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**EVALUATION OF THE QUALITY INDICES OF THE FINAL EFFLUENTS
OF TWO WASTEWATER TREATMENT PLANTS IN BUFFALO CITY
METROPOLITAN MUNICIPALITY IN THE EASTERN CAPE PROVINCE**

BY

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DECLARATION

I, the undersigned, declare that this thesis submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Microbiology in the Faculty of Science and Agriculture, School of Biological and Environmental Sciences, and that the work contained herein is my original work with exemption to the citations and that this work has not been submitted at any other University in partial or entirety for the award of any degree.

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DEDICATION

This Project is dedicated to God Almighty for making my dream for academic excellence a reality in my life. I appreciate and bless Your Holy name.

LIST OF ABBREVIATION

- Al³: Aluminum ion
- AlCl₃: Aluminum Chloride
- BNR: Biological Nutrient Removal
- BOD: Biological Oxygen Demand
- BOD₅: Five-day biochemical oxygen demand
- cDNA: complementary DNA
- CFU: Colony-forming unit
- ClO₂: Chlorine Dioxide
- COD: Chemical Oxygen Demand
- Ct: Concentration & Contact time
- DEPC: Diethylpyrocarbonate
- DNA: Deoxyribonucleic acid
- dNTP: Deoxyribonucleotide
- DO: Dissolved Oxygen
- dsRNA: Double-stranded RNA
- F/M: Food to Microorganism
- Floc: Flocculation
- H₂SO₄: Sulfuric acid
- Hadv: Human Adenovirus
- LB: Luria broth
- mS/m: Millisiemens/meter
- N₂: Nitrogen gas
- NaCl: Sodium chloride

NaOH: Sodium Hydroxide

NH₃: Ammonia

NH₃⁺: Ammonia

NO₂⁻: Nitrites

NO₃⁻: Nitrate

NSF: non-sorbitol fermenters

NTU: Nephelometric Turbidity Units

OCl: Hypochlorite ions

PCR: Polymerase Chain Reaction

ppm: Parts per million

RAS: Return Activated Sludge

RNA: Ribonucleic acid

rRNA: ribosomal RNA

TCBS: Thiosulphate Citrate Bile Salt Sucrose agar

TSS: Total Suspended Solids

WWTPs: Wastewater Treatment Plants

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ABSTRACT

Wastewaters can be sources of pollution to surface water and the environment with severe implications for public health. Most treatment plants in the Buffalo City Municipality in the Eastern Cape Province discharge their treated effluent into the surface waters which directly and indirectly impacts on the quality of surface waters in the region. The objective of this study was to determine the microbiological and physicochemical qualities of the final effluents of two wastewater treatment plants in the Buffalo City Municipality in the Eastern Cape Province of South Africa over a period of 12 months (September 2012 to August 2013).

The qualities of the final effluents of WW-Ama Wastewater Treatment Plant with respect to phosphate (3.9 mg/l - 20.6 mg/l), free chlorine (0.05 mg/l - 0.71 mg/l), chemical oxygen demand (COD) (4.7 mg/l - 211 mg/l), and faecal coliform (0 - 2.92×10^4 CFU/100 ml) were not in compliance with the permissible limits set for effluent discharged to surface water by South Africa guidelines for effluent discharge. Other physicochemical parameters like biological oxygen demand (BOD) (2.2 mg/l - 9.0 mg/l), total dissolve solid (TDS) (253 mg/l - 336.3 mg/l) and turbidity (4.8 NTU - 43.20 NTU) with no SA regulatory set limits were compared to other regulatory standards and they do not comply with the limits. Also, at the second WWTP's, the WW-Dim Treatment Plant effluent quality for free chlorine (0.06 mg/l - 7.2 mg/l), BOD (0.1 mg/l - 7.4 mg/l), and turbidity (4.02 NTU - 24.3 NTU) also did not comply.

For microbiological qualities, counts of presumptive *E. coli* and *Vibrio* ranged between 0 - 2.92×10^4 CFU/100 ml and 0 - 9.93×10^3 CFU/100 ml for *E. coli* and *Vibrio*

respectively at the WW-Ama plant and $0 - 1.86 \times 10^4$ CFU/100 ml for and $0 - 1.44 \times 10^3$ CFU/100 ml for *E. coli* and *Vibrio* respectively at the WW-Dim plant. About 41.7% of the samples analysed for *E. coli* and 16.7% for *Vibrio* for WW-Ama plant complied with the set limit of 1000 CFU/100 ml while 83.3% (*E. coli*) and 91.7% (*Vibrio*) of the WW-Dim samples complied in with the set limit. Also, DNA of the confirmed *E. coli* and *Vibrio* isolates were used for confirmation of their identities using species specific polymerase chain reaction (PCR) methods. About 300 confirmed *E. coli* and *Vibrio* isolates were pathotyped. The prevalence of the *E. coli* pathotypes were of the following orders: uropathogenic *E. coli* (9%), enteroaggregative *E. coli* (3.7%), neonatal *E. coli* (1.7%) and enteropathogenic *E. coli* (0.7%). None of the targeted *Vibrio* pathotypes i.e *Vibrio parahaemolyticus*, *Vibrio fluvialis* and *Vibrio vulnificus* were detected in either plant suggesting that the confirmed isolates are members of other *Vibrio* species besides those targeted.

The incidences of enteric viruses in the final effluents were also investigated using real - time PCR. Target viruses included enteric viruses Adenovirus, hepatitis A virus (HAV), enterovirus, norovirus and rotavirus. Norovirus, enterovirus and hepatitis A virus were not detected in any sample from the treatment plants, while adenovirus and rotavirus were detected in all the plants with the WW-Ama plant having the highest detection rate and concentration of the viruses. The detection rate for Adenovirus was 67% for the WW-Ama Treatment Works and 17% for the WW-Dim samples; while for Rotavirus, the detection rate was 42% for WW-Ama and 17% for WW-Dim Sewage Treatment Works effluents.

Antibiogram of the bacterial isolates were determined using the disk diffusion method. A total of 107 confirmed *E. coli* and 100 confirmed *Vibrio* spp. were used for this assay. Results of antibiotic sensitivity test revealed that 63.6% of the *E. coli* isolates were resistance to ampicillin while 49.5% were resistant to tetracycline and cephalothin. The least resistances were observed against gentamicin (3.7%) and cefotaxime (1.9%). No resistance was observed against meropenem. For the *Vibrio* spp, resistance was most frequently observed against tetracycline (38%) ampicillin (26%), chloramphenicol (16%), cefotaxime (14%), trimethoprim-sulfamethoxazole (13%) and the least resistance observed was against ciprofloxacin (1%).

This study demonstrates that poorly treated wastewater effluent can be a source of eutrophic water with high nutrient levels and pathogenic bacteria and enteric viruses as well as antibiotic resistance determinants that could impact negatively on human health. The finding of this study also suggests that WWTPs have to be properly monitored and controlled to ensure compliance to set guidelines. This could be attained through the application of appropriate treatment processes, which will help to minimize possible dangers to public environment health.

CHAPTER ONE

INTRODUCTION

Freshwater is essential for the daily life of all aquatic and terrestrial organisms, including humans. Although water is normally a recyclable resource, it needs careful management and protection because of its vulnerability to over exploitation and pollution (Department of Environmental Affairs and Tourism, 2006). Water is an important resource for human survival and the need arises to monitor and protect this valuable gift (FAO, 1993). Every nation has made efforts to protect their various water bodies through water policies, monitoring and treatment (Stringer, 1997). The avoidance of contamination of water assets and the assurance of open wellbeing by defending water supplies against the spread of sicknesses, are the two principal purposes behind treating wastewater. This is refined by removing substances that contain a high demand for oxygen from the wastewater treatment system using physical, biological and chemical methods to make a tolerable quality of wastewater effluents before being released back to a receiving water body (Akpor and Muchie, 2011; Conservation Ontario, 2001).

Many, if not near all developing countries, nevertheless, have contaminated rivers and water courses that are environmentally deplorable because of their significant hazards to health. Thus, these countries, as an obligation, must reduce these grossly polluted water bodies, which requires the procurement of fundamental wastewater treatment amenities to overcome ecological degradation (Oliveira and von Sperling, 2011).

Water is progressively turning into a restricted asset in South Africa and the shortage of this asset influences national, local municipal and provincial growth in unfavourable territories. South Africa is a parched and semi-dry nation with high water push because of the low mean yearly precipitation (Ilemobade, Adewumi and Van Zyl, 2009). The strains exerted by human actions on the available quality water resource stems from industrial and mining activities. Mining can lead to increased salinity, change of pH (water acidity), high sediment load, and increased metal content. The contributions of industrial activities vary based on the industrial process; however, they can incorporate toxic and dangerous chemicals, with consequential elevated salinity, and decrease in water nutrients and increased dregs. Expanded urbanization and deterioration in the standard of wastewater management is also a contributing factor. Practically no treatment of wastewater happens in some places; for example, in casual communities where treatment is accessible, sewer reticulation might be lacking or defectively kept up, bringing about uncontrolled discharges through leakages and flooding to the environment. High organic and nutrient load from urban runoff contributes to urban water bodies such as the urban streams and impoundments. The outcome is elevated nutrient, microbial contamination and organic load. To forestall this situation calls for earnest activity for sufficient and enhanced urban wastewater treatment to curtail the harmful effect, as well as the expense arising from harm to the basic local water assets (DEAT, 2006).

Eddy (2003) confirmed that South African water resources are very limited, with demand expected to exceed supply by 2020. This will possibly lead to residential

pollution load associated with discharging putrefied sewage effluent into water bodies escalating to a point where the water environment will no longer be able to assimilate it adequately. At this point, natural purification processes will collapse and will result in a serious deterioration of water quality. To forestall this situation, wastewater treatment processes must be evaluated and ensure that standards are met before the waste water is discharged into the environment. With South Africa's effort to standardize effluent quality, general and special effluent standards were promulgated for all industries to comply with, except those in possession of exemption permits. Notwithstanding these efforts, however, South Africa's water resources quality and wastewater final effluent have continued to deteriorate (Department of Water Affairs, 2011; Eddy, 2003).

The dwindling state of the municipal wastewater and sewage treatment infrastructure in South Africa continues to constitute the greatest causative reasons for the various contamination issues faced in many regions of the country and a real threat to wellbeing issues in deprived communities (Mema, 2004). Presently, many wastewater works have attained their configuration limits. They are in a poor state and not appropriately working, bringing about significant wastewater spillages and related environmental and health hazards. Mass framework improvement, asset administration and water quality administration should be the priority intervention areas by the concerned authority (DWAF, 2008). Contamination of water assets happens either from the point-source discharges (for instance, releases from sewage works and industrial activities) or from dissolved contributions through air, surface overflow or land. *On-site* sanitation, for instance, can prompt distribution of a high level of contamination from nitrogenous

pollutant in groundwater and inadvertent spillage or waste discharge will constitute a grave danger. Littering of urban areas, spilling of dangerous material on a journey, inappropriate storage or mishandling are all examples of pollution. Pollution of surface waters is more visible and easier to detect than groundwater contamination, which is both harder to detect and to cure than surface water pollution. Sullyng of surface water bodies and aquifers by contaminated subsurface release, for instance, can easily be recognized as a problem (DEAT, 2006). According to the Green report (2011) most of the final effluents of the wastewater treatment plants of the municipality districts do not meet the set effluent standards. The treatment plants are said to be obsolete and need urgent upgrading (DWAF, 2011). The nature of wastewater effluents influences the debasement of the accepting water bodies. This is on account of the fact that untreated or insufficiently treated wastewater effluent can bring about eutrophication in the receiving water bodies and, furthermore, make ecological conditions that support expansion and spread of waterborne pathogens (Akpore and Muchie, 2011), including toxic organic and inorganic compounds (Crockett, 1997). Recreation water clients and any person coming into touch with the contaminated water are at risk, though the risk might be negligible where water is intended for landscape irrigation (National Research Council, 2012; Akpor and Muchie, 2011).

It is a common knowledge that various micro-organisms play many beneficial roles in wastewater systems (Akpore and Muchie, 2011) and are, therefore, useful in reducing volumes of sludge sewage effluent in either wastewater Treatment Plants or *on-site* wastewater treatment systems such as the Septic Tanks (Szymanski and Patterson,

2003). Studies have shown that there are a number of exceptional organisms believed to be dangerous in contributing to several waterborne disease epidemics (Akor, 2011). Wastewater effluents as a case in point have been indicated to hold a mixed bag of anthropogenic mixes, a hefty portion of which have endocrine-upsetting properties (Sowers, 2009). Faecal coliforms and more specifically *Escherichia coli* (*E. coli*) are the most commonly used bacterial indicators of faecal pollution. This indicator group is used to evaluate the quality of wastewater effluents, river, sea beaches, raw water for drinking water supply, treated drinking water, water used for irrigation, aquaculture and recreation water (DWAF: Department of Water Affairs, 1996e). The presence of *Escherichia coli* serves as an affirmation of the presence of faecal contamination by warm-blooded animals or faecal pollution which may not necessarily be of human origin. Other indicators employed for effluent quality testing include human enteric viruses which are also considered as indicators of faecal contamination (Ashbolt, Grabow and Snozzi, 2001).

Until recently, South Africa initiated a Green Drop certification programme for wastewater treatment works. The effort was to evaluate the performance of the wastewater treatment plants in the country to ensure that there is improvement in their services. Compliance to best practices often attracts a Green Drop status award (Henning, 2010). In many areas of South Africa, wastewater effluents from industrial, agricultural and domestic sources are discharged into the marine environment with little or no regulation. However, in the Eastern Cape of South Africa, the final destination of effluents from many regulated and non-regulated sources is the aquatic

environment. Quality water availability in the Eastern Cape, and in many other parts of the world, has become a concern as the demands on the natural water resources are on the increase (Keirungi, 2007). Environment pollution has increased radically, to the point that in many places the society can no longer cope with these pressures with the result that several economic activities have become threatened (Zamxaka, Pironcheva and Muyima, 2004).

South Africa's coastal waters have very high levels of pollution. There are as many as 67 discharge points through which as much as 1.3 million m³ of wastewater is discharged daily into the marine environment. The daily discharge is 62% greater than what it was five years ago. Most of these discharges are released into the surf zone with fewer points of discharge into estuaries and offshore water bodies. As many as 23 points discharge into the surf zone in the Western Cape alone, where 275 000 m³ of wastewater (90% of which is domestic effluent) are discharged each day. Offshore discharges along the KwaZulu-Natal coast amount to 500 000 m³/day, of which 61% is industrial and the rest is domestic effluent. The Eastern and Northern Cape have considerably lower amounts of wastewater discharge than either of the other two coastal provinces. The seriousness of contamination from wastewater to the marine environment has kept on growing from the time when it was reported (DEAT, 2006).

There is a permitted discharge level under the South African Water Act (Act 54 of 1956) which was promulgated in 1956. Section 21 of this Act requires permission for all effluent dischargers, including that from sewage works. The General and Special Standards were subsequently published in the Government Gazette in 1984 in

accordance with this Act, which set effluent discharge quality limits for such discharges. This was the Uniform Effluent Standard approach. This was later revised in The National Water Act (Act 36 of 1998) and was subsequently promulgated in 1998, providing the tool to effect these changes. This Act adopted the Receiving Water Quality Objectives (RWQO) approach through the provision of sets of water quality guidelines. This approach takes into account the impacts on the receiving water as well as the impacts on other water users (Eddy, 2003; Jimenez and Asano, 2008). The South Africa water quality guidelines (DWAF, 1996) described water parameters that require monitoring, depending on their intended use and requires that these guidelines be observed to ensure safe water quality.

Some of the parameters in the National Water Act state the required levels and concentration of physicochemical parameters and faecal coliform count for effluent discharge (DWAF, 2013). The Green Drop status report (DWAF, 2011) revealed that most wastewater treatment works in Eastern Cape are not performing optimally and are in dire critical state and therefore require immediate attention. From the Green Drop report, it was evident from the data that compliance was very poor with regards wastewater quality. Similar reports also confirmed that the proposed study site, Buffalo City Local Municipality, is one of the best performing districts in wastewater treatment (DWAF, 2011). A large percent of the treatment plants were categorized as either low risk treatment plants or medium risk treatment plants (DWAF, 2012). The extensive monitoring programme was recognized as necessary for effective treatment. However, the DWAF is concerned about the prevalence of the recorded microbiological non-

compliance. This could be due to ineffective disinfection. Nevertheless the municipal authority is required to give attention to the improvement of this component of effluent quality (DWAF, 2012).

1.1 Statement of the problem

Studies have shown that the majority of wastewater plants in South Africa experience issues with regard to providing sufficient treatment and sterilization with the result that this poses environmental and health risks arising from poorly treated wastewater effluent (Mackintosh and Colvin, 2003). The Green Drop report for 2010-2011 (DWAF, 2011) indicates that municipal wastewater facilities in the Eastern Cape are not up to the set standard. Most treatment plants in Buffalo City Municipality under the Eastern Cape discharge poorly treated effluent into the surface water which directly and indirectly impacts on the quality of water and its aesthetic use. Contamination from poor effluent can cause water quality impairments both at the site and downstream where recreational activities may be taking place

1.2 Hypothesis

We hypothesise that the final effluents of the two selected wastewater treatment works in Buffalo City Local Municipality do not efficiently remove bacteria and human viral pathogens from wastewater. It is further hypothesis that there may be a correlation between the physicochemical indicators of the final effluent of the treated wastewater and its microbiological indicators.

1.3 Research questions

- Is the faecal bacteria load of the selected final effluent within the acceptable limits as indicated by the Department of Water Affairs and Forestry (DWAF)?
- Do the physiochemical parameters of the final effluents meet the set standards for wastewater quality?
- What is the prevalence of the enteric bacteria and viruses in the final effluent wastewater treatment?

1.4 Objectives

- To determine the physicochemical qualities of the final effluent of the wastewater from two wastewater treatment plants;
- To assess the prevalence of faecal coliform from two selected wastewater treatment plants;
- To assess the prevalence of *Escherichia coli* and their pathotypes in the selected wastewater treatment plants;
- To assess the prevalence of *Vibrio* pathotypes in the final effluent of the selected wastewater treatment plants;
- To determine the antibiogram profile of *E. coli* and *Vibrio. spp*
- To assess the occurrence and distribution of enteric viruses in the selected wastewater treatment plants.

