das A (2008). Water pollution by industrial effluents in India: discharge scenario and case for participatory ecosystem specific local regulation. Futures 40(1): 56 - 69.

Rajendran P, Muthukrishnan J, Gunasekaran P (2003). Microbes in heavy metal remediation. Indian Journal of Experimental Biology. 41: 935 - 944.

Rao MS, Gopalkrishnan R, Venkatesh BR (2001). Medical geology an emerging field in environmental science. In National symposium on role of earth sciences. Integrated and Related Social issues GSI Special. Publication No.65 (II).

Re V, Faye SC, Faye A, Faye S, Gaye CB, Sacchi E, Zuppi GM (2011). Water Quality Decline in Coastal Aquifers under Anthropic Pressure: The Case of a Suburban Area of Dakar (Senegal), Environmental Monitoring and Assessment 172 (1 - 4): 605 - 622.

Röllin HB, Rudge CV, Thomassen Y, Mathee Odland J.Ø (2009). Levels of toxic and essential metals in maternal and umbilical cord blood from selected areas of South Africa—results of a pilot study. Journal of Environmental Monitoring 11(3): 618 - 627.

Ryan MP, Adley CC (2010). *Sphingomonas paucimobilis*: a persistent Gram-negative nosocomial infectious organism. Journal of Hospital Infection 75(3): 153 - 157.

SAGES (2013). Requirements for the Purification of Wastewater or Effluent. Gazette No 9225, Regulation No 991, 18 May.

Samad M, Mahmud Y, Adhikary R, Rahman S, Haq M, Rashid H (2015). Chemical profile and heavy metal concentration in water and freshwater species of Rupsha River, Bangladesh. American Journal of Environmental Protection 3(6): 180 - 186.

Samad M, Mahmud Y, Adhikary R, Rahman S, Haq M, Rashid H (2015). Chemical profile and heavy metal concentration in water and freshwater species of Rupsha River, Bangladesh. American Journal of Environmental Protection *3*(6): 180-186.

SANCOR (1984). Report of the ad hocworking committee. Lusher JA (Ed.). Established by the Marine Pollution Committee of the South African National

Committee for Oceanographic Research, South African Network for Coastal and Oceanic Research, 25.

Sandeep KP, Shweta T (2008). Physico-chemical analysis of ground water of selected area of Ghazipur city- A case study. Nature and Science 6(4): 25 - 28.

Sangeeta P, Neha P (2015). Monitoring of Seasonal Variation in Physicochemical Water Parameters in Nalasopara Region. Journal of Ecosystem and Ecography 5(1): 1.

Santona L, Castaldi P, Melis P (2006). Evaluation of the interaction mechanisms between red muds and heavy metals. Journal of Hazardous Materials *136*(2): 324-329.

Sato T, Qadir M, Yamamoto S, Endo T, Zahoor A (2013). Global, regional, and country level need for data on wastewater generation, treatment, and use. Agricultural Water Management 130: 1 - 13.

Savenije HH, Van der Zaag P (2000. Conceptual framework for the management of shared river basins; with special reference to the SADC and EU. Water policy *2*(1):9 - 45.

Savini V, Catavitello C, Bianco A, Balbinot A, D'antonio D (2009). Epidemiology, pathogenicity and emerging resistances in *Staphylococcus pasteuri*: from mammals and lampreys, to man. Recent patents on anti-infective drug discovery 4(2): 123 - 129.

Seanego KG (2014). Ecological Status of the Sand River after the Discharge of Sewage Effluent from the Polokwane and Seshego Wastewater Treatment Works (Doctorate Dissertation, University of Limpopo).

Sedibeng District Municipality (2010). Integrated Development Plan. Vereeniging: SDM.

Sekhar KC, Chary NS, Kamala CT, Raj DS, Rao AS (2004). Fractionation studies and bioaccumulation of sediment-bound heavy metals in Kolleru Lake by edible fish. Environment International 29(7): 1001 - 1008.

Sharma S, Dastidar MG, Sreekrishnan TR (2003). Biological removal of zinc from wastewater using Aspergillus sp. European Journal of Mineral Processing and Environmental Protection 3(1):1 - 8.

Shun-Xing L, Hua-Sheng H, Feng-Ying Z, Nan-Sheng D, Fang L (2007). Influence of nitrate on metal sorption and bioaccumulation in marine phytoplankton, *Dunaliella salina*. Environmental toxicology 22(6): 582 - 586.

Sibanda T, Chigor VN, Koba S, Obi CL, Okoh AI (2014). Characterisation of the physicochemical qualities of a typical rural-based river: ecological and public health implications. International Journal of Environmental Science and Technology 11(6): 1771 - 1780.

Sibeya C (2016). Assessment of the Performance of Wastewater Treatment Plants: A case study of Gammams and Otjomuise Wastewater Treatment Plants in Windhoek, Namibia. (Masters Dissertation, University of Zimbabwe).