1.5 Organization of the dissertation

Chapter One: This chapter introduces the motivation behind this research and provides a brief description of the state of wastewater treatment plants in South Africa. It describes the essentials of good quality water and the imminent adverse impact of polluted water resulting from anthropogenic activities.

Chapter Two: This chapter provides the review of literature.

Chapter Three: This chapter is the methodology section. It highlights the steps taken in sample collection, physicochemical and microbiological analysis. Included also is the statistical method used for the data analysis.

Chapter Four: The chapter reports all results of the physicochemical analysis done, bacteriological counts, molecular identification and pathotyping as well as the result of the viral component reported.

Chapter Five: This chapter discusses each of the physicochemical and microbiological tested. The results from chapter four are fully discussed for each of the parameters tested.

Chapter Six: This chapter offers conclusion on the findings of this study. It answers the research questions and the hypothesis.

CHAPTER TWO

LITERATURE REVIEW

It is common knowledge that water forms an integral part of any living organism. The importance of water is not limited to the sustenance of living cells but extends to and beyond everyday human activities. The domestic, recreational and industrial uses spotlight water as a unique resource that requires attention at any point in time. The end use of the water is wastewater which is unfit for any other use (Rached and Brooks, 1996). Most of this wastewater, if disposed of into the environment untreated, will result in contamination of water bodies and any other environmental element it comes in contact with. It is, therefore, essential that wastewater be treated optimally for proper disposal into the environment (Helmer and Hespanhol, 1997).

Wastewater treatment plants (WWTPs) are specially designed technologies for effective removal of biological oxygen demand and nutrients. Traditional wastewater treatment lessens the number of enteric organisms, but since decreases in treatment processes vary extensively, wastewater effluents can, in any case, hold high tallies of faecal microorganisms (Koivunen, Siitonen and Heinonen-Tanski, 2003). Influent wastewaters vary largely from plant to plant in both flow and composition. In terms of regulation, there is a wide disparity in the discharge regulations that WWTPs effluent must meet depending on the regulatory jurisdiction and the point of discharge. That is, whether the effluent will be discharged into freshwater, salt water or will be reused for irrigation. For the regulations currently in vogue, many plants now use tertiary

treatment in addition to secondary treatment, thus indicating a growing shift in treatment which raises the effluent quality before it is discharged into the receiving environment (PG&E New Construction, 2003). Also, the nature of the final effluents is dependent on the effectiveness of the treatment process involved and the treatment technologies employed in the treatment process.

Globally, due to rapid industrialization and increasing population, domestic and industrial wastewaters are becoming large sources of effluents that are discharged into receiving water bodies daily and the quality of wastewater effluents will determine the degree or level of degradation of the receiving water bodies and the impact of such degradation and spread of various waterborne diseases. It will additionally focus on the level of physical changes, dissolved oxygen to accepting water, discharge of poisonous materials, biomagnification or bioaccumulation in amphibian life, and high nutrient loads. In an effort to shield public health and anticipate harmful ecological effects, rules and policies regulating treated wastewater prior to being released into receiving waterbodies are implemented on countrywide and global levels (Akpoy, 2011).

As earlier pointed out, effluent quality can vary from plants to plants and the differences make it difficult to determine the true picture of the contaminants released into the environment (Omar and Barnard, 2010). As a possible effort to address this problem, Lokhande, Singare and Pimple (2012) noted that countries around the world are battling to settle at an effective regulatory regime to control the release of industrial effluents into their environments. Elevated nutrient concentrations are associated with

physical and chemical parameter changes that can stimulate eutrophication, leading to the growth of algae and increased biomass of phytoplankton (Mahananda, Mohanty and Behera, 2010). Three major disturbing impacts include diminished biodiversity, changes in species composition and predominance and harmfulness impacts (Ballance and King, 1999; Zuma, 2010). Other effects include dissolved oxygen depletion; increased biomass of phytoplankton, toxic or inedible phytoplankton species; increases in blooms of gelatinous zooplankton; increased biomass of benthic and epiphytic algae; changes in macrophyte species composition and biomass; decreases in water transparency (increased turbidity); colour, smell, and water treatment problems; increased incidences of fish kills; loss of desirable and reduction in harvestable aquatic fish species and shellfish; and decreases in perceived aesthetic value of the water body (Zuma, 2010).

A study carried out in Malawi by Phiri et al. (2005) identified the adverse influence of poor effluent discharges into water bodies. Their assessment of various physiochemical parameters revealed poor quality of effluents discharged into the river downstream which resulted in acidifying of the river. Coeurdassier et al. (2005) experimented with snails to determine the toxicity of the effluents on the organism. Varying effluents concentration were used but in all cases the snails survived except for observed fecundity in eggs production (Coeurdassier et al., 2005). However, the study was able to show that effluent, depending on its quality, can either cause mortality, impaired growth, or reduced rate of reproduction (Coeurdassier et al., 2005). This demonstrates the adverse effect of poorly treated effluents on their victims. It is in this way

prescribed that the inconsiderate transfer of the wastes ought to be debilitated in spite of the fact that the expense may be high; however, the continued release of effluents in the river may bring about extreme aggregation of the contaminants and, unless the authorities actualize the laws administering the disposal of wastes, the adverse effect on human lives and other organisms may be higher in cost (Phiri et al., 2005). It is widely accepted that there is the call for protection of aquatic environments from the consequences of deadly substances and releases. Most developed nations have legislations planned to control effluent discharges in order to diminish the risk to the environment (Coourdassier et al., 2005).

The monitoring system employed in the US and UK helps to recognize, analyze, and manage the impact of the effluents discharged from the composite mixtures of pollutants on the ecosystem (Abrantes et al., 2009). In South Africa, a monitoring system called the South African River Health Programme (RHP) is used to survey the condition or health of the river system. The justification for utilizing biological checking is that the respectability of biota possessing river biological systems gives an immediate, all-encompassing and coordinated measure of the trustworthiness or health of the river in general (CSIR, 2011). Furthermore, there exists another monitoring system which ensures, encourages, and rewards wastewater treatment best practices. The Green Drop system serves to checkmate poor practise among wastewater treatment facilities toward achieving effluent of quality standard (DWAF, 2011).

South Africa's water resource is such that it needs adequate monitoring. It is a known fact that water resource is scarce in South Africa (25 Degrees in Africa, 2010). The

agricultural sector, mining sector, and domestic users rely heavily on these scarce water resources and produce large amounts of wastewater which, therefore, calls for removal and proper treatment of the constituent of the wastewater pollutants produced by these sectors, ranging from organic to inorganic pollutants, before being released back to the environment. It is for this reason that Velasco (2001) saw the importance of proper monitoring of the treatment of the wastewater to ensure strict compliance and to safeguard the surface water bodies. The negligence of wastewater treatment plants by South Africa to deliver high microbiological quality effluents is a subject of incredible apprehension regarding the contamination of water assets, as noted by Dungeni, van Der Merwe and Momba (2010), and this could easily imply ineffective implementation of the country's regulations.

Water quality monitors always check for water conditions to ensure safe quality water. Monitoring activities also extend to wastewater quality which is critical because of the adverse effects on the environment, receiving water bodies and the end users. A recent report on the Eastern Cape Province by Green Drop (DWAf, 2011) shows poor quality of final effluent produced after treatment in Buffalo City District Municipality with the attention of the municipal authority already drawn to the potential danger posed by the WWTPs.

The nature of the final effluents is dependent upon the effectiveness of the treatment process involved and treatment technology employed in the treatment process. Wastewater improperly treated using inappropriate treatment technology can result in a poorly managed pollutant load in the wastewater. This contributes to excess nutrients in

the final effluents thus enhancing the growth of harmful organisms and the production of potent toxins by specific groups of organisms (El-Bestawy, Hussein, Baghdadi and El-Saka, 2005).

There have been reports of poor sewage discharge in KwaZulu Natal Province, for instance, killing a large population of fish and destabilizing aquatic ecosystems. In the Western Cape Province, high levels of toxic elements were reported to be found in groundwater due to seepage from the WWTPs. Investigations suggested that pollution of water resources was because of design shortcomings, overloaded limit, and broken equipment and apparatus/machinery of the municipal wastewater and sewage treatment plants (Mema, 2004). In other areas, serious pollution results from poorly maintained and operated sewage works and infrastructure where a large unlicensed solid waste site was located on the river bank, and effluent discharges from industries such as from the tanneries in the Lower Kei catchment area into the Gcuwa tributary which runs through Butterworth (DWAF, 2004). Also of concern is the threat to groundwater quality posed by poor management or maintenance of urban effluent treatment works in Komga, Butterworth, King William's Town, and East London (DWAF, 2004).

In spite of high free chlorine residual concentration in treated effluents, the continued existence and incidence of coliform bacteria were significantly higher at three wastewater plants in Gauteng Province compared to one other wastewater treatment plant, which only showed the survival of *E. coli*, at a much lower detection rate (Dungeni, van Der Merwe and Momba, 2010). Generally, poor effluent, going by the findings of Dungeni, Merwe and Momba (2010), is produced by plants in Gauteng.

Mema (2004) reported that over 80% of Eastern Cape-treated effluent does not meet the minimum permissible discharge standards and cities in such areas will undoubtedly experience increased health and hygiene challenges. Momba, Osode and Sibewu (2009) clearly noted the situation in Buffalo City and Nkokonbe Municipalities of the Eastern Cape Province where the maximum safe limit for effluent discharge is not met. High presence of *Aeromonas hydrophila* and *Escherichia coli* were observed suggesting the inefficiency of the wastewater treatment plant as the reason; hence, both environmental and public health problems were observed in the area. Momba, Osode, and Sibewu (2009) also examined the plants for the efficient removal of somatic and F-RNA coliphage which is associated with enteric viruses, and found the organisms are being discharged into the receiving water without thoroughly going through the treatment process. Report of groundwater contamination by Mema (2004) showed the negative effect of poor effluent management, poor planning, poor enforcement of environmental laws and lackadaisical attitude of authorities to address the issue. In KwaZulu Natal, industrial effluents contributed to the high level of faecal coliform in the water catchment with the people prone to water borne diseases (Mema, 2004). Apart from the coliform bacteria, high levels of heavy metals have been reported by Morrison, Fatoki, Linder and Lundehn (2004) in the Eastern Cape, where some discharged WWTPs final effluent did not comply with the permit issued by the Department of Water Affairs and Forestry (Jackson et al., 2002).

Information pertaining to characterization of final effluent treatment technology employed at the studied sites is briefly reviewed. The two most commonly used

wastewater treatment technologies in South Africa are activated sludge and bio filters (trickling filter) (Keirungi, 2007). Biological treatment systems reviewed under the scope of this thesis are as discussed.

2.1 Activated sludge treatment systems (conventional biological wastewater system)

The systems used for wastewater treatment can be classified according to where the bacterial communities grow. Suspended growth processes are those in which bacteria are maintained in a liquid suspension, and attached growth processes or bio-films are those in which bacteria grow on a supportive inert material such as rocks or plastics. Both can be carried out through aerobic or anaerobic processes. The activated sludge process is a common method of aerobic wastewater treatment. The procedure lessens the amount of dissolved organic matter from wastewater, utilizing microorganisms developing in aeration tanks (Rech, 2008). This system was invented in the beginning of the 20th century and received its name due to the necessary conditions for an activated mass of microorganisms to grow in a reactor in order to remove certain pollutants. Generally, aeration and mixing mechanisms assist in providing the necessary oxygen for bacterial growth. In activated sludge, an interesting characteristic is the formation of bacterial flocs which can be settled and recycled into the reactor or removed from the system. As a consequence, activated sludge processes usually generate a clear effluent. Activated sludge is probably the most widely used biotechnological process and has a number of features which make it unique:

- The mixture of substrates in terms of compound composition and particle sizes

- The high level of bacterial diversity
- The vast changes as far as influent stream, composition, temperature and concentration
- The capability to eliminate distinctive impurities containing phosphorus, sulphur, nitrogen and carbon amongst others
- The variety of possible reactor configurations

The simplest activated sludge system configuration consists of an aeration tank and a clarifier from which sludge is sent back to the aeration tank. In this case, COD removal would be achieved as aerobic decomposition of organic matter is enhanced through aeration.

As mentioned before, the possible combinations of different reactors as well as the design lead to different treatment properties and performances. For example, when nitrogen removal is required, a configuration in which anoxic and aerobic reactors alternate must be given. In that case, ammonia oxidation would be performed in the aeration tank, and pre-denitrification would be carried out in the anoxic reactor, releasing nitrogen gas as a result. The activated sludge process has advantages and limitations which can vary depending on the configuration design of the reactors. Without going deep into the complexity of the different configurations of the system, the general limitations and problems presented by the activated sludge process are mainly the costs of construction and maintenance (highly skilled personnel are required for its control), the energy consumed during aeration, the dependency of chemical additives like external organic carbon for denitrification or phosphorus precipitation

agents (costly) and the decrease in performance efficiency under low temperature conditions (Caballero, 2011).

The system is, however, not without some advantages and these include:

- lowest sludge production of any activated sludge process
- capacity to attain high-quality effluent
- putting in place of pre-engineering bundle plants with insignificant site preparation
- consistency with insignificant administrator consideration
- nitrification possible at wastewater temperatures more noteworthy than 15 °C
- moderately unobtrusive area space requirements
- moderately small initial expense
- capacity to handle moderate-shock hydraulic loadings with negligible issues

Major disadvantages include:

- soaring power utilization and energy fee
- skilled administrators, high process and upkeep prerequisites
- capability of high flow variation to decrease viability of BOD removal and suspended solids (SS)
- likely solidifying issues in cool atmospheres
- possible for rising sludge because of denitrification in final clarifier in hotter months
- potential for blower commotion and sludge taking care of smell

- possibility that pre-engineering plants may require extra parts or modifications to meet specified effluent limitations (Licis, 1995).

The fusion nitrification, biological nitrogen removal, and/or biological phosphorus removal are employed in today's activated sludge processes. These designs employ reactors in series, worked under aerobic, anoxic and anaerobic conditions, and may use internal recycle pumps and piping (Redda, 2008). Nitrification being one of the important processes in the activated sludge requires optima conditions like oxygen concentration, temperature, BOD₅/TKN ratio, ammonia/nitrite concentration, pH, and the presence of poisonous chemicals. The process of controlling nitrification is dependent on dissolved oxygen (DO) concentration which remains one of the most critical variables. Oxygen half-saturation constant is 1.3 mg/L. For the continuous nitrification process to continue oxygen ought to be generally dispersed in the aeration tank of an activated sludge system and its level must not be less than 2 mg/L.

The aeration and sedimentation tanks of the activated sludge process influence, to various levels, the removal/inactivation of pathogens and parasites. During the aeration phase, biological (e.g., inactivation by antagonistic micro-organisms) and physical (e.g., temperature, sunlight) factors, possibly aeration, have an effect on pathogen/parasite survival. Floc formation during the aeration stage is in addition instrumental in eliminating undesirable microorganisms. During the sedimentation stage, certain organisms (e.g., parasites) go through sedimentation, whereas the floc-entrapped microbial pathogens settle readily in the tank. The activated sludge is reasonably efficient when compared with other biological treatment processes in

removing pathogenic microorganisms and parasites from incoming primary effluent.

Trickling filter in general is less efficient than the activated sludges for the removal of indicators such as coliforms and pathogenic (e.g., *Salmonella*) bacteria. The removal efficiency may vary from 80 percent to more than 99 percent. Bacteria are removed via inactivation, grazing by ciliated protozoa (grazing is particularly effective for free-swimming bacteria), and adsorption to sludge solids or encapsulation within sludge flocs, or both, followed by sedimentation. Of all the biological treatment processes, the activated sludge is the most productive process for virus expulsion in sewage. It gives the idea that the vast majorities of the virus particles (90 percent) are solids-related and are eventually moved to sludge. Activated sludge capacity to remove viruses is identified with the ability to remove solids. Hence, a significant number of the virus particles found in the effluents are solids-related. Virus particles are additionally inactivated by environmental as well as through biological factors. Endeavours have been evaluated in gauging the role of both relationships to solids and inactivation to removal of virus in activated sludge. Following 10 hours of aeration, 25% are removed through adsorption to sludge flocs, and 75% are removed by inactivation. In this way, removal is not sufficient through inactivation alone for removing the greater part of the viruses with a maintenance time differing from 6 to 12 h. Research done in India has demonstrated that enteric viruses were removed at a rate of 90–99% (Bitton, 2005; Redda, 2008; Gedalanga, 2010; Wang, 2005).

2.2 Bio filters (trickling filters)

Trickling filters, well-known as biofilters (Boyd and Mbelu, 2009; U.S. Environmental Protection Agency, 2004), are the most regularly utilized sort of settled media filters for conventional wastewater treatment. This is disseminated over a bed, generally made of rock or plastic, and streams over the media by gravity. Oxygen is normally provided by natural or forced ventilation. Flow distributors or sprayers distribute the wastewater evenly onto the surface of the medium. As the wastewater moves by gravity through the medium, soluble and colloidal organic matter is metabolized by the biofilm that forms on the medium. Excess biomass sloughs from the medium and is carried with the treated wastewater to the clarifier, where the solids settle and separate from the treated effluent. At this point, the treated wastewater may be discharged or recycled back to the filter medium for further treatment. Significant points of interest concerning trickling filters contrasted with activated sludge systems include:

- simplicity
- low working and upkeep costs
- lessened sludge generation
- increased shock resistance.

The shortcomings include:

- fairly lower BOD removal (a reduced amount of 85% contrasted with 90% for activated sludge)
- higher initial cost

- require more land area
- probably have to be secured in chilly climates
- possible smell issues

(Johnson and Durme, 1987; Babu, 2007; WAMTechnology, n.d.; Licis, 1995; U S EPA, 2002).

High organic loading may lead to filter clogging as a result of excessive growth of slime bacteria in biofilms. Excessive biofilm growth can also cause odour problems in trickling filters. Clogging restricts air circulation, resulting in low availability of oxygen to biofilm microorganisms (Bitton, 2005). Ordinary trickling filter systems now obtainable must be able to produce effluent with total suspended solids and biology chemical demand of good quality but generally they have poorer removal efficiency for TSS and BOD, they are delicate to low temperatures, and possibly will be plagued by mosquitoes and flies. Performance of a lab-scale recycled rubber particles (RRP) biofilter was compared with a conventional gravel system and a peat biofilter system for treatment of septic tank effluent. During the study, the RRP biofilter provided a similar or better performance than other systems in terms of organic removal and hydraulic capacity. After the start-up period, RRP biofilters achieved removal efficiencies for BOD₅, TSS and ammonia nitrogen of 96%, 93% and 90%, respectively. Then again, the peat biofilter was unsuccessful hydraulically and the gravel system indicated elevated TSS concentrations in the effluent. RRP gave high surface area and enough time for biological treatment. Besides, RRP provided a non-toxic media for biofilm attachment in the biofilter. RRP was observed to provide

ammonia adsorption capacity. The results showed that RRP has the potential to be used as substitutes for natural aggregates such as gravel in septic system drain fields. The RRP biofilter can be used as an alternative to septic systems for sites where an existing septic system has failed or at a site having a high groundwater table or small plot size that is not suitable for the installation of conventional septic systems (Oh, 2012).

The septic system reliability is somewhat better than suspended growth package plants because of the more effective way of capturing and controlling suspended solids. Nitrification is achievable at low loading rates in warm climates. Factors affecting performance include influent wastewater characteristics, hydraulic and organic loading, medium type maintenance of optimal dissolved oxygen levels, and recirculation rates. The process is characteristically vulnerable to climatic conditions because of the cooling effect of the wastewater as it passes through the medium. Appropriate protection, low effluent recirculation, and enhanced circulation techniques can diminish the effect of cold climates. Restricted denitrification is prominent in nitrifying filters when oxygenation is poor and inside dead zones (anaerobic parts) of the filter. Faecal coliform diminishments are poor. Their nitrogen and phosphorus removal is so small it would be impossible to advocate their extensive application in nations with strict effluent quality standards, albeit trickling filters are in fact practical and alluring on the grounds that they are not difficult to work with and they use up less energy (U S EPA, 2002; Helmer, Hespanhol and Supply, 1997).

The removal of pathogens and parasites by trickling filters is generally low and erratic. Bacterial removal is inconsistent, depending on the operation of the trickling filter.

Removal of *Salmonella* by trickling filters is lower than the activated sludge process. The removal of viruses by trickling filters is also generally low and erratic. Filtration rate affects the removal of viruses, and probably other pathogenic micro-organisms. It may be that viruses passing through filters simply do not make good contact with the adsorptive surfaces and that many viruses are eventually eluted. Similarly, removal of bacterial phage is inconsistent and varies, depending on the season of the year. Laboratory experiments showed that at medium filtration and at low rate, the removal of viruses, coliforms, faecal streptococci, and BOD and COD, was greater than at a higher rate. In New Zealand, Texas and Japan comparable findings were made; the removal proficiency of trickling filters is poorer for viruses than for indicator bacteria. The removal process involved in elimination of viruses by trickling filters is feebly comprehended. A few specialists have recommended that viruses are removed by adsorption to the biofilm material. Cysts and oocysts removal by trickling filters is likewise by and large low and inconsistent. Another study also found no huge distinction was found between activated sludge and trickling filters as regards the removal of *Giardia* cysts and *cryptosporidium* oocysts (Bitton, 2005; Ngari, Kotut and Okemo, 2011; Dahling, Safferman and Wright, 1989; Berg, 1973; Lin, 1974).

2.3 Physicochemical

Much of the current concern with regards to environmental quality is focused on water because of its importance in maintaining human health and that of the ecosystem. Fresh water is a finite resource, essential for agriculture, industry and even human existence. Without fresh water of satisfactory amount and value, sustainable growth won't be

feasible (Mahananda, Mohanty and Behera, 2010). Extensive literature has shown the deterioration of water quality due to various pollutants and nutrients from different contaminable sources released into the water body. This brings gradual changes in physiochemical characteristics of the water (U.S. EPA, 1985).

Parameters such as temperature, turbidity, pH, dissolved oxygen, colour, and organic and inorganic metallic substances have shown to impact the natural biomass of surface water. High doses of this pollutant can destroy the natural habitat, creating a new one capable of supporting foreign organisms (Beddow, 2010; Civil-Guy, 2010).

2.3.1 pH

pH is a logarithmic expression of the hydrogen ion concentrations and reflects the degree of acidity (pH less than 7) or alkalinity (pH greater than 7) of the water (DWAF, 1996a). The pH of pure water at temperature of 24° C is 7.0 (Plessis, 2008; Pescod, 1992). pH is an essential variable governing the science of natural water systems. The pH of water specifically influences physiological capacities of plants and animals and is, along these lines, an essential indicator of the health of a water system (Wilde and Ed, 2008b). pH plays important roles in maintaining conducive conditions for biochemical and metabolic reactions to take place (Zuma, 2010). Biological treatment of wastewater happens at neutral pH. All in all, the ideal pH for bacterial development is approximately 7, albeit several could be obligatory acidophilic (e.g. *Sulfolobus*, *Thiobacillus*) and flourish at pH 2. Fungi lean toward acidic conditions with a pH of 5 or lower. Cyanobacteria develop ideally at pH higher than 7. Bacterial development for the most part brings about a diminishing of the pH of the medium

through the release of acidic metabolites (e.g., H_2SO_4 , organic acids). Then again, a few microorganisms know how to increase the pH value of their environment milieu (e.g., denitrifying microbes, algae growth). pH influences the action of microbial enzymes. It influences the ionization of chemicals and in this manner assumes a part in the transfer of nutrients and poisonous chemicals into the cell (Bitton, 2005). High or low effluent pH issues can happen for different reasons. Low effluent pH (<7.0) may be because of both organic over-burdening and low oxygen conditions, or because of nitrification when the treatment alkalinity (buffer capacity) is low. High effluent pH is constantly due to extensive algae growth (Richard and Bowman, 1998). The pH changes are also controlled by temperature, the organic and inorganic ions and biological activity. The pH plays crucial roles in toxicity and availability of metals and non-metallic ions, e.g. ammonium. Industrial effluents and increased biological reaction activities due to sewage treatment work effluents can lead to pH changes. If not buffered properly, low pH levels can allow for the formation of toxic substances, leading to species diversity and structure alterations (Zuma, 2010). The more normal concern is changes in pH brought about by release of municipal or industrial effluents because polluted effluent ordinarily relates with increased photosynthesis in a stream and contamination may cause a long-term increase in pH (Michaud, 1991).

Low pH values in a stream influence aquatic life and weaken recreational uses of water. A change in pH from that regularly experienced in un-impacted streams influences the biota. High pH qualities could additionally modify the danger of different toxins in the stream. A case in point, ammonia is substantially more harmful

in alkaline water than acid on the grounds that free alkali (NH_3) at high pH values (pH > 8.5) is more poisonous to aquatic biota than when it is in the oxidized form (NH_4^+). It likewise "strips" out into the environment and is lost from the water. A reduction in pH might additionally diminish the solvency of certain vital components, for example, selenium. Human populaces from territories contaminated by acid downpour are in danger of being liable to selenium deficits. Low pH likewise increases the dissolvability of numerous different elements, for example, Aluminium (Al), Boron (B), Copper (Cu), Cadmium (Cd), Mercury (Hg), Manganese (Mn) and Iron (Fe). Ammonia, formed only at high pH values (pH > 8.5), is extremely toxic to fish and other aquatic life at high concentration (> 2.0 mg/l N) (Morrison et al., 2001a).

2.3.2 Electrical conductivity (EC) and total dissolved solids (TDS)

Electrical conductivity is a measure of the ability of water to conduct an electrical current (Plessis, 2008). Conductivity forms a critical tool in an inexact measure of the amount of dissolved solids in a liquid specimen. Since dissolved ions create conductivity, conductivity has been indicated to have an immediate connection to the measure of TDS in a sample. The concentration (mg/L) of TDS in a water example could be "approximated" by multiplying conductivity by 0.64 (WDNR, 2010). It also estimates total dissolved solids in water and is used to assess salinity effects on most aquatic fauna and flora.

Electrical conductivity (EC) is a dependable indicator of the total dissolved solids (salts) substance of the water. The use of irrigation water on soils adds to the salt concentration of soil. Concentration of these salts will bring about an increase in

osmotic potential in the soil solution meddling with the extraction of water by the plants. Lethal effects might additionally result with an increase in saltiness. Electrical conductivity (EC) of water is measured in units of milliSiemens per metre, mS/m. Other non-SI units which are still used include $\mu\text{S}/\text{cm}$ which is numerically equal to mmho/cm and dS m⁻¹ (DWAF, DHE and WRC, 2001; Alberta Environment, 2000) and dS m⁻¹ (Alberta Environment, 2000). Conversion to mS/m is as follows: $\text{mS}/\text{m} = \mu\text{S}/\text{cm} \times 0,1$ (DWAF, DHE and WRC, 2001).

Conductivity measurement is affected by:

- The nature of the different ions, their relative concentration and the ionic quality of water
- Dissolved CO₂
- Turbidity
- Temperature (for specific work, the conductivity must be determined at 25 °C (CPCB, n.d.)

Total dissolved solids: Total dissolved solids are directly proportional to electrical conductivity (Plessis, 2008). TDS stands for total dissolved solids, and represents the total concentration of dissolved substances in water. TDS is made up of inorganic salts and, in addition, a little measure of organic matter. Normal inorganic salts that could be found in water are sodium, potassium, magnesium and calcium, which are all cations, and carbonates, nitrates, chlorides, bicarbonates and sulphates, which are all anions. Cations are positively charged particles and anions are negatively charged particles. (Safe Drinking Water Foundation, 2009; Health Canada, 1991; Lokhande, Singare and

Pimple, 2012). Materials dissolved in water are measured as total dissolved solids (TDS), conductivity or as salinity.

Total dissolved solids are a measure of the quantity of all compounds dissolved in water (Plessis, 2008). Salinity is measured as either TDS (Total Dissolved Solids), which measures the amount of dissolved salts in the water, or as EC (Electrical Conductivity), which is the property of a substance which empowers it to serve as a channel or medium for electricity. Salty water conducts electricity more promptly than purer water. A test sample EC could be changed over to TDS and vice versa. Salinity is a measure of the dissolved salts in the water. Salinity is generally most noteworthy throughout times of low flows and increases as water levels diminish (Hunter-Central Rivers Waterwatch, n.d.). Virtually all natural water contains varying concentrations of TDS and hence the TDS of natural water frequently rely on the attributes of the geological formations that the water was, or is, in contact with. TDS are liable to aggregate in water as they travel downstream on the grounds that salts are constantly being included through manmade and natural processes, while almost none are removed by precipitation or manual techniques. The properties of the TDS are represented by the attributes of the constituent inorganic salts dissolved in the water. Thusly TDS is likewise nearly identified with other water quality constituents, for example, the total hardness and the corrosion and scaling capability of water (Plessis, 2008). Effluent water from tanneries and food industries might additionally help increased saltiness concentration in water bodies (DWAF, 1996). Despite effluent

quality regulations, salinisation and eutrophication are two of the important problems threatening water supplies in South Africa (Slabbert, 2007).

Total dissolved solids in water supplies begin from common sources; sewage, urban, mining and agricultural runoff; and industrial wastewater (Health Canada, 1991; Slabbert, 2007). Total dissolved solids are not appreciably removed using conventional water treatment processes. The addition of chemicals during conventional water treatment generally increases the TDS concentration. Certain treatment processes, such as lime–soda ash softening and sodium exchange zeolite softening, may slightly decrease or increase the TDS concentration, respectively. Demineralization processes are required for significant TDS removal. In order to monitor the quality of outlet water, parameters such as TDS are being compared between the inlet and outlet water (Health Canada, 1991; Gray and Becker, 2002). High TDS increase sediments rate which reduces the light penetration into water and ultimately decreases the photosynthesis (Prasad and Rao, 2011). A high content of dissolved components influences the density of water, impacts osmoregulation of freshwater in organisms, diminishes dissolvability of gasses (like oxygen) and utility of water for drinking, irrigational, and industrial purposes. Water might be characterized as focused around the concentration of TDS as attractive for drinking (up to 500 mg/L), admissible for drinking (up to 1,000 mg/L), helpful for watering system (irrigation) (up to 2,000 mg/L), not valuable for drinking and irrigation (over 3,000 mg/L) (Lokhande, Singare and Pimple, 2012). As far as watering system (irrigation) is concerned, the two central point to be considered when deciding water's suitability for that utilization are saltiness

measured by electrical conductivity (EC) or the concentration of total dissolved solids (TDS). While a suitable concentration of salts is crucial for aquatic plants and creatures, saltiness that is past the typical normal level for any types of creature will result in stress to or even demise of that organism. Salinity additionally influences the accessibility of supplements to plant roots. Water with a TDS level in excess of 500 mg/L is inadmissible for a watering system for some plants and tastes obnoxious for drinking. Due to the sensitivity and tolerance of different plants to TDS, plants can be used as indicators of soil salinity (Hunter-Central Rivers Waterwatch, n.d.). TDS concentration of <500 mg/l, with no noticeable effect on soil or crops, indicates that the quality of the wastewater is generally good for agriculture with reference to TDS after secondary treatment. Also high salt concentration in wastewater can result in unfavorable ecological effects on aquatic biota. The level of TDS concentrations automatically influences the quality of the received water body. High TDS might be poisonous to freshwater animals by creating osmotic stress and influencing the osmoregulatory capacity of the organisms (Igbiosa and Okoh, 2009). It may have antagonistic effects or impacts upon fresh water flora and fauna which are not salt tolerant. Large amounts of salinity additionally have implications when utilizing water for animals watering (Hunter-Central Rivers Waterwatch, n.d.).

Alterations in the concentration of the TDS can influence aquatic entities at three levels, to be specific:

- effects on and adjustments of individual species
- effects on group structure

- effects on microbial and environmental processes, for example, rates of metabolism and nutrient cycling.

The rate of change of the TDS concentration, and the span of change, gives off an impression of being more paramount than absolute changes in the TDS concentration, especially in systems where the organisms may not be adjusted to fluctuating levels of TDS. Occasional timing of the change in TDS concentration might additionally have essential synergistic impacts with water temperature on the aggregate group composition and functioning. Organisms adjusted to low-saltiness habitats are by and large delicate to changes in the TDS concentration (DWAF, 1996).

2.3.3 Turbidity

Turbidity is a measure of the capacity of light to pass through water, that is, a measure of the water's cloudiness. Measuring cloudiness gives an evaluation of suspended solids in the water (Hunter-Central Rivers Waterwatch, n.d.; Plessis, 2008). It is brought about by the presence of suspended matter which normally comprises of a mixture of inorganic matter, for example, clay and soil particles, organic matter (Plessis, 2008), silt, finely divided organic matter, plankton and other microscopic organisms, organic acids, and dyes (Wilde and Ed, 2008a). Turbidity is reported in nephelometric turbidity units (NTU). It is determined by looking at the intensity of light scattered by the water sample to the intensity of light scattered by a standard reference in the turbidity meter (DWAF, DHE and WRC, 2001). Turbidity estimations likewise consider algae growth and plankton present in the water. High turbidity influences submerged plants by keeping sufficient light from reaching them for photosynthesis. It

likewise has the ability to fundamentally increase water temperature. Water temperature needs to remain fairly constant so aquatic fauna can survive (Hunter-Central Rivers Waterwatch, n.d.). At the effluent of wastewater treatment plants, turbidity is a quantitative measure of remaining undissolved solids, showing glitches within the treatment process (WTW, n.d.). Soil particles constitute the major part of the suspended matter leading to the turbidity in most natural water. Release of sewage and other wastes can fundamentally lead to increases in turbidity (DWAF, 1996b). Other sources of turbidity are caused by runoffs from point sources (e.g. effluent) and non-point (e.g. irrigation schemes). Higher turbidity can affect benthic, invertebrates and fish communities (Zuma, 2010). In spite of the fact that high turbidity is frequently an indication of poor water quality, crystal clear water does not generally ensure healthy water. Exceptionally clear water can connote extremely acidic conditions or abnormal amounts of saltiness. Suspended solids aid turbidity and residue load and generally require sedimentation or filtration for removal (Licis, 1995).

Colour and smell serve as indicators of the level of contamination of a waste stream, and their presence in wastewater demonstrates inadequate pre-treatment preceding release (Licis, 1995).

Turbidity has no health effects but influences the microbial quality of water body.

However, turbidity can interfere with disinfection and provide a medium for microbial growth. Turbidity may indicate the presence of disease causing organisms. These organisms include bacteria, viruses and parasites that can cause symptoms such as nausea, cramps, diarrhoea, and associated headaches (TLC, 2007; DWAF, 1996b;

Plessis, 2008). Another report by Keegan, Wati, Blackbeard and Monis (2011) on effects of turbidity and particles states that:

- water with turbidity > 1 NTU may pose a risk for disinfection through protection of virus particles,
- clays, humic and fulvic acids have no effects on disinfection,
- particle size has an effect on chlorine disinfection, and
- particle size has no discernible effect on chloramines disinfection.

Turbidity is paramount in light of the fact that it influences the worthiness of water to consumers, the determination and effectiveness of treatment processes, especially the proficiency of disinfection with chlorine since it exerts a chlorine demand, protects microorganisms and, furthermore, stimulates the growth of micro-organisms. In all processes in which sterilization is utilized, the turbidity should dependably be low - ideally underneath 1 NTU. It is proposed that for water to be cleaned the turbidity ought to be reliably less than 5 NTU and preferably have an average estimation of less than 1 NTU (WHO, 1997; Bitton, 2005; Gedalanga, 2010; Jackson et al., 2011; LeChevallier and Au, 2004). Low turbidity subsequently minimizes the required chlorine measurements and diminishes the development of chloro-organics that regularly result in taste and smell issues and trihalomethane. Because of the numerous preferences connected with water of low turbidity and the relative simplicity of monitoring, it is regularly utilized as an indicator of potential water quality issues (DWAF, 1996b; Mazibuko, 2012). The appreciation of turbidity as a pointer of the ecological soundness of water bodies has expanded over the previous decade, bringing

about a developing interest for high-quality and target turbidity estimations. Turbidity should be low in drinking water if proper chlorination is required (Hussain, 2010).