Silver S (1996). Bacterial resistances to toxic metal ions – a review. Gene 179: 9 - 19.

Snyman HG, Van Niekerk AM, Rajasakran N (2006). Sustainable wastewater treatment–what has gone wrong and how do we get back on track. In Proceedings of the water Institute of South Africa (WISA) Conference. 21 -25 May, Durban.

Stanbridge HH (1976). History of Sewage Treatment in Britian. 5. Land Spreading. Institute of Water Pollution Control, Maidstone, Kent, United Kingdom.

Stolberg F, Borysova O, Mitrofanov I, Barannik V, Eghtesadi P (2006). Global international waters assessment Caspian Sea, regional assessment report, Sweden: University of Kalmar.

Strauss M, Blumenthal UJ (1990). Human waste use in agriculture and aquaculture. Utilization, practices and health perspectives, executive summary. International Reference Center for Waste Disposal Report 9: 90.

Strydom JP, Britz TJ, Mostert JF (1997). Two-phase anaerobic digestion of three different dairy effluents using a hybrid bioreactor. Water SA *23*(2): 151 - 156.

Subhashini V, Swamy AVVS, Hema KR (2003). Phytoremediation emerging and technology for the uptake of cadmium the contaminated soil by plant species. International Journal of Environmental Research 4: 0976 - 4402.

Sukumaran D, Saha R, Saxena RC (2015). Performance Evaluation of Prevailing Biological Wastewater Treatment Systems in West Bengal, India. 3(1):1 - 4.

Syed Zaghum Abbas, Mohd Rafatullah, Norli Ismail and Japareng Lalung (2014). Isolation, Identification, characterization, and evaluation of cadmium removal capacity of Enterobacter specie. Journal of Basic Microbiology 54: 1279 - 1287.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012). Heavy metal toxicity and the environment. In Molecular, clinical and environmental toxicology133 - 164. Springer, Basel.

Tebbutt THY (1983). Relationship between natural water quality and health. Paris: UNESCO.

Tempelhoff J, Munnik V, Viljoen M (2007). The Vaal River Barrage, South Africa's hardest working water way: an historical contemplation. TD: The Journal for Transdisciplinary Research in Southern Africa 3(1):107 - 133.

Tempelhoff JW (2006). Water and the human culture of appropriation: the Vaal River up to 1956. TD: The Journal for Transdisciplinary Research in Southern Africa 2(2): 431 - 452.

Teplitski M, Eberhard A, Gronquist MR, Gao M, Robinson JB, Bauer WD (2003). Chemical identification of N-acyl homoserine lactone quorum-sensing signals produced by Sinorhizobium meliloti strains in defined medium. Archives of microbiology 180(6): 494 - 497.

The World Water Organization (TWWO) (2010). Water Facts and Water Stories from Across the Globe.”

Thebe TA, Mangore EN (2012). Wastewater production, treatment, and use in Zimbabwe. Department of Civil and Water Engineering, National University of Science and Technology, Bulawayo.

Topare NS, Attar SJ, Manfe MM (2011). Sewage/wastewater treatment technologies: a review. Scientific Reviews and Chemical Communications 1(1).

Tshwane University of Technology (2009). Integrated water management of the Vaal River catchment. Pretoria: Civil Engineering..

Tyagi VK, Chopra AK, Durgapal NC, Kumar A (2007). Evaluation of Daphnia magna as an indicator of toxicity and treatment efficacy of municipal sewage treatment plant. Journal of Applied Sciences and Environmental Management 11(1).

Uaboi-Egbenni PO, Okolie PN, Martins O, Teniola O (2010). Studies on the occurrence and distribution of heavy metals in sediments in Lagos Lagoon and their effects on benthic microbial population. African Journal of Environmental Science and Technology 4(6).

Ujang Z, Buckley C (2002). Water and wastewater in developing countries: present reality and strategy for the future. Water Science and Technology 46(9):1 - 9.

United Nation (2012). Population connected to wastewater collecting system.

UN Department of Economic and Social Affairs, Division for Sustainable Development (UNDESA-DSD), (2004). Sanitation Country Profile: Russian Federation.

UNECE (2003). Environmental Performance Reviews: Georgia. UN Publication, NewYork and Geneva.

UNEP (2008). UN collaborative programme on reducing emissions from deforestation and forest degradation in development countries (UN-REDD) framework document; van der werf GR, Morton DC, DeFries RS, Olivier JG, Kasibhatla PS, Jackson RB, Collatz JG, Randerson JT 737 - 738.

United Nations Environmental Protection/Global Program of Action (2004). Why The Marine Environment Needs Protection from Heavy Metals, Heavy Metals 2004, UNEP/GPA Coordination Office.

United Nations World Water Assessment Programme (2003). The World Water Development Report 1: Water for People, Water for Life. UNESCO: Paris, France.