Primary production is reduced in turbid water as a result of decreased photosynthesis due to light scattering. Turbidity > 5 NTU can cause reduction of primary production. Primary production decrease reduces food availability at multiple trophic levels in the aquatic ecosystems (Zuma, 2010). In addition, an increase in turbidity impacts on feeding patterns of filter feeders, causes physiological damage and limits habitat for certain invertebrate species. Turbidity also influences the chemical composition of natural water because the particles are generally charged, thus forming adsorption and desorption surfaces (Slabbert, 2007). Apart from many physical and chemical parameters, turbidity and suspended solids appear crucial to virus survival.

Chlorination successfully inactivates viruses if the turbidity of the water is less than or equivalent to 1.0 nephelometric turbidity unit (NTU), likewise ozonation when there is lower turbidity and total organic carbon. It requires free chlorine residual of 1.0 or greater for 30 minutes, and a pH of less than 8.0 (LeChevallier and Au, 2004; Rosenblum et al., 2012).

2.3.4 Temperature

This is a measurement of the intensity (not amount) of heat stored in a volume of water. Surface water temperatures naturally range from 0 °C under ice cover to 40 °C in hot springs. Natural sources of heat include: solar radiation, transfer from air and condensation of water vapour at the water surface, sediments, precipitation, surface runoff and groundwater. Temperature is the primary influencing factor on water density

and viscosity. Temperature affects the solubility of many chemical compounds and biological reactions, and can accordingly influence the impact of toxins on aquatic life. Higher temperatures hoist or increase the metabolic oxygen demand which, in conjunction with diminished oxygen solvency, affects numerous species (Ministry of Environment, Lands and Parks and Geographic Data BC, 1998; Licis, 1995; Khan et al., 2006). Temperature of a conduit is important in light of the fact that it influences the amount of dissolved oxygen in the water. The amount of oxygen that will dissolve in water increases as temperature decreases. Water at 0 °C will hold more oxygen for every litre, while at 30 °C it will hold up less oxygen per litre. Temperature also affects the rate of photosynthesis of plants, the metabolic rate of aquatic animals, rates of development, timing and success of reproduction, mobility, migration patterns and the sensitivity of organisms to toxins, parasites and disease. Life cycles of aquatic organisms are often related to changes in temperature. Temperature ranges for plants and animals might be influenced by manmade structures, for example, dams and weirs and release of water from them (Hunter-Central Rivers Waterwatch, n.d.; Shon, Vigneswaran and Snyder, 2006). Generally, higher temperatures increase reaction rates and solubility up to the point where temperature gets sufficiently high to hinder the action of most microorganisms (around 35 °C) (Licis, 1995). In wastewater treatment, temperature, pH, and dissolved oxygen concentration are especially important to consider when outlining/designing for nitrification (Redda, 2008). Temperature among other parameters affects the stability of the nitrification process in wastewater treatment operations. Nitrification process failures are brought about, in addition to different elements, by temperature changes in the sewage. This can prompt

challenges in keeping up the required rates of nitrogen removal as nitrification throughout winter can be a problem as nitrification can be less efficient (Caballero, 2011; Conroy, 2006). Temperature also plays a significant role in the oxidation process (Conroy, 2006). At low temperatures of 11 °C, COD, BOD, and TSS removal rates obtained were high respectively (Oh, 2012). High TSS can result in a build-up in surface water temperature on the grounds that the suspended particles retain heat from sunlight. This can result in dissolved oxygen levels falling significantly further (in light of the fact that hotter water can hold less DO), and can hurt aquatic life from various perspectives. It was found that inhibitory concentrations of free ammonia and free nitrous acid are a function of temperature (Redda, 2008). High temperature diminishes the solvency of gasses in water which is eventually communicated as high BOD/COD (Prasad and Rao, 2011). The synergistic effect of temperature and oxygen is a key factor in substrate competition between bacterial groups and improved microbial characterization of activate sludge in wastewater treatment operations, especially under oxygen limited conditions. Therefore, total bacteria concentrations are reduced as temperature increases because the organisms are not able to cope with the low oxygen concentrations. Relatively low temperatures (24 °C-25 °C) are favourable conditions for *Nitrobacter*, while *Nitrospira* was more adapted to higher temperatures (28 °C-29 °C). These two organisms are important in the nitrification process (Gedalanga, 2010).

Most microorganisms survive well at low temperatures (5 °C) and quickly die at high temperatures (>40 °C). Temperature is frequently thought to be the transcendent component deciding viral inactivation. Temperature impacts may particularly be of

concern in mild regions where the temperatures are low throughout a substantial part of the year. Some viruses and bacteria are considered to be heat resistant. Freezing is considered to inactivate bacteria and protozoa to some degree while viruses are unaffected. There are known bacteria that can proliferate over a wide range of temperatures in water with the optimum temperature for pathogenic species being 30 °C. These are thermophilic and grow well at temperatures up to 45 °C. Then again, generally high amounts of biodegradable organic carbon together with warm temperatures and low residual concentrations of chlorine can allow the growth of some bacteria and nuisance organisms in some surface water. High temperatures can harm the virus capsid or nucleic acids, which may avert the adsorption of the virus to its host and may inactivate chemicals/enzymes needed for replication (Höglund, 2001; Wochinger, 2012; Seidel, 2003; Fong and Lipp, 2005; Gorchev and Ozolins, 2008).

WWTPs must be aware of cultivation conditions because there are several environmental parameters that may influence microalgae growth, such as pH, temperature, light supply, and dissolved oxygen concentration (Paul, 2012). The presence of algal blooms in some lakes is often associated with an increase in water temperature throughout the warm season (Paul, 2012).

2.3.5 Biological oxygen demand (BOD)

Biological oxygen demand (BOD) gives a pointer of the measure of organic substances of biological origin (proteins, sugars, fats and oils) and biodegradable manufactured organic chemicals in wastewater. The purpose of this test is to determine the potential of wastewater and other water to deplete the oxygen levels of

receiving water. It is used to determine the efficiency of treatment plants at removing organic material, and government regulatory agencies use it to determine how efficient the plants are and how the effluent will affect receiving water. A correlation between the BOD of influent wastewater and treated effluent gives a measure of the effectiveness of a treatment plant in balancing out organic matter (Licis, 1995; Mantech, n.d.). The concentration of organic matter is usually high in wastewater, i.e. the biochemical oxygen demand (BOD) is high, even after primary treatment. If wastewater is discharged untreated, the biological processes will result in depletion of the receiving water's oxygen supply, with subsequent negative effects on aquatic life. In biological treatment, heterotrophic bacteria are used in a confined aerated environment to remove BOD from the effluent. The microorganisms likewise use organic matter for development, consequently the generation of biomass or sludge in a biological treatment step (Mantech, n.d.; Norström, 2005). A high concentration of BOD is found in water bodies with excessive algal growth or a high level of organic matter. Low levels are associated with cleaner and clearer water bodies with a good level of decomposed material.

An easy determination of the nature of waste-water treatment plants as far as contamination quality is concerned, i.e. biochemical oxygen demand (BOD), is troublesome or even incomprehensible utilizing the chemical determination method because of the time taken to acquire the concentration of organic matters (Rastogi et al., 2003a).

In the laboratory procedure for determining the BOD concentration, the amount of biomass generated is considered negligible (or inconsequential). The experimental conditions and the actual measured concentration of BOD do vary. Therefore, several measurements of the same sample (at the same dilution) are usually required to obtain reliable and reproducible results from the BOD. One difficulty in the BOD testing is the interference of nitrification. The typical BOD test determines the carbonaceous BOD and excludes the impacts of nitrification. The nitrification exert more oxygen demand than the carbonaceous process (Ellis, 2004). Also, a 5-day length of time is the most critical point of limit where fast input is needed for environmental monitoring and/or process control. An essential variable in this time deferral is the low solvency of oxygen, which rapidly turns into the rate-restricting reagent in the catabolism of organic material. Other impediments with the BOD₅ measure include: limited linear working range (additionally because of the low dissolvability of oxygen); confounded lengthy techniques; faulty correctness and flawed reproducibility - the standard technique. However, the conventional BOD method requires not only 5 days, but also experience and skills (Catterall et al., 2001; Chen, Zhang and Wang, 2007).

The control of WWTPs can be very troublesome or even outlandish using the conventional method for BOD because of its high time consumption (3–5 days) (Rastogi et al., 2003b). Advancements have been made in BOD measurement to principally minimize the time and nullify the measurement challenges. Four new techniques are currently employed in BOD testing: BOD-BART™, Biosensors, Ferricyanide-mediated method and luminous bacterial immobilized chip method (Kale

and Mehrotra, 2009). Other various variations, modifications and techniques are reported by Jouanneau et al. (2014). Study has shown the achievability and guarantee of the ferricyanide-interceded approach as a suitable, fast option to the conventional 5-day BOD assay which gave a BOD reading within 1 hour as compared to the 5-day BOD (Catterall et al., 2001). Luminous bacterial immobilized chip method has shown that the BOD of industrial waste-water having low–moderate–high biodegradable organic matter could be evaluated for BOD load within a brief time period (5-10mins) (Rastogi et al., 2003a). Biosensors have been tested in measuring of BOD and the sensor response was reported to be within 15 minutes and the results were reproducible within $\pm 5\%$ with good sensitivity and stability (Chen, Zhang and Wang, 2007). The new methods of real measurement are relatively easy to use, technologically reliable and information about them are easily available for operators to make quick judgment (Jouanneau et al., 2014).

2.3.6 Chemical oxygen demand (COD)

An option to the BOD test for deciding the oxygen devouring capability of a wastewater specimen is the chemical oxygen demand (COD) test. One advantage of the COD test over other tests, such as the BOD test, is that it is relatively fast to carry out; for example the BOD test takes place over a five-day incubation period whilst the COD tests can be carried out in 2 hours; hence, the COD test provides a much quicker indication of water quality. Chemical oxygen demand (COD) measures non-biodegradable as well as biodegradable organics. The degree between BOD₅ (oxygen demand utilizing a five-day test) and COD gives a marker of the simplicity of

biological treatment. The carbonaceous oxygen demand is oxidized chemically in the COD test. The organic matters are commonly measured in terms of chemical oxygen demand (COD). On the off chance that untreated wastewater gets released to the environment, their natural biological stabilisation can prompt the exhaustion of the oxygen level and the development of septic conditions. If the COD surpasses the required limits, a solution is required to amend the circumstances (e.g. improve operation at the treatment plant). Like BOD, the units for COD are in milligrams of oxygen per litre (mg L^{-1}). The advantage of this test is that it is quick and reproducible. The disadvantage is that not all of the measured COD can be degraded biologically. Therefore, there is still a need to ascertain what the biodegradable portion of the oxygen demand is since that is how the performance of biological wastewater treatment systems (e.g. activated sludge, trickling filters, anaerobic digesters, rotating biological contactors, oxidation ponds and lagoons) are evaluated. In addition, the BOD, not COD, is the component that is expected to induce an oxygen demand in the receiving stream (Ellis, 2004; Licis, 1995). COD wastes typically are not promptly biodegradable and frequently hold compounds that hinder living organism in wastewater treatment plants. The carbonaceous contaminants form the greater part of the problem of wastewater treatment. The degree of contamination is given as the “COD value” or the chemical oxygen demand. This is an indication of how much oxygen is needed to oxidize all the carbonaceous contaminants to carbon dioxide by chemical means. The COD is best removed by anaerobic breakdown followed by aerobic breakdown (ENBITEC Environmental Solution, n.d.; Terry, 2010). The COD concentration varies due to the various organic and inorganic wastes entering WWTPs.

Some influents have very high COD levels while some have low COD levels. Pre-precipitation and flocculation are, therefore, of interest today, as flocculation of the organic suspended solids facilitates the biological treatment and also reduces the need for aeration in the aeration basin thereby saving a great amount of energy. The use of magnesium chloride was found to be a good removal of COD. Also, an advance oxidation process has been recommended (Karat, 2013; Molahalli, 2011). Anaerobic-aerobic systems have been remarkably employed in industrial and municipal wastewater treatment for a long time. While formerly most treatment of wastewaters had been done in conventional anaerobic-aerobic treatment plants, lately high rate anaerobic-aerobic bioreactors have been progressively utilized for wastewaters with high chemical oxygen demand (COD) (Chan, Chong, Law and Hassell, 2009).

2.3.7 Dissolved oxygen

Dissolved oxygen (DO) monitoring in wastewater is basic for the productive operation of WWTPs. Consistent and dependable DO observing can enhance plant proficiency (accordingly bringing down working expenses) and in addition diminish the danger of undesirable smell (Y S I Environmental, 2006). DO test measures the concentration of oxygen dissolved in water or wastewater. The concentration of DO in a water specimen is essentially impacted by:

- Atmospheric Pressure: As pressure increases, DO also increases (i.e. water holds less oxygen as you increase altitude)
- Salinity: As water salinity increases, DO decreases (i.e. as water gets saltier, it holds less oxygen)

- Temperature: As water temperature increases, DO decreases (i.e. as water gets warmer, it holds less oxygen) (Kiepper, 2010; Clean Water Team (CWT), 2004)

Activated sludge wastewater treatment methods are hard to control as a result of their unpredictable and nonlinear operation; on the other hand, the control of the dissolved oxygen level in the reactors assumes an imperative part in the operation of the system. The dissolved oxygen concentration must be kept up at 2 mg/l in an aerobic tank of a pre-denitrification process with influent unsettling influences and an alternating dissolved oxygen level must be kept up in an alternating activated sludge process (Holenda, Domokos, Rédey and Fazakas, 2008).

Biochemical reactions for wastewater treatment are primarily reactions enabled by microbial metabolic processes. The reactions that occur within aerobic systems mineralize carbonaceous material through oxidation and scavenge chemical nutrients. Dissolved oxygen (DO) concentrations are water treatment factors for carbonaceous and an important factor in waste-solid bacterial uptake. Oxygen administration for organic waste treatment processes differs in diverse treatment unit-processes. Oxygen prerequisites for solids treatment are diverse for organic waste chemicals, and administration of oxygen levels is normally needed for cyclic changes in waste stacking. Carbonaceous solids in wastewater create a Biochemical Demand (BOD) for oxygen through reactions that are enabled by bacteria. Biochemical processes in oxygenated environments are important for biological nutrient removal (BNR). The biochemical nitrification rates are very subject to DO level with constraining DO level

as high as 2.5 mg/litre for appended and suspended colonies. This results in higher accessibility of dissolved oxygen, prompting enhanced treatment and lessened creation of sludge. Exorbitant development of specific filamentous micro-organisms is characteristic of specific operational issues in the plant, for example, low DO, low F/M (Food to Microorganism) ratio (i.e., low organic loading rate), high concentration of sulphides in wastewater, nitrogen and phosphorus deficiencies, and low pH. The overgrowth of these filamentous bacteria in activated sludge systems is linked to low dissolved oxygen in the aeration tank. Also, on the off chance that the amount of free or DO accessible in the wastewater process gets to be excessively low, the aerobic bacteria that routinely treat the sewage will be killed. The treatment process won't work productively and septic conditions will occur (Bitton, 2005; Godshall, 1996; US EPA, OW, 2012). It was noted that the impact of release on water quality is the increase in dissolved oxygen concentration that corresponds with the increase in the release of the well treated effluent (Mladenov, Strzepek and Serumola, 2005).

2.3.8 Nitrates and nitrites

Nitrogen-containing compounds act as nutrients and are the most noxious pollutants of water (Bellos, Sawidis and Tsekos, 2004). Nitrate reactions (NO_3^-) in fresh water can cause oxygen consumption. Aquatic organisms relying upon the supply of oxygen in the stream will die. The significant routes through which nitrogen enters into the waterways are municipal and industrial wastewater, septic tanks, and animal wastes. Bacteria in water rapidly change from nitrites (NO_2^-) to nitrates (NO_3^-). Nitrate (NO_3^-) and nitrite (NO_2^-) are naturally occurring inorganic ions that are part of the nitrogen

cycle. The tracking of $\text{NO}_2\text{-N}$ in the effluent of any WWTP, together with nitrate (NO_3) and COD, gives a great sign of the execution of the nitrogen removal process (Dalton, 2012). Since the second step of nitrification is quick, the nitrite concentration in the effluent of a WWTP is typically low (around 0.1 mg/l) (Munch, Lant and Keller, 1996). Enhancement of nitrite in the system normally suggests that the microbiological procedures are disturbed, i.e. they are repressed because of harmful substances or to conditions for the nitrite oxidizer.

In a wastewater treatment plant, ammonia is normally oxidized to nitrites and then to nitrates (Pollice, Tandoi and Lestingi, 2002). If the nitrate/nitrite concentration surpasses as far as the required limits, intervention is essential to correct the circumstances (e.g. guarantee source protection, guarantee that the treatment plant can viably remove nitrate/nitrite, and optimize operation at the treatment plant).

Nitrification is the conversion of ammonia (NH_3^+) to nitrate (NO_3^-). This involves a two-step process that is done with oxygen and two types of bacteria, *Nitrosomonas* (ammonia-oxidizers) and *Nitrobacter* (nitrite-oxidizers), known collectively as the nitrifiers. The denitrification stage is the conversion of nitrate (NO_3^-) to nitrogen gas (N_2). Heterotrophic bacteria consume the nitrate as an oxygen source under anoxic conditions to break down organic substances. During nitrification of ammonia to nitrate in the aeration tank many hydrogen ions will be discharged and these impacts on the pH - should never permit the pH of the aeration tank to drop underneath 6.5.

Biological activity will be repressed and dangerous alkali (ammonia) can drain directly through the system. Ammonia releases likewise put a high oxygen demand on

the accepting streams. Prior to the chlorination of the treated effluent, the pH level should never be out of range and the concentration of ammonia should be extremely low and nitrite should not show at all while more of nitrate can be allowed. Elevated amounts of nitrite in the system show potential issue with the nitrification cycle. *Nitrosomonas* (ammonia-oxidizers) bacteria are harder to kill than *Nitrobacter* (nitrite-oxidizers) bacteria (Arp and Stein, 2003). In the event that the *Nitrobacter* bacteria are killed off, the *Nitrosomonas* bacteria will keep chipping away at the ammonia and this jammed the cycle with high levels of nitrite. An effluent with high nitrite concentrations will be hard to sanitize due to the enormous chlorine demand it poses. The denitrification stage follows the nitrification stage (Gee, Pfeffer and Suidan, 1990; Hanaki, Wantawin and Ohgaki, 1990; Scott, 2014).