US Department of Health and Human Services (2008). Agency for Toxic Substances and Disease Registry. Toxic substance portal: Formaldehyde. 4770 Buford Hwy NE, Atlanta, GA 30341

United State Environmental Protection Agency (USEPA) (2004). Guidelines for Water Reuse. EPA/625/R-04/108, USEPA, Washington, DC.

United State. Environmental Protection Agency (1996). Inductively Coupled Plasma-Atomic Emission Spectrophotometry, Method 6010B, Revision 2.0, SW-846 Manual, 3rd edition, Office of Solid Waste and Emergency Response

USEPA (1992). U.S. Environmental Protection Agency, Office of Groundwater and Drinking Water. Status Report on the Development of Draft MCLGs for Disinfectants and By-Products. Washington, D.C.

USEPA (1995). Technical guidance manual for determining total maximum daily loads. Book II: streams and rivers, part I; BOD, DO and Nutrients/Eutrophication.

Vaishali P, Punita P (2013). Assessment of seasonal variation in water quality of River Mini, at Sindhrot, Vadodara. International journal of environmental sciences 3(5): 1424.

Valavanidis A, Vlachogianni T (2010). Metal pollution in ecosystems. Ecotoxicology studies and risk assessment in the marine environment. Department. of Chemistry, University of Athens University Campus Zografou, 15784.Athens, Greece.

Van der Hoek W (2004). A framework for a global assessment of the extent of wastewater irrigation: the need for a common wastewater typology. Wastewater use in irrigated agriculture: Confronting the livelihood and environmental realities. Wallingford, UK: CABI Publishing11 - 24.

Van Vuuren L (2009). The state of water in South Africa – Are we heading for a crisis? The Water Wheell 8(5): 31 - 33.

Varol M, Şen B (2012). Assessment of nutrient and heavy metal contamination in surface water and sediments of the upper Tigris River, Turkey. Catena 92: 1 - 10.

Verandani S, Vardhan D (2012). Study of groundwater quality of Ulhasnagar city in Thane district, Maharashtra, India using multivariate and microbial analysis. Research Journal of Chemistry and Environment 16: 58 - 64.

Vodela JK, Renden JA, Lenz SD, McElhenney WH, Kemppainen BW (1997). Drinking water contaminants (arsenic, cadmium, lead, benzene, and trichloroethylene). Interaction of contaminants with nutritional status on general performance and immune function in broiler chickens. Poultry Science 76(11): 1474 - 1492.

Vodela JK, Renden JA, Lenz SD, MchelHenney WH, Kemppainen BW (1997). Drinking water contaminants. Poultry Science 76: 1474 - 1492.

Volesky B (1990). Biosorption and biosorbents. In biosorption of heavy metals, CRC press, Florida 3 - 6.

Volesky B, Naja G (2007). Biosorption technology: starting up an enterprise. International Journal of Technology Transfer and Commercialisation 6:196 - 211.

Waisberg M, Joseph P, Hale B, Beyersmann D (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192(2): 95 - 117.

Wakelin SA, Macdonald LM, Rogers SL, Gregg AL, Bolger TP, Baldock JA (2008). Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. Soil Biology and Biochemistry 40(3): 803 - 813.

Wang J, Chen C (2006). Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. Biotechnology advances 24(5): 427 - 451.

Wang SL, Xu XR, Sun YX, Liu JL, Li HB (2013). Heavy metal pollution in coastal areas of South China: a review. Marine pollution bulletin 76(1): 7 - 15.

Water Resource Department of Land and Water Bureau (WRDLWB), Ministry of Land, Infrastructure, Transport and Tourism, Japan (2012). Water Resources in Japan, 2012.

Water wheel (2009). The state of water in South Africa. Water wheel 8(5): 31 - 33.

Watson C, Pulford ID, Riddell-Black D (2003). Screening of willow species for resistance to heavy metals: comparison of performance in a hydroponics system and field trials. International Journal of Phytoremediation 5(4): 351 - 365.

WHO (1982). Rapid assessment of sources of air, water and land pollution, WHO offset Publication, No. 62, England.

World Bank (2012). The World Bank data; GNI per capita, Atlas method (current US$).

Yabuuchi E, Kosako Y (2015). "Sphingomonas". Bergey's Manual of Systematics of Archaea and Bacteria. John Wiley and Sons.

Yakimov MM, Golyshin PN, Lang S, Moore ER, Abraham WR, Lünsdorf H, Timmis KN (1998). *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. International Journal of Systematic and Evolutionary Microbiology 48(2): 339 - 348.

Yi L, Jiao W, Chen X, Chen W (2011). An overview of reclaimed water reuse in China. Journal of Environmental Sciences, *23*(10): 1585 - 1593.

Zamxaka M, Pironcheva G, Muyima NYO (2004). Microbiological and physico-chemical assessment of the quality of domestic water sources in selected rural communities of the Eastern Cape Province, South Africa. Water SA 30(3): 333 - 340.