2.3.9 Phosphates

Phosphorus, as soluble orthophosphate, is an important supplement in all biotic processes, including activated sludge treatment. The presence or trace of concentrations of dissolved phosphate is frequently involved in creating eutrophication issues in lakes, reservoirs, other restricted water bodies and coastal waters (Zhao and Sengupta, 1998; Sengupta and Pandit, 2011). However, accelerated eutrophication resulting from unnatural excessive discharge of nutrients to water systems is defective for if the phosphate/phosphorous concentration surpasses acceptable limits, mediation is obliged to redress the circumstances (e.g. guarantee source protection, guarantee that the treatment plant can successfully treat phosphates and optimize operation) (Emanti Management, 2011). Techniques for phosphorus removal from wastewater are in

general absorption, biological and physical/chemical treatment (Sengupta and Pandit, 2011; Lee et al., 2007).

In an enhanced biological phosphate evacuation (EBPR) process, wastewater is dealt with in an anaerobic/aerobic consecutive pattern. Throughout the anaerobic phase of treatment, polyphosphate-accumulating organisms (PAO) take up the released phosphorus. Hydrolysis of intracellular polyphosphate gives the energy required to remove the organic acid from the waste. During the subsequent aerobic stage, more of the compound of phosphate is consumed to produce energy for bacterial growth.

As the amount of phosphate expelled from wastewater throughout the aerobic stage is more noteworthy than that discharged throughout the initial anaerobic stage, phosphate is accumulated in bacterial cells and in the end expelled from the system alongside the waste sludge (Liu, Zhang and Fang, 2005).

2.3.10 Free chlorine residual

Free chlorine residual is an indication of the efficiency of the disinfection process and is thus a rapid indicator of the probable microbiological safety or otherwise of the treated water. The use of free chlorine is favoured universally for the following advantages. Absence of residual chlorine either means that the water is not treated with chlorine or that insufficient chlorine is used to disinfect the water. Where the untreated water contains pathogenic micro-organisms, the absence of free residual chlorine indicates that there is a risk of microbial infection. If the free chlorine residual does not meet the required limits, intervention is required to rectify the situation (e.g. optimize disinfection) (Emanti Management, 2011). Chlorine may be applied in two

ways: gaseous form or liquid form. In gaseous form, chlorine gas is dissolved in water and a solution is formed. This solution is mixed with water according to the dose ascertained in the laboratory test.

Chlorine is the most broadly utilized disinfectant of wastewater because of its ability to inactivate most pathogenic micro-organisms rapidly, the process of application is easy, it can be stored easily, and the optimum dose can be easily found by the break point of the chlorine (Fayyad and Al-Sheikh, 2001). Some of the chlorine disinfection processes are:

Chloramines: The free chlorine is not stable in water. To make this stable, some amount of ammonia is mixed with water along with chlorine. As a result of a chemical reaction, some compounds are formed which are known as chloramines. The following are the benefits of adding ammonia along with chlorine. It makes chlorine stable in water. It reduces the amount of chlorine necessary for treatment. It becomes more powerful in killing bacteria. It reduces the irritating effect of chlorine. Other study reports on chloramines showed that organic chloramines examined had little or no effect on the viability of *E. coli* and another reported no evidence of inactivation (Donnermair and Blatchley, 2003; Amiri, Mesquita and Andrews, 2010).

Chlorine dioxide: Sometimes chlorine dioxide (ClO_2) is used for removal of bacteria. It is produced by passing chlorine gas through sodium chlorite in a closed container. It is very unstable and it used very quickly. In comparison to liquid chlorine, the slaughtering impact of ClO_2 on microorganisms and algae growth is about the same as or superior to that of liquid chlorine and the inactivation impact of ClO_2 on viruses and

animal plankton is more remarkable than that of liquid chlorine. The viruses were eliminated off by ClO_2 in a more extensive pH range (Junli et al., 1997a; b; GE Power & Wate, 2012).

Bleaching powder: Bleaching powder is also known as calcium hypochlorite [$\text{Ca}(\text{OCl})_2$]; when it is mixed with water, hypochlorite ions (OCl^-) are formed. These ions again combine with hydrogen ions (H^+) present in water thus hypochlorous acid is formed. This phenomenon is known as hypo-chlorination. The hypochlorous acid and hypochlorite ions are both responsible for the killing of bacteria. The bleaching powder is available in white powder which contains usually 35 percent chlorine.

The speed at which these reactions happen is controlled by pH, temperature, and oxidation/reduction potential. As pH increases, the chemical reactivity of chlorine abates; as temperature increases, reactions move on more quickly. The oxidation reactions of chlorine with such inorganic reducing compounds are for the most part exceptionally quick. Some dissolve organic materials additionally react quickly with chlorine, yet it can take hours for the organic-chlorine reactions to finish (GE Power & Wate, 2012; Ayyildiz, Ileri and Sanik, 2009). As a result of this, free chlorine was found to form more trihalomethane (THMs) than chloramines and chlorine dioxide (Hua and Reckhow, 2007). The effect of organics and particles on chlorine sterilization of grey water is measured by total coliform inactivation. The viability of purification is attributed to particle size. Bigger particles protected aggregate coliforms from inactivation and sterilization viability diminished with expanding particle size

(Winward, Avery, Stephenson and Jefferson, 2008; Narkis et al., 1995; GE Power & Wate, 2012).

Chlorine additionally dispenses with slime microorganisms, moulds, and algae growth that normally develop in water supply repositories, on the dividers of central pipes and storage tanks. Just chlorine-based disinfectants leave a gainful "residual" level that remains part of treated water, serving to secure it throughout circulation and storage.

When chlorine is added to water, a portion of the chlorine reacts first with organic materials and metals in the water and is not accessible for disinfections because of their chlorine demand. The remaining chlorine concentration after the chlorine demand is accounted for is called total chlorine. The free chlorine is the chlorine available to inactivate disease causing organisms (Hussain, 2010; Tree, Adams and Lees, 2003). It is shown that reduction in demand of tertiary treated sewage allows other disinfectants like bromine to become the superior disinfectant at the same weight dosage as chlorine (Sun William, 1973).

Of the three chlorine compounds (HOCl , OCl^- , and NH_2Cl), hypochlorous acid is the most effective for the inactivation of microorganisms in water and wastewater. The presence of interfering substances in wastewater reduces the disinfection efficacy of chlorine, and relatively high concentrations of chlorine (20–40 ppm) are required for adequate reduction of viruses. In wastewater effluents, no free chlorine species are available after a few seconds of contact. Chlorine, specifically HOCl , is generally quite efficient in inactivating pathogenic and indicator bacteria. In spite of the fact that there is wide variation in the resistance of enteric viruses to chlorine these

pathogens are far more impervious to this disinfectant than are vegetative microorganisms. This clarifies why viruses are often detected in chlorinated secondarily treated effluents. Chloramines are substantially less proficient than free residual chlorine as regards viral inactivation. In the presence of HOCl at pH 6, the Ct for *E. coli* is 0.04, compared with a Ct value of 1.05 for poliovirus type 1 and 80 for *G. lamblia*. Studies have shown that free chlorine inactivated enteric microorganisms much quicker than did inorganic chloramines. Besides, the bactericidal effect of chloramines increases with temperature and hydrogen ion concentration. Comparable results were found with respect to viruses and protozoan cysts, mycobacteria, some enteric viruses and protozoan cysts (Bitton, 2005; Deborde and von Gunten, 2008; Donnermair and Blatchley, 2003; Amiri, Mesquita and Andrews, 2010; Gagnon et al., 2004). Also chlorination has been reported to contribute to the selection of chlorine-resistant pathogenic bacteria, and regrowth of pathogenic bacteria after chlorination in reclaimed water with a long retention time and this could threaten public health during wastewater reuse (Li et al., 2013).

2.4 Enteric bacteria

Enteric microorganisms are microscopic organisms in the family *Enterobacteriaceae*. These microbes live ordinarily in the guts of numerous creatures, including people, and some are pathogenic, bringing about ailments in certain animal species (McMahon, 2014). For the purpose of this write up, enteric bacteria is focused only on two families: *Enterobacteriaceae* (pathogenic *E. coli*) and *Vibrionaceae* (*Vibrio*). The term ‘enteric bacteria’ will be used to refer to either of the two families. A successful disease of the human digestive system by enteropathogenic microbes relies on the capacity of microscopic organisms to append and colonize the intestinal epithelium and, in a few cases, to attack the host cell, survive intracellularly and spread from cell to cell (Reis and Horn, 2010). Enteric bacteria are natural to wastewater and because of their likely danger to the public there is need for strict monitoring of treatment works. WWTPs are typically intended to proficiently remove biological oxygen demand compounds and nutrients; however, occasionally have they been arranged particularly to expel pathogenic micro-organisms from wastewaters (Pescod, 1992; Agency, 1999). Routine wastewater treatment diminishes the amounts of enteric organisms, but limitations faced during treatment processes can extensively lead to variation such that wastewater effluents are still found to contain high numbers of faecal micro-organisms. Richness of faecal coliform microorganisms is a feeble index of the presence of human pathogens in wastewater entering coastal waters. Regardless of this, utilization of fecal coliform for quality purposes is universal (Valiela, Alber and LaMontagne, 1991). Certain clonal groups of *E. coli* with virulence qualities of uropathogenic strains were reported surviving the wastewater treatment processes. The presence of this pathogenic

E. coli were found in high percentage in the environment suggesting that they may have originated from effluents (Anastasi et al., 2010; Anastasi, Matthews, Stratton and Katouli, 2012). Pathogenic strains of *Vibrio* have been reported to be isolated from effluent in a WWTP (Cañigral et al., 2010; Igbinosa, Obi and Okoh, 2009; Igbinosa, 2010; Igbinosa and Okoh, 2010). Efficient expulsion of pathogens from wastewaters is a basic task, since sewage released directly untreated may multiply pathogen pollution of surface water and bring about waterborne contaminations (Koivunen, Siitonen and Heinonen-Tanski, 2003). It has been reported that conventional municipal wastewater treatment without effective tertiary treatment, in the same way as filtration or disinfection, may constitute a danger for public health from enteric bacteria (Koivunen, Siitonen and Heinonen-Tanski, 2003). Some enteric bacteria have been found to survive better during the activated sludge system as well as the trickling filter treatment processes (Wéry et al., 2008; Stevik, Aa, Ausland and Hanssen, 2004). The inactivation rates of enteric bacteria by chlorine treatment has been found to be adequate where effluent treatment is efficient (Tyrrell, Rippey and Watkins, 1995) while the absence of organic matter reduces the resistance of the bacteria in treated effluent during the disinfection process (Virto et al., 2005). Disinfection by chlorination was reported to be effective against coliform bacteria and antibiotic resistant bacteria (Staley, Crosa, DeWalle and Carlson, 1987). Other studies have reported that disinfection did not contribute to the reduction of antibiotic resistant bacteria in effluent (Munir, Wong and Xagorarakis, 2011). *V. cholerae* was reported to have survived in about a lethargic stage under sub-optimal temperature and supplement concentrations, holding up for suitable conditions for recuperation (Rojas and Hazen, 1989). The currently used faecal

indicator test is considered not a good indicator of microbial contamination water because of its limitations as a predictive factor for the presence of other pathogens (Hazen, 1988; Tyagi, Chopra, Kazmi and Kumar, 2006). New molecular-based systems have demonstrated that joined utilization detection methods of conventional and alternative indicators for faecal contamination increases the identification of both the sensitivity and specificity of faecal contamination and related pathogens (Savichtcheva and Okabe, 2006). The use of the molecular approach has further aided the monitoring of enteric bacteria in the environment which shows superiority to faecal coliform assays in term of sensitivity (Field, Bernhard and Brodeur, 2003). In recent years, the use of alternative microbial faecal indicators such as faecal anaerobes (i.e., *Bacteroides spp.*, *Bifidobacterium spp.*, *Clostridium perfringens*), and viruses (phage), and chemical indicators (i.e. faecal sterols, caffeine, and optical brighteners) have become popular because these can also provide sensitive and accurate measurement of faecal pollution of water in the environment (Ahmed, Goonetilleke and Gardner, 2008).

2.5 Enteric Virus

It has gotten to be progressively obvious lately that viruses are a leading cause of water borne gastroenteritis (Mara and Horan, 2003; Lin and Ganesh, 2013). Various studies have demonstrated that enteric viruses are present at an abnormal rate in wastewater, especially after the treatment process (Nordgren et al., 2009). Human enteric viruses are currently listed on the United States Environmental Protection Agency Contaminant Candidate List (USEPA CCL) as emerging contaminants. To date, no regulations have been implemented for the monitoring of wastewater viral concentration before being

discharged into a water body. Human enterovirus (ev), Human adenovirus (HAdv), norovirus, rotavirus and hepatitis-A infection (HAV) are part of the enteric viruses causing infections of importance in this study. These infections have been connected with a few waterborne ailments, for example, intense gastroenteritis, conjunctivitis and respiratory disease in both developed and developing nations over the world. There are a few courses whereby general society can get contaminated, including direct contact (fecal-oral course or dermal contact) and food-borne ailments and pollution (Simmons and Xagorarakis, 2011; Lin and Ganesh, 2013). A combine sewage overflow was reported to be releasing a significant high level concentration of viruses into the receiving water bodies and the occurrence was more during the wet weather than the dry weather period (Rodríguez, 2007; Fong, Phanikumar, Xagorarakis and Rose, 2010). The release of infectious enteric viruses in the final effluent has also been demonstrated (Simmons and Xagorarakis, 2011; Pusch et al., 2005; Haramoto, Katayama, Phanuwat and Ohgaki, 2008). Insufficiently treated wastewater likewise serves as a wellspring of human enteric viruses in the environment (Okoh, Sibanda and Gusha, 2010). The efficacy of the activated sludge treatment to adequately eliminate enteric viruses in treatment plants has been reported (Prado, Fumian, Miagostovich and Gaspar, 2012; Arraj, Bohatier, Laveran and Traore, 2005; Clarke, Stevenson, Chang and Kabler, 1961).

Trickling filters are generally less effective in removing pathogens than the conventional activated sludge (Henze, 2008; Umbreit, 1966). The report claims that enteric viruses were isolated more frequently from trickling filter effluents than from

the raw sewage influents (Umbreit, 1966). The removal rate of enteric viruses in trickling can vary from 0 to 94% and additional secondary treatment steps (sedimentation) can increase the removal rate to 99.9% (Henze, 2008). Further treatment of treated unchlorinated effluent from trickling filters with additional treatment processes of chemical coagulation-flocculation (with lime at a pH >11), filtration, activated carbon adsorption stage and a final post-disinfection with chlorine can, if properly sequenced in a treatment train and under optimum operating conditions, produce a final product of acceptable microbiological quality (National Research Council, 1982; Pepper, Gerba and Gentry, 2014). This system is fully operational in California with the resultant effect that no viral or bacterial pathogens are found in the final effluent (Nelson, Sheikh and Cooper, 2003).

Enteric viruses are for the most part more impervious to free chlorine than enteric microorganisms, with CT values for 99% inactivation going from about 2 to more than 330mg/min l-1 (LeChevallier and Au, 2004). Enteric viruses stay alive longer than faecal microbes in natural freshwater and longer in cooler climates than hot climates (Mara and Horan, 2003; Lin and Ganesh, 2013).

Human adenoviruses (HAdvs) are a standout amongst the most well-known pathogenic group connected with a few clinical disorders, for example, respiratory, conjunctivitis, and gastroenteritis sicknesses (Kuo et al., 2010). Norovirus (Nov) is the main cause for nonbacterial, intense gastroenteritis in grown-ups, bringing about various episodes around the world. This virus has two virulent strains, GI and GII, with GII strain being the major cause for most outbreaks (Nordgren et al., 2009). Hepatitis A infection

(HAV) is the fundamental cause of intense hepatitis worldwide and has been connected with numerous outbreaks interfaced with sewage tainting of shellfish or to tainted water utilized for drinking, a watering system, vegetable washing or recreational utilization or through the faecal-oral route (Kokkinos, Filippidou, Karlou and Vantarakis, 2010; Morace et al., 2002). Rotaviruses have been perceived as the paramount reason for intense irresistible gastroenteritis among newborn children and youngsters worldwide since their finding in the 1970s. Rotavirus diseases are the significant reason for looseness of the bowels of babies and adolescent youngsters causing gastroenteritis. Several of these waterborne episodes of grown-up gastroenteritis brought about by rotaviruses had been accounted for. Rotaviruses are shed in amazingly high numbers in the faeces of contaminated people, in particular 10^{11} virus particles are shed per gram of stool (He et al., 2008). The genus enterovirus, which includes poliovirus, coxsackievirus A and B, echovirus, and other enteroviruses, can lead to a broad spectrum of manifestations, ranging from asymptomatic infection to serious disease and fatality. The presence of enteroviruses in the environment is a risk to public health (Puig et al., 1994). Enteroviruses are resistant to most concentrations of chlorine used in sewage treatment and they are tolerant to cold and warm temperatures. This makes them ideally suited for survival in the environment. Stability of enteroviruses in the environment is therefore dependent on temperature, humidity, and UV radiation. In order to inactivate 90% of poliovirus in a salt water environment, 671 days at 4 °C is required. On the other hand, an increase in the temperature up to 25 °C reduces the inactivation period by 25 days (Woods, 2010).

Bacteria used as indicators for pathogenic microorganisms in water are not considered adequate as enteric virus indicators. Analysis to identify the presence of enteric viruses as indicators of faecal contamination is important. These will serve as complementary to bacterial indicators, and to reflect the general survival conditions of enteric viruses. The fact that enteric viruses are tolerant to wastewater treatment makes them suitable indicators for the evaluation of treated effluents.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant description

Two wastewater treatment plants (WWTPs) in Eastern Cape of South Africa were examined for selected physicochemical and microbiological properties. These WWTPs are described below.

3.1.1 The WW-Dim Sewage Treatment Works

The WW-Dim Sewage Treatment Works is situated in the Eastern Cape Province of the Buffalo City Municipality with the geographical coordinate of Long. 27°23'47" S and Lat. 32°85'36" E. The plant receives municipal domestic sewage, heavy industrial wastewater, and run-off water. The wastewater treatment plant operates an activated sludge system with design capacity of about 8 ML/day which is considered to be a medium sized treatment plant (DWAF, 2009). The plant treats an average of dry weather flow of 7000 m³/day and an average wet weather flow of 21 000 m³/day.

The influent inlet works has a flow recorder, three grit channels and two screens. There are two aeration tanks, each equipped with three vertically mounted mechanical aerators, two anaerobic tanks and two clarifiers. A splitter box controls the flow of the raw sewage and Return Activated Sludge (RAS) to the aeration tank. In addition, an automated mechanical inlet Huber Screen was installed at the treatment plant in April 2013. This screen assists with the removal of rags and other foreign material from

sewage before it enters the treatment process. Sludge recycling is done through the RAS pump station which helps to haul the sludge from the sedimentation tanks to the aeration tanks. The waste mixed liquor from the aeration tanks is eventually pumped into the sludge lagoons. Chlorination is done by means of a water pressure operated, wall mounted, gas chlorinator in a baffled resistant concrete contact tank. Thereafter the final effluent is discharged into the Mdizeni stream, which is a tributary of the Keiskamma River.

3.1.2 The WW-Ama Central Treatment Work

WW-Ama Central Treatment Works is located on a geographical location of Long. 33°00'59" Sand Lat. 27°51'48" E. The plant is a medium size one with treatment design capacity of 5 ML/Day. The Bio-filter/PETRO (pond enhanced treatment and operation) process treatment system is employed (DWAF, 2009). In an email on the 28th March 2014, L. Jack stated that the final effluent is discharged into the Umzonyana stream.

3.2 Sample collection

Samples were collected on a monthly basis from the final treated effluent (FE) and discharge point (DP). The WW-Ama Centre Treatment Works discharge points are not accessible. Samples were collected in one litre Nalgene bottles previously cleaned by washing in non-ionic detergent, rinsed with tap water and finally rinsed with deionised water and autoclaved prior to usage. Sodium thiosulphate (10%) was added to sampling bottles required for bacteriological, virological analysis and BOD. Also, sulphuric acid (Table 3.1), a preservative, was added to the phosphate nitrate and nitrite bottles.

Samples were then transported in cooler boxes containing ice packs to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory at the University of Fort Hare, Alice, South Africa for analysis. Samples were processed within six hours of collection. Note that the sampling frequency and number of samples are as recommended in the Quality of Domestic Water Supplies Volume 2: Sampling Guide (DWAF, DHE and WRC, 2000). Table 3.1 below shows the methods of preserving the samples collected for various analytes.

Table 3.1:- Methods of preservation for water samples.

Analysis	Preservative	Maximum holding period
Ortho phosphate	2 milliliters sulfuric acid per litre	7 days
Nitrate (NO₃-)	0.8 milliliter sulfuric acid per litre (1 ml was used for the 1.7 litre sample bottles used)	7 days
Nitrate (NO₂-)	0.8 milliliter sulfuric acid per litre (1 ml was used for the 1.7 litre sample bottles used)	7 days
Faecal coliform	4 degrees Celsius, For samples with chlorine residual, Na ₂ SO ₃ solution was added	6 hours
Biochemical Oxygen Demand and Chemical Oxygen Demand	2 millilitres sulphuric acid per litre	7 days
Dissolved Oxygen, Temperature, pH	Must be determined immediately at collection site	

(Fulhage, Sievers and Porter, 1993)

3.3 Physicochemical analysis

Effluent samples were collected in sampling bottles employing the grab sampling method and analyzed as recommended by the South Africa National Standard (SANS, 2011). The sample bottles were filled to the mark, leaving ample air space in the bottle

to facilitate mixing by shaking, before examination. Sample bottles were filled without rinsing and care was taken so as not to contaminate the inner surface of the stopper or cap. The bottle cap was replaced immediately.

The BOD samples were collected in 300 ml containers and a glass stopper was carefully placed in order to avoid trapping the sample with air. For bacteriological and virology analysis, samples of water were collected in pre-sterilized bottles containing 10% sodium thiosulphate and preserved at 4 °C. The following physicochemical parameters were measured on site as concentrations can significantly change if samples were transported or stored in storage:

- Dissolved oxygen (DO)
- Temperature
- pH
- Conductivity
- Turbidity

Temperature, pH, electrical conductivity (EC), total dissolved solid (TDS), and dissolved oxygen (DO), were all determined *on site* using the multi-parameter ion specific meter (Hanna-BDH laboratory supplies). Turbidity was determined *on site* using a microprocessor turbidity meter (HACH Company, model 2100P). The concentrations of orthophosphate as P, nitrate, nitrite, and chemical oxygen demand (COD) were determined in the laboratory by the standard photometric method using the Spectroquant Pharo 100 Photometer (Merck Pty Ltd). Samples for COD analysis were

digested with a Thermoreactor model TR 300 (Merck Pty Ltd) prior to analysis using the Spectroquant Pharo 100 photometer. Biological Oxygen Demand (BOD₅) was measured using the procedure BOD₅ days. The instruments were calibrated to ensure accurate results following the manufacturer's instructions supplied with the equipment. Other parameters, like the chemical oxygen demand, phosphate, nitrate and nitrite were analyzed in the laboratory using standard methods as describe by Merck. Merck cell tests (Merck, VWR International, Poole, UK) were used for the following tests: chemical oxygen demand (COD) (100-1500mg L⁻¹), Nitrite nitrogen (NO₂-N) (0.02 – 1.00 mg L⁻¹), Nitrate nitrogen (NO₃-N), (0.5-20 mg L⁻¹), Phosphate phosphorous (PO₄-P) (0.05-5.0 mg L⁻¹).

3.3.1 Quality control

Facility: Tests were done in a well-ventilated laboratory to reduce contamination which permits a more stable operation in the fume and decreased moisture problems with media and instruments. The work areas were kept clean and free of unnecessary chemicals. At the end of the tests, the work bench surfaces were wiped with an appropriate disinfecting solution (typically a bleach solution). Spillages were cleaned with a sorbent material to soak up the spill and the used sorbent was disposed of in the proper disposal container (Biohazard bag for on-campus disposal of biohazardous materials).

Laboratory equipment and instrumentation: Two incubators were used for testing *E. coli*, *Vibrio* and faecal coliform. The temperatures were maintained at 37± 0.5 °C for *Vibrio*, *E. coli* and *E. coli* O157:H7 while faecal coliform was maintained at 44.5 ± 0.5

°C. A glass thermometer with its bulb and stem submerged in water kept in a beaker inside the incubator was used to validate and monitor the incubator temperature. The water levels in the beakers were intermittently checked to ensure that the bulb and stem of the thermometers were always submerged.

Sample blanks were used to check for any form of contamination so as to ensure quality assurance. All blanks were analysed for the same parameters under study. Sample blanks used were Trip blanks, Field blanks and Equipment blanks.

Trip blank: A reagent bottle was filled with distilled water and placed with other sampling bottles.

Field blank: A sampling bottle was filled with distilled water *on site*. This was check for any form of contamination during sampling collection.

Equipment blank: Distilled water was run through the sampling equipment and stored away in a sampling bottle.

Selective media were tested with test organisms which would be expected to grow, differentiating them from others. Test strains were bought from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ).

All media used for bacterial analyses were performance checked. Sterility was assessed as well as the proper reaction with appropriate positive and negative control organisms. Additionally, all bacterial tests were conducted with positive controls run simultaneously with each assay. Negative controls were also done with faecal coliform and *E. coli* analysis.

3.3.2 Procedure for sample preparation for analysis

Samples were made ready in preparation for serial dilutions. In certain cases where there was excessive chlorine dosage in the effluent, the raw samples were not diluted before filtration. All sample dilutions were homogenized before filtering. The filtered samples were placed on selective agars for the target organisms. The plates were allowed 15 minutes to dry in an inverted position and were incubated promptly at the appropriate temperature and condition.

After incubation, counting was done in triplicate plates at a suitable range (0-300 colonies) using manual counting and a tally register was done recording results per dilution plate counted.

3.4 Bacteriological analysis

3.4.1 Enumeration and identification of faecal indicator bacteria (FIB)

Analysis of faecal indicator bacteria counts was determined by membrane filtration according to SANS (2011). Concentration of bacterial pathogens is usually performed by membrane filtration, although turbidity of water can severely inhibit the volume of water that can be passed through the filter and therefore suitable dilution should be made (Rogers and Haines, 2005).

3.4.2 Faecal coliforms

Faecal coliforms were examined by a membrane filtration method. The enumeration of faecal coliform involved effluent water samples serially diluted (for samples considered to have low levels of chlorine) (APHA, AWWA and WEF, 2012; SANS, 2011). This

technique involves the filtration of a measured volume of sample (100 ml) through a filter membrane (47 mm, 0.45 µm pore size), the contents of which are then transferred onto m-FC agar and incubated at 44.5 °C for 24 hours. The target colonies appearing as blue or magenta in colour were counted and reported as CFU/100 ml (SANS, 2011).

3.4.3 *Escherichia coli* (*E. coli*)

E. coli coliforms chromogenic Agar (Conda, Madrid) was used for the isolation of *E. coli*. It is used for the differentiation of *E. coli* from the rest of *Enterobacteriaceae*. *E. coli* is easily distinguishable due to the dark blue-greenish blue colony colour.

E. coli was examined as described above. The filters were placed on *E. coli* coliforms chromogenic agar and incubated at 37 °C for 24 hours. The target colonies appeared as dark blue in colour and were counted and reported as CFU/100 ml SABS (2011).

3.4.4 Presumptive *E. coli* O157:H7 enumeration and isolation

E. coli O157:H7 chromogenic agar base is a selective and differential medium for the detection of *E. coli* O157:H7. The chromogenic mixture allows for easy detection of the presence of *E. coli* O157:H7 by colony coloration that grows pale pink. The addition of Potassium tellurite and cefixime are highly selective for *E. coli* O157:H7 and inhibit most contaminating bacteria including other *E. coli* strains and coliforms.

E. coli O157:H7 was examined as described above. The filters were placed on *E. coli* O157:H7 chromogenic agar base (Conda, Madrid) supplemented with cefixime tellurite and incubated at 37 °C for 24 hr. The target colonies appeared as pale pink in colour and it was counted and reported as CFU/100 ml presumptive *E. coli* O157:H7.

3.4.5 *Vibrio* bacteria

Thiosulfate citrate bile salts sucrose agar (TCBS) is a selective media for isolation of *Vibrio* from samples. Sodium thiosulfate provides sulphur, and ferric citrate is the indicator for H₂S production. Sucrose is the carbohydrate energy source. Bromothymol blue and Thymol blue are pH indicators. Sodium chloride promotes growth (*Vibrio* grows well in salty media). Sucrose-negative species, such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*, produce blue-green colonies. Most *Vibrio* ferment sucrose and yellow colonies are formed as a result of acid production.

Enumerations of presumptive *Vibrio* pathogens were carried out by the method described above on sterile TCBS agar plates as described by Bopp et al. (1999). Presumptive *Vibrio* bacteria was isolated from the plates, purified and subjected to molecular identification. Polymerase chain reaction (PCR) was used to confirm the identities of the *Vibrio* species using the species specific primers as described by Tarr et al. (2007).

Salt tolerance: presumptive isolates from the TCBS culture were inoculated into 1 tube each of 1% tryptone broth containing 2% NaCl and incubated 18-24 h at 35-37°C. Profuse growths in tubes are considered as positive. All *Vibrio* spp. can grow at a salt concentration of 3% NaCl (Kaysner and DePaola, 2004). Various species have different salt tolerance that can be used for identification. This test helps to eliminate presumptive colonies from the TCBS plate which resemble *Vibrio*, e.g. *Proteus*.

3.4.6 Isolates preservation

The isolates were taken as presumptive from the selective media on which they were grown based on their phenotypic identification and sub-cultured for purification. Presumptive *E. coli* O157:H7 and *E. coli* isolates were prepared in Luria broth and stored in 15% glycerol at -80 °C. *Vibrio* was prepared in 2% salt Tryptone Soy broth and stored in 15% glycerol stock.

3.4.7 *E. coli* O157 latex agglutination assay

Each of the presumptive isolates was streaked into a Sorbitol Mac-Conkey Agar supplemented with cefixime and tellurite plate from a 24 hour growth culture and incubated for 18 hours at 37 °C. Sorbitol-negative or non-Sorbitol fermenters (NSF) were tested for agglutination by the Prolex *E. coli* O157 latex test reagent kit (Pro-lab, Canada).

3.4.8 Sensitivity and Specificity Testing for *E. coli* and *Vibrio*

A stock of the positives controls for *E. coli* (ATCC 29522) and *Vibrio* strains collections (Table 3.2) was screened as pure culture using the selective medium for each target organism. The PCR-based detection system was used to identify those strains that would be recognized as *E. coli* and *Vibrio*. For this experiment, pure cultures were grown overnight in TSB broth and then spread plated onto the selective agar plates. A portion of the broth samples and isolates from the agar plates were used for PCR detection and confirmation. Detection experiment using this complete process was conducted to determine whether the selective growth media would prevent the false-positive detection of these organisms.

3.4.9 Control isolates

Six *E. coli* strains purchased from DSMZ were used. The strains comprised of Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), Uropathogenic *E. coli* (UPEC), Enteropathogenic *E. coli* (EPEC), and Neonatal *E. coli* (NMEC) were used as positive controls. Three *Vibrio* pathotypes were worked upon as against the four proposed. The limitation was due to the unavailability of the fourth reference strain. The positive controls include *Vibrio fluvialis*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*.

Table 3.2:- Reference strains and reference number.

Reference strains	Reference number
<i>Escherichia coli</i>	ATCC 29522
NMEC	DSM 10819
EPEC	DSM 8695
UPEC	DSM 4618
ETEC	DSM 10973
EAEC	DSM 10974
EIEC	DSM 9025
<i>Vibrio parahaemolyticus</i>	DSM 11058
<i>Vibrio vulnificus</i>	DSM 11507
<i>Vibrio fluvialis</i>	DSM 19283

3.4.10 Environmental isolates

A total of 540 environmental isolates of *E. coli* O157:H7, 843 isolates of *E. coli* and 786 isolates of *Vibrio* were examined.

3.4.11 Genotypic identification of *E. coli*, *E. coli* O157:H7 and *Vibrio* isolation of genomic DNA

Isolates were grown in Luria broth (LB) for all *E. coli* isolates and TSB broth for *Vibrio* isolates. LB medium is a rich medium that is commonly used to culture members of the *Enterobacteriaceae*. Bacteria from the freeze storage were inoculated overnight for crude DNA extraction. Frozen cells were kept on ice to reduce thawing by scraping the ice surface with a loop. It was possible to transfer a few bacteria to the tube with broth media.

ZR Fungal/Bacterial DNA MiniPrep by Zymo Research was used to isolate genomic DNA following the manufacturer's instruction; Genomic extract was immediately used in the molecular identification of the isolated organisms.

3.4.12 Polymerase Chain Reaction (PCR)

Primers specific for the confirmation of the *E. coli* and *Vibrio* isolates were used in the polymerase chain reaction. The primer sequences are listed in Table 3.3 and Table 3.4. In this study, the conventional PCR was used. It was essential to test the analytical sensitivity and specificity of the PCR on micro-organisms *in vitro* prior to application to the environmental samples. The positive controls for each pathotypes were tested; the assays were able to detect the targets genes.

The PCR reaction was carried out in 25 μ l reaction volume containing 12.5 μ l of 2 \times PCR master mixes (Fementas), 10 pmol of 1 μ l for each primer (Inaqba, SA) stated in Table 3.3 and Table 3.4 and 6.5 μ l of Nuclease free water. A total volume of 5 μ l

genomic DNA was used in each PCR reaction. The reaction was carried out in a Bio-Rad Thermo-Cycler (Bio-Rad, SA).

3.4.13 Genotypic identification of *E. coli* pathotypes

The identification and prevalence of the 8 recognized *E. coli* pathotypes were assessed targeting the genes used as shown in Table 3.3 below.

Table 3.3:-Primer pairs, expected amplicon size, PCR cycling conditions and the corresponding references for characterization of *E. coli* pathotypes.

Target strains	Target genes	Primer sequence (5'→3')	Amplicon size (bp)	References
EPEC	eae	TCA ATG CAG TTC CGT TAT CAG TT GTA AAG TCC GTT ACC CCA ACC TG	482	(Vidal et al., 2005)
ETEC	lt	GCA CAC GGA GCT CCT CAG TC TCC TTC ATC CTT TCA ATG GCT TT	218	
EIEC	ipaH	CTC GGC ACG TTT TAA TAG TCT GG GTG GAG AGC TGA AGT TTC TCT GC	933	
EAEC	Eagg	AGA CTC TGG CGA AAG ACT GTA TCATG GCT GTC TGT AAT AGA TGA GAA C	194	(Omar and Barnard, 2010)
DAEC	daaE	GAA CGT TGG TTA ATG TGG GGT AA TAT TCA CCG GTC GGT TAT CAG T	542	(Vidal et al., 2005)
UPEC	pap	GACGGCTGTA CTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	(Abe et al., 2008)
NMEC	IbeA	ibeA-F- TTACCGCCGTTGATGTTATCA ibeA-R- CATTAGCTCTCGGTTACGCT	171	(Watt and Lanotte, 2003)
<i>E. coli</i>	uidA	AAA ACG GCA AGA AAA AGC AG ACG CGT GGT TAA CAG TCT TGC G	147	(Dungeni, van Der Merwe and Momba, 2010)

The optimized cycling conditions used were as follows:

- The reaction conditions for UPEC and NMEC were as follows: initial denaturation at 94 °C for 2 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at the melting temperature of each primer at 55 °C for 1 min, and extension at 72 °C for 1 min, followed by final 5-min extension period at 72 °C.
- For EAEC and EPEC: initial denaturation at 95 °C for 15 min followed by 35 cycles of denaturation at 94 °C for 45sec, annealing at the melting temperature of each primer at 55 °C for 45sec, and extension at 68 °C for 2 min, followed by a final 5-min extension period at 72 °C.
- For ETEC: initial denaturation at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at the melting temperature of each primer at 58 °C for 30 min, and extension at 72 °C for 20 sec, followed by a final 5-min extension period at 72 °C.
- For EIEC: initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 30, annealing at the melting temperature of each primer at 60 °C for 20 sec, and extension at 72 °C for 1 min, followed by a final 5-min extension period at 72 °C.
- For DAEC: initial denaturation at 94 °C for 2 min followed by 30 cycles of denaturation at 92 °C for 30 sec, annealing at the melting temperature of each primer at 59 °C for 30 sec, and extension at 72 °C for 5 min, followed by a final 5-min extension period at 72 °C.

3.4.14 Genotypic identification of *Vibrio* species

The confirmation of the *Vibrio* species was done using the set of primers stated in Table 3.4 below:

Table 3.4:-Primer pairs, expected amplicon size, PCR cycling conditions and the corresponding references for characterization of *Vibrio*. spp.

Target species	Primer	Sequences (5'-3')	Target gene	Amplicon size (bp)	Reference
<i>All Vibrio spp.</i>	V. 16S-700F	CGG TGA AAT GCG TAG AGA T	16S rRNA	663	(Tarr et al., 2007)
	V. 16S-1325R	TTA CTA GCG ATT CCG AGT TC			
	<i>V. parahaemolyticus</i>	Vp. flaE79F GCA GCT GAT CAA AAC GTT GAG T			
	Vp. flaE934R	ATT ATC GAT CGT GCC ACT CAC			
<i>V. vulnificus</i>	Vv. hsp-326F	GTC TTA AAG CGG TTG CTG C	hsp60	410	
	Vv. hsp-697R	CGC TTC AAG TGC TGG TAG AAG			
<i>V. fluvialis</i>	Vf-toxR F	GAC CAG GGC TTT GAG GTG GAC GAC	toxR	217	(Chakraborty et al., 2006)
	Vf-toxR R	AGG ATA CGG CAC TTG AGT AAG ACTC			

The cycling condition used was as stated below:

The thermal cycling profile was as follows: a 15 min initial denaturation at 93 °C followed by 35 cycles of 92 °C for 40 sec, 57 °C for 1 min, and 72 °C for 1.5 min and a final soak at 72 °C for 7 min (Tarr et al., 2007).

3.4.15 Agarose gel electrophoresis

Gel electrophoresis was performed on the PCR product and ran on a 2% w/v agarose gel at 95 V for approximately one hour.

Procedure:

2 % agarose (Thermo Scientific Top Vision) in 0.5x TBE buffer was made (the agarose was dissolved by boiling the solution in microwave oven). 0.5 µg/ ml EtBr (laboratory prepared) was added for staining the DNA molecules. The agarose-EtBr solution was poured into the gel tray of the electrophoresis apparatus containing the combs and allowed to set for about 20 minutes. 3µℓ of each PCR product added to 2µℓ of loading dye was loaded into the gel wells. 3µℓ of 100bp DNA molecular size marker (Thermo Scientific) was loaded into the flanking wells. The electrophoresis was run at 95V for approximately 1 hour and 30 minutes. The gel was visualized on Alliance 4.7 (Uvitec, UK) and stored on disks as JPEG files.

3.5 Antimicrobial susceptibility test

Antimicrobial susceptibility testing was done using the standard disc diffusion method on Mueller-Hinton agar (MH) (Conda, Madrid) as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012). Fresh colonies (about 18 hrs old) from nutrient agar culture plates were picked into test tubes containing 5 ml sterile normal saline. The turbidity of the suspension was adjusted to 0.5 McFarland standards. Sterile swabs were then deepen into the bacterial suspensions and used to inoculate the MH agar plates by spreading uniformly on the surface of the agar. The plates were left to air dry for 5 minutes after which the antibiotic discs were impregnated on the bacterial

lawn using a disc dispenser and the plates were then incubated within fifteen minutes of bacterial inoculation at 35 ± 2 °C for 18 to 24 hrs (Hudzicki, 2013). Zones of growth inhibition were measured to the nearest millimeter using the caliper and results interpreted according to the guidelines of Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing breakpoints for Enterobacteriaceae (CLSI, 2012). *Escherichia coli* ATCC 43895 was included as a positive control.

Selection of antimicrobial is based on the type of organism being tested and source of the isolates (CLSI, 2012). Also, the antibiotics were selected as representatives of different classes of antibacterial drugs, to better depict the behaviour of the examined strains against these molecules. The Antimicrobial susceptibility test for *E. coli* isolates was determined using the following antibiotic discs were used: ampicillin (10 µg), cefotaxime (30 µg), gentamicin (10 µg), meropenem (10 µg), tetracycline (30 µg), and cephalothin (30 µg) (Davies Diagnostics, SA) (CLSI, 2012). The antibiotic susceptibility testing for *Vibrio* isolates was determined using the following antibiotic discs: ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), cefotaxime (30 µg), Trimethoprim-sulfamethoxazole (1.25/23.75 µg), and ciprofloxacin (5 µg) (CLSI, 2010).

3.6 Virological analyses

3.6.1 Concentration of water samples for viral detection

Viruses in effluent sample were concentrated following the adsorption-elution method as described by Haramoto et al. (2009) with some modifications. Five millilitres of 250mM AlCl₃ was passed through a Millipore HA filter after five minutes (0.45µm

pore size and 47mm diameter) to form a cation (Al^{3+}) coated filter that was attached to a 250 ml Millipore Sterile filtration system on 3 station filtration manifolds. A total of 1, 250 litres of the water sample was passed through the filter. A volume of 200 ml of 0.5mM H_2SO_4 was then filtered through the membrane and viral particles were eluted with 10 ml of 1mM NaOH in a petri dish. Eluates were placed in a Centriprep Centrifugal Filter Unit with Ultracel-50 membrane containing 0.1 ml of 50mM H_2SO_4 and 0.1 ml of 100 × Tris-EDTA (TE) buffer for neutralization before further concentration. The Centriprep YM-50 ultra-filtration device (Millipore) was centrifuged to obtain a final volume of approximately 700 μ l. In exceptional cases where elutes were turbid, the centrifuging time was increased and the clogged membrane was cleared with sterile forceps. Further filtration and concentration of more effluent sample was done to have a final volume of 1.4 ml concentrate. The concentrated samples were stored at -80 °C till ready for use.

3.6.2 Extraction of viral nucleic acids

Viral nucleic acids were extracted from 200 μ l of concentrated effluent samples by the use of a Zymo gDNA and Zymo viral RNA extraction kit using the spin column technique according to the manufacturer's instructions (Zymo Research). All samples were tested for the presence of Rotavirus, Adenovirus, and Hepatitis A Virus nucleic acids by real time PCR.

3.6.3 Quantification of viral genome by real time PCR reaction

RNA viruses were quantified in a two-step protocol where RNA was first transcribed into cDNA in a separate reverse-transcription step. Briefly, 10 μ l of template RNA, 1 μ l

of Random Hexamer Primer, 1 μ l dNTP mix, 2.5 μ l DEPC-treated water, 4 μ l 5X RT buffer, 0.5 μ l Ribolock RNase inhibitor and 1 μ l RevertAid Premium Reverse Transcriptase (Fermentas Life Sciences) were added in the indicated order into a 0.5 ml PCR tube on ice. The mixture was briefly vortexed to ensure total mixing and thereafter centrifuged. The tubes were then incubated at 25 °C for 10 min followed by 30 min at 60 °C. The reaction was terminated by heating at 85 °C for 5 min. An aliquot of 5 μ l of the resultant cDNA was used as template in a quantitative real time PCR reaction containing reagents in the same proportions with those used for Adenovirus. Fluorescence data were collected at the end of the annealing step.

For rotavirus, prior to reverse transcription, sample RNA was subjected to denaturation at 95°C for 5 min followed by flash chilling in ice for 2 min, to separate the rotavirus dsRNA (Jothikumar, Kang and Hill, 2009).

3.6.4 Detection of enteric viruses

Since the PCR reaction amplifies DNA directly, and enteroviruses are RNA viruses, the RNA was first converted to complement DNA through an initial step called reverse transcription described above. This was accomplished through the use of an enzyme called reverse transcriptase. The enzyme can read the RNA sequence and synthesize a complementary strand of DNA (cDNA). After this, the real time PCR was initiated. This two-step process is called Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) (Seidel, 2003). Quantification of enteric virus by qPCR was done following a one-step reaction in a 96-well plate. The wells were loaded with 20 μ l of a reaction buffer containing 12.5 μ l of 2x TaqMan universal PCR MasterMix Applied

Biosystems, 400nM forward primer, 400nM reverse primer, and 250nM TaqMan probe and PCR grade water. Then, 5 μ l aliquots of sample cDNA were added with the mixture to give 25 μ l total reaction mixtures. The thermal cycling protocols used for the viruses are given below:

Enterovirus: Taq activation at 95 °C for 10 min; 45 cycles of denaturation at 94 °C for 15 sec, annealing at 58 °C for 1 min, and extension at 72 °C for 20 sec.

3.6.5 Detection of rotavirus (RoV)

The RT-PCR method was used for the detection of Rotavirus virus. The steps taken were as done for enteric virus. The thermal cycling protocols used for the respective viruses are given below:

Rotavirus: Taq activation at 95 °C for 15 min; 45 cycles of denaturation at 95 °C for 15 sec, annealing at 55 °C for 30 sec, and extension at 72 °C for 30 sec.

3.6.6 Hepatitis A virus (HAV) detection

The real time PCR method was used for the detection of Hepatitis A virus. The steps taken were as done for enteric virus. The thermal cycling protocols used for the respective viruses are given below:

HAV: 10 min at 95 °C for Taq activation, and 45 cycles of denaturation at 95 °C for 15 sec, annealing at 60 °C for 1 min, and extension at 70 °C for 1 min.

3.6.7 Adenovirus (AdV) detection

Positive controls for Adenovirus were extracted and DNA obtained from the cell lysate of Adenovirus type 40 Dugan strain (ATCC VR-931) was used in the PCR reactions.

The RT-PCR cycling protocol and reaction component concentrations were optimized for detection of the hexon gene of the virus.

Extraction of HAd DNA was performed using a Zymo DNA extraction kit according to the manufacturer's instructions and stored at -80 °C until use. PCR amplification was carried out in 0.2 ml volumes containing 25µl of reaction mixture. Quantification of AdV by qPCR was done following a one-step reaction in a 96-well plate. The wells were loaded with 20µl of a reaction buffer containing 12.5µl of 2x TaqMan universal PCR MasterMix Applied Biosystems, 400nM forward primer, 400nM reverse primer, and 250nM TaqMan probe and PCR grade water. Then, 5µl aliquots of sample DNA were added with mixing to give 25µl total reaction mixtures. Amplification was performed on a StepOne Plus real time PCR System thermal cycler (Applied Biosystems) with preliminary denaturation; 15 min at 95 °C for Taq activation, followed by 45 cycles of denaturation at 95 °C for 10 sec, annealing at 55°C for 30 sec, and extension at 72 °C for 20 sec (Sibanda and Okoh, 2012). The primers and probes used for the real time PCR are shown in the Table 3.5.

Table 3.5:- Primers and Probes for One-step Real-time RT-PCR and qPCR

Enteric Virus	Primers and Labelled TaqMan Probe	Reference
Hepatitis A Virus	HAV68 (F): 5'-TCA CCG CCG TTT GCC TAG-3' HAV240 (R): 5'-GGA GAG CCC TGG AAG AAA G-3' HAV150 (P): 5'-FAM-CCT GAA CCT GCA GGA ATT AA-MGBNFQ-3'	(Costafreda, Bosch and Pintó, 2006; Pintó, Costafreda and Bosch, 2009)
Rotavirus	JVK (F): 5'-CAGTGGTTGATGCTCAAGATGGA-3' JVK (R): 5'-TCATTGTAATCATATTGAATACCCA-3' JVK (P): 5'-FAM-ACAACCTGCAGCTTCAAAGAAGWGT-BHQ-3'	(Jothikumar, Kang and Hill, 2009)
Adenovirus	JTVX(F) 5'-GGACGCCTCGGAGTACCTGAG-3' JTVX(R) 5'-ACIGTGGGGTTTCTGAACTTGTT-3' JTVX(P) 5'-FAM-CTGGTGCAGTTCGCCCGTGCCA-MGBFQ-3'	(Jothikumar et al., 2005)
Enteroviruses	EV1 (F): 5'-CCCTGAATGCGGCTAAT-3' EV1 (R): 5'-TGTCACCATA AGCAGCCA-3' EV-BHQ (P): 5'-FAM-ACGGACACCCAAAGTAGTCGGTTC-BHQ-1-3'	(Gregory, Litaker and Noble, 2006; Noble et al., 2006)
Abbreviations: F, forward/sense; R, reverse/antisense; P, probe; FAM, 6-carboxyfluorescein (reporter dye); MGBNFQ, minor groove binder/nonfluorescent quencher; TAMRA, 6-carboxy-tetramethylrhodamine (quencher dye); BHQ, black hole quencher.		

3.6.8 Detection of viral serotypes

Adenovirus Serotypes

Serotype-specific PCR assays and PCR conditions as described by Metzgar, Osuna and Yingst (2005) for species B to E serotypes and species F serotypes by Tiemessen and Nel (1996) were used for the serotypes identification. The primers used here are shown

in Table 3.6 below. For quality assurances, the specific virus strains were used as controls.

Table 3.6:-Primers for Detection of Adenovirus Serotypes

Species	Serotype	Primer	Sequence (5' to 3')	Target Region
B	Ad3	Ad3F Ad3R	GGTAGAGATGCTGTTGCAGGA CCCATCCATTAGTGTCATCGGT	Ad3 hexon
	Ad7	Ad7F Ad7R	GGAAAGACATTACTGCAGACA AATTTTCAGGCGAAAAAGCGTCA	Ad7 hexon
	Ad21	Ad21F Ad21R	GAAATTACAGACGGCGAAGCC AACCTGCTGGTTTTGCGGTTG	Ad21 hexon
C	Ad1	AdCF Ad1R	TGCTTGCGCTHAAAATGGGCA CGAGTATAAGACGCCTATTTACA	AdC fibre Ad1 fibre
	Ad2	Ad2R	CGCTAAGAGCGCCGCTAGTA	Ad2 fibre
	Ad5	Ad5R	ATGCAAAGGAGCCCCGTAC	Ad5 fibre
	Ad6	Ad6R	CTTGCAGTCTTTATCTGAAGCA	Ad6 fibre
E	Ad4	Adeno4.U3 Adeno4.L1	CAAGGACTACCAGGCCGTCA TTAGCATAGAGCATGTTCTGGC	Ad4 hexon
F	Ad40	AdF1 K402	ACTTAATGCTGACACGGGCAC CAC TTA ATG CTG ACA CG	Fiber
	Ad41	K403	ACT GGA TAG AGC TAG CG	

Rotavirus Groups A, B and C

Human rotaviruses, Group A, B and C were detected by PCR amplification of the inner capsid protein VP6, as described by Lai et al. (2005) using the primers listed in Table 3.7.

Table 3.7:-Primers for Detection of Rotavirus Groups

Species	Primer	Sequence (5' – 3')	Amplicon size (bp)
A	Beg9 End9	GGCTTTAAAAGAGAGAATTTCCGTCTGG GGTCACATCATAACAATTCTAATCTAAG	1062
B	GRB-1F GRB-1R	CTATTCAGTGTGTCG TGAGAGG CGTGGCTTTGGAAAATTCTTG	498
C	G8S G8A	GGCATTTAAAAAAGAAGAAGCTGT AGCCACATGATCTTGTTTACGC	1063

3.6.9 Sensitivity and Specificity Testing

All the individual primers/probes sets were tested individually against the DNA and cDNA extracted from stock viruses purchased from ATCC individually (Table 3.8). The primers/probes set amplified the target viruses only and no cross reactions were found with the primers/probes. This method was as described by Huang et al. (2009).

The sensitivity of our Real PCR assay was evaluated with the nucleic acid of the viral stock culture of Rotavirus, Coxsackie A virus, Hepatitis A virus and Adenovirus DNA from a serial 7-fold dilution of the genetic extracts. To validate the real-time PCR assays prior to application to effluents samples, the detection limit and amplification efficiency of each reaction were as determined by Simmons and Xagorarakis (2011).

3.6.9.1 *Detection limit of real time PCR and quality control*

The detection limit of the real time PCR was determined by using quantified Adenovirus DNA from ATCC. The quantified DNA was diluted in Nuclease free water in a dilution series of 1:10 to find the lowest detectable concentration. This was performed three times.

In each DNA isolation and PCR set up, controls were used to monitor the protocols. Human Adenovirus 40 ATCC VR-931-Strain Dugan was used as Positive PCR Control (PPC) and nuclease free water was used as Negative PCR Control (NPC). In addition each PCR set up was run by including a purified DNA from Adenovirus 40 as a positive template control (PTC), and water as “No template control” (NTC). The controls were consistently evaluated before the other results and, if they passed, an evaluation of the other reactions followed. If the control gives the expected results in each run, this indicates that the isolation and PCR reaction were accurate

3.6.10 Viral controls

Each virus test included two controls, a positive control consisting of a spiked sample containing a viral concentration near the detection limit of the method and a negative control consisting of PCR-grade water and Mastermix. The control strains used as shown in Table 3.8 below was obtained from ATCC and preserved at -80 °C.

Table 3.8:-Control strains

Virus	Reference Number	Strain
Human Rotavirus	ATCC VR-2274	Strain 248
Human Adenovirus 40	ATCC VR-931	Strain Dugan
Human Adenovirus 41	ATCC VR-930	Strain Tak (73-3544)
Human Adenovirus 2	ATCC VR-846	Strain Adenoid 6
Human Adenovirus 6	ATCC VR-6	Strain Tonsil 99
Human Adenovirus 7	ATCC VR-7	Strain Gomen
Human Adenovirus 3	ATCC VR-3	Strain GB
Human Adenovirus 1	ATCC VR-1	Strain Adenoid 71
Adenovirus T 21	ATCC(R) VR- 256	Strain AV 1645
Human Adenovirus 4	ATCC VR-1572	Strain R1-67
Adenovirus 5	ATCC VR-1516	
Hepatitis A Virus	ATCC VR-1357	Strain PA21
Coxsackie virus A2	ATCC VR-1550	Strain Fleetwood

3.6.11 Interpretation of PCR results

The results from the real time PCR were interpreted according to the software guidelines from the manufacture (Software ABI™ StepOnePlus Real-Time PCR System, Applied Biosystems). All reactions with signals from the target probe were assigned positive. All results with no signal from the target probe and with amplified

internal amplification Control (IAC) were designated negative. Furthermore, target probes without signals were considered to lack the target.

3.7 Statistical methods

This post hoc exploratory study examined the relationships between concentrations of bacterial indicators and a variety of physicochemical parameters. Concentrations of enteric viruses, *E. coli*, faecal coliform and *Vibrio* bacteria were compared and contrasted based on differences in sampling location months and physicochemical parameters using descriptive statistics. A one sample t-Test was then performed to determine the level of significance between the data as it compares to the regulatory standards, which parameter factors had the strongest influence, and the total amount of variation that could be explained by the study. The t-Test and Wilcoxon Signed Rank Test were used to determine if any significance difference existed between the effluent monitored parameters and the set standards. The t-Test was used when observed parameters were normally distributed, while the Wilcoxon Signed Rank Test was employed with non-normal distribution. IBM SPSS statistics 22 software, a programme by IBM, Armonk, NY, USA (2014), was used to determine the t-test statistic using a 95% level of confidence, $\alpha = 0.05$ and $P = 0.05$. The mean difference is regarded as highly significant and statistically significant if P value is lower than 0.05 and non-significant if P value is higher than 0.05.

Note that the presentations of the results in the discussion section follow the steps below:

(FE=value, DP=value, P = 95% level of confidence)

Value = Mean difference

Final effluent (FE) and Discharge point (DP)

Mean difference = Mean of measured parameter – Mean of standard limit.

CHAPTER FOUR

RESULTS

This chapter presents the results of the physicochemical, bacteriological and virological analyses. Summary of Tables are included in this chapter, with explanations of the results.

4.1 Physicochemical characteristic of the wastewater effluent

Results for the evaluated physicochemical parameters of effluents of the two wastewater treatments plants are compared against the South African recommended standards for effluent discharge (Table 4.1) and the results are shown in Tables 4.2 and 4.3.

4.1.1 pH

The pH of the final effluent samples of WW-Ama WWPT ranged between 7.35 and 8.56 as shown in Table 4.2. The pH range was 4.16 to 7.82 at the WW-Dim discharge point and 3.89 to 7.50 at the WW-Dim Final Effluent point as shown in Table 4.3. The average value of the pH during the monitoring period from the WWTP for WW-Dim Treatment Plant was 6.42 at the final effluent point and 6.70 at the discharge point of the outflow, while it was 7.95 for WW-Ama central work. The recommended pH level is between 5.5 – 9.5 for general limit and 5.5 – 7.0 for special limit. The measured pH at both WWTPs is within the specified limits. Low pH level was observed twice at the WW-Dim WWTP for the month of September 2012 and May 2013. This was due to the extremely high chlorine dosage at the plant for those months. The mean pH values for

the final effluent and the discharge point were within both the recommended special and general limits of pH value of 5.5 – 7.5/9.5, with a statistically significant difference at pH 5.5 (FE = 0.92, $p = 0.01$; DP=1.20, $p=0.003$) and at pH 9.5 (FE = -3.78, $p<0.05$, DP = -3.47, $p<0.05$). The WW-Ama Treatment Plant has pH within the set standard. The mean pH values for the final effluent was within the recommended general limit of pH value of 5.5 – 9.5, with a statistically significant difference at pH 5.5 (FE=2.45, $p<0.05$) and pH 9.5 (FE = -1.54, $p<0.05$).

Table 4.1:-List of recommended parameters and limits

PARAMETERS, UNITS	Regulatory Guidelines		
	General limit	Special limit	Reference
Total Dissolved Solid (mg/l)	450		(DWAF, 1996b)
pH	5.5-9.5	5.5-7.5	(DWAF, 2013)
Electrical Conductivity (mS/m)	70 above intake to a maximum of 150	50 above background Receiving water, to a maximum of 100	
Temperature (°C)	natural ambient water temperature of the receiving water resource should not increase by more than 2 - 3 degrees Celsius		
Free chlorine (mg/l)	0.25	0	
Nitrite (NO₂⁻) (mg/l)	15	1.5	
Nitrate (NO₃⁻) (mg/l)	15	1.5	
Phosphate (P) (mg/l)	10	1	
Chemical Oxygen Demand (COD) (mg/l)	75 after removal of algae	30	
Biochemical Oxygen Demand (BOD)	3-6		(European Parliament, 2006)
Dissolved Oxygen (mg/l)	≥5		(WHO, 2006)
Turbidity (NTU)	<5 NTU		(WHO, 2008)

Note: - There are no South Africa regulatory set guidelines for effluent quality discharge for Total Dissolved Solids (TDS), Biochemical Oxygen Demand (BOD), Turbidity and Dissolved Oxygen.

Table 4.2:- The average means of the measured physiochemical parameters for WW-Ama Central Treatment Works.

Parameters	September 2012	October 2012	November 2012	December 2012	January 2013	February 2013	March 2013	April 2013	May 2013	June 2013	July 2013	August 2013	
pH	7.4 ± 0.2	7.4 ± 0.1	8.1 ± 0.1	8.6 ± 0.2	7.4 ± 0.1	8.3 ± 0.2	8.2 ± 0	8.4 ± 0.1	8.4 ± 0.1	8.3 ± 0.2	7.4 ± 0.1	7.6 ± 0	**
Turbidity (NTU)	16.7 ± 0.60	16.7 ± 0.8	7.6 ± 0.4	11.1 ± 0.3	5.4 ± 0.2	11.9 ± 0.1	13 ± 1.2	4.8 ± 0.1	9.9 ± 0.1	43 ± 0.3	42.3 ± 3.8	26.3 ± 0.6	
EC(µS/cm)	525 ± 4	397 ± 2	454 ± 1	404.7 ± 4	419.7 ± 4.9	428.3 ± 5.9	439 ± 5.6	437 ± 4.7	464 ± 33.7	442 ± 16.1	394.3 ± 3.8	505.3 ± 3.2	**
TDS (mg/l)	336 ± 2	254 ± 1	291 ± 1	259 ± 2.6	268.3 ± 3.8	274.3 ± 4.2	282 ± 2.5	279 ± 3.2	298 ± 21.8	282 ± 10.4	253 ± 2.6	323.7 ± 2.1	**
DO (mg/l)	5.5 ± 0.1	6.2 ± 0.40	5.0 ± 0.2	9.6 ± 0.3	4.4 ± 0.1	4.8 ± 0.3	4.7 ± 0.1	5.0 ± 0.1	4.5 ± 0.1	4.0 ± 0.4	3.9 ± 0.1	5.0 ± 0.1	
Temp.(°C)	24 ± 2	19 ± 1	23 ± 0	22 ± 0.2	28 ± 0.4	21 ± 0.4	25 ± 0.4	24 ± 0.6	24 ± 0.6	19 ± 0.3	19 ± 0.6	17 ± 0.7	
Free chlorine (mg/l)	0.71 ± 0.1	0.13 ± 0.10	0.05 ± 0	0.05 ± 0	0.17 ± 0	0.24 ± 0	0.25 ± 0	0.13 ± 0	0.1 ± 0	0.05 ± 0	0.14 ± 0.1	0.05 ± 0	
BOD (mg/l)	4.36 ± 0.26	4.95 ± 0.40	4 ± 0.1	8.99 ± 0.5	3.4 ± 0.2	3.76 ± 0.3	3.93 ± 0.1	4.0 ± 0.2	3.23 ± 0.2	3.08 ± 0.8	3.42 ± 0.1	4.35 ± 0.6	
Nitrite (mg/l)	0.77 ± 0.02	0.60 ± 0.1	0.46 ± 0	0.4 ± 0	0.25 ± 0	0.2 ± 0	0.23 ± 0	0.37 ± 0	0.2 ± 0	0.22 ± 0	0.2 ± 0	0.21 ± 0	**
Nitrate (mg/l)	12.50 ± 4.39	4.47 ± 0.40	5.1 ± 0.4	5.17 ± 1	4.6 ± 0.6	4.83 ± 0.5	6.13 ± 1.3	5.2 ± 1.4	4.8 ± 1.7	4.77 ± 0.5	0 ± 0	4 ± 0.1	**
Phosphate (mg/l)	1.56 ± 0.09	4.80 ± 0.10	4.4 ± 0	6.57 ± 0.3	5.35 ± 0	16.47 ± 1.9	16.77 ± 0.8	3.89 ± 0.1	4.29 ± 0.1	4.97 ± 0.1	4.21 ± 0.1	20.57 ± 0.5	
COD (mg/l)	39 ± 8	45 ± 1	71 ± 5	42.3 ± 7	35.7 ± 7	61.8 ± 5	4.67 ± 5	59 ± 8.7	163.33 ± 135.1	173 ± 66.6	199 ± 75	211 ± 74.9	**

Note:-Values reported are the mean for triplicate samples per site ± standard deviation. ** indicate parameters with significant differences between the standard limit and mean value when $p < 0.05$.

Table 4.3:-The average means of the measured physiochemical parameters for WW-Dim Sewage Treatment Works.

Parameters	September 2012		October 2012		November 2012		December 2012		January 2013		February 2013		March 2013		April 2013		May 2013		June 2013		July 2013		August 2013		
	FE	DP	FE	DP	FE	DP	FE	DP	FE	DP	FE	DP	FE	DP	FE	DP	FE	DP	FE	DP	FE	DP	FE	DP	
pH	3.9 ± 0.1	4.2 ± 0.2	6.8 ± 0.1	7.2 ± 0.2	7 ± 0	7 ± 0	7.4 ± 0		6.8 ± 0.1	7.0 ± 0	7.4 ± 0	7.8 ± 0	6.9 ± 0	7.4 ± 0.2	7.5 ± 0.2	7.6 ± 0.1	4.8 ± 0.1	6.5 ± 0	6.5 ± 0	6.6 ± 0	5.7 ± 0.1	5.8 ± 0	6.2 ± 0.1	6.6 ± 0.1	**
TURBIDITY (NTU)	12.8 ± 1.50	13.1 ± 1	5.8 ± 0.4	5.8 ± 0.5	4.0 ± 0.4	4.2 ± 0.6	12.8 ± 0.1		5.5 ± 0.5	5.7 ± 0.2	5.5 ± 0.3	5.1 ± 0.3	6.0 ± 1.7	6 ± 0	5.6 ± 0.6	5.0 ± 0.3	5.7 ± 1	8.6 ± 0.7	17 ± 0.3	16 ± 0.2	24.3 ± 0.6	27.3 ± 4.2	21 ± 1.7	23.3 ± 1.5	
EC(µS/cm)	179 ± 2	176 ± 2	155 ± 3	158 ± 1	166 ± 1	165 ± 1	161.8 ± 14.8		138.5 ± 3.1	136.3 ± 0.3	135.7 ± 1	135.1 ± 4.5	149 ± 3.8	150 ± 1.1	147 ± 2	145 ± 0.3	199 ± 4.6	169 ± 0.6	159 ± 14.6	149 ± 0.4	150.3 ± 5	148 ± 3	173.7 ± 3.4	171.6 ± 0.9	**
TDS (mg/l)	114 ± 2	112 ± 0	99 ± 2	101 ± 1	106 ± 0	106 ± 0	103.8 ± 9.4		88.6 ± 2	87.3 ± 0.1	86.8 ± 0.6	86.5 ± 2.8	95 ± 2.5	94 ± 0.7	94 ± 1.4	93 ± 0.3	127 ± 3.1	108 ± 0.4	102 ± 9.2	96 ± 0.3	96.2 ± 3.3	94.7 ± 1.9	111 ± 2.2	110 ± 0.7	**
DO (mg/l)	7.9 ± 0.1	9 ± 0.2	8 ± 0.1	8.7 ± 0.10	7.9 ± 0	8.5 ± 0.1	7.5 ± 0.1		7.5 ± 0.1	8.6 ± 0	7.1 ± 0.1	8.4 ± 0	6.9 ± 0.1	8.1 ± 0	8.1 ± 0.1	8.9 ± 0.1	8.4 ± 0.1	9.7 ± 0	8.3 ± 0	9.7 ± 0.1	9.3 ± 0	10.1 ± 0	9.4 ± 0.2	10.0 ± 0.1	
TEMP.(°C)	20 ± 2	19 ± 1	25 ± 4	25 ± 1	25 ± 1	24 ± 1	21 ± 0.2		22 ± 0.2	21 ± 2.6	24 ± 2.3	23 ± 1	25 ± 0.3	29 ± 2.5	18 ± 0.3	20 ± 0.9	16 ± 0.5	16 ± 0.3	16 ± 0.6	15 ± 0.5	13 ± 0.1	14 ± 0.4	14 ± 0.8	15 ± 0.4	
FREE CHLORINE (mg/l)	2.31 ± 0.5	2.1 ± 0.3	0.31 ± 0	0.33 ± 0	0.36 ± 0	0.28 ± 0	0.09 ± 0		0.22 ± 0	0.17 ± 0.1	0.21 ± 0	0.26 ± 0.1	0.16 ± 0	0.15 ± 0	0.14 ± 0	0.13 ± 0	7.18 ± 0.4	0.43 ± 0.1	0.14 ± 0	0.09 ± 0	0.06 ± 0	0.06 ± 0	0.13 ± 0	0.16 ± 0	
BOD (mg/l)	0.13 ± 0.10	0.42 ± 0.2	5.12 ± 1.80	4.84 ± 0.60	4.48 ± 0.3	4.9 ± 0.5	6.76 ± 0.1		4.96 ± 0.2	7.36 ± 0.3	1.84 ± 2.1	7.35 ± 0.1	4.9 ± 0.8	6.59 ± 0.1	5.1 ± 1.7	6.1 ± 1.4	0.37 ± 0.2	3.75 ± 0.5	7.39 ± 0.4	8.84 ± 0.4	6.65 ± 0.5	7.08 ± 0.1	7.02 ± 0.3	7.51 ± 0.4	
NITRITE (mg/l)	0.18 ± 0	0.18 ± 0	0.09 ± 0.10	0.17 ± 0	0.01 ± 0	0.15 ± 0	0.43 ± 0		0.16 ± 0	0.16 ± 0	0.18 ± 0	0.19 ± 0	0.04 ± 0	0.07 ± 0	0.17 ± 0	0.22 ± 0	0.18 ± 0	0.17 ± 0	0.24 ± 0	0.14 ± 0.1	0.18 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	**
NITRATE (mg/l)	17.87 ± 2.2	15.63 ± 1.5	12.5 ± 0.4	11.63 ± 0.4	16.5 ± 1.6	14.87 ± 1.5	8.03 ± 1.6		10.87 ± 0.3	11.7 ± 2	11.37 ± 0.7	11.8 ± 0.6	18.7 ± 1.7	17.23 ± 1	15.87 ± 1.9	17.37 ± 1.2	13.97 ± 1.6	12.77 ± 1.6	10.47 ± 1	10.3 ± 1	14.53 ± 2.9	15.97 ± 1.2	14.37 ± 2	21.73 ± 0.2	
PHOSPHATE (mg/l)	3.3 ± 0.1	3.31 ± 0.1	2.29 ± 0.20	2.43 ± 0.10	3.22 ± 0.1	3.15 ± 0.2	2.66 ± 0.1		1.29 ± 0.1	1.55 ± 0.1	1.52 ± 0.1	1.54 ± 0.2	9.8 ± 0.3	11.33 ± 0.3	11.37 ± 1.2	68.33 ± 4.2	2.8 ± 0.1	2.74 ± 0	3.19 ± 0	3.29 ± 0.1	3.02 ± 0	3.02 ± 0.1	2.79 ± 0	3.11 ± 0.6	**
COD (mg/l)	82 ± 28	339 ± 26	23 ± 1	20 ± 4	11 ± 3	15 ± 3	29.67 ± 15.3		27 ± 36.4	31.67 ± 6.4	10.33 ± 5	17 ± 5.3	54 ± 47.3	42 ± 3.5	88.33 ± 9.5	68.33 ± 4.2	47.33 ± 5	70 ± 21.1	13 ± 17.3	39.67 ± 55.1	22.33 ± 16.8	47 ± 19.1	33.67 ± 13.3	7 ± 3.5	

Note: Values reported are the mean for triplicate samples per site ± standard deviation, FE: Final effluent, DP: Discharge point. ** indicate parameters with significant differences between the standard limit and mean value when $p < 0.05$.

4.1.2 Dissolved Oxygen

The average value of dissolved oxygen during the monitored period was 8.02 mg/l at the final effluent point and 9.04 mg/l at the discharge point of the WW-Dim WWTP, while it was 5.21 mg/l for WW-Ama Central Works. The DO for WW-Ama varied between 3.91 mg/l and 9.60 mg/l, while it varied from 8.09 mg/l to 10.11 mg/l for WW-Dim discharge point and 6.93 mg/l to 9.36 mg/l at the WW-Dim Final Effluent point (Tables 4.2 and 4.3). There is no specified limit for effluent in the current regulation. Seventy five (75) percent saturation was previously specified as a special standard (DWAF, 1984). Saturated water is expected to have about 8 – 9 mg/l of dissolved oxygen at 20 °C. WW-Ama WWTP had a low concentration level of dissolved oxygen while the WW-Dim WWTP had sufficient level of dissolved oxygen.

4.1.3 Biochemical oxygen demand

The average value of BOD during the monitoring period was 4.6 mg/l and 5.9 mg/l at the final effluent point and discharge point respectively for WW-Dim and 4.29 mg/l for WW-Ama. The BOD of the WWTP effluents ranged from 3.1 mg/l to 9.0 mg/l for WW-Ama, while it ranged from 0.4 mg/l to 8.8 mg/l for WW-Dim discharge point and 0.1 mg/l to 7.4 mg/l at the WW-Dim final effluent point. There is no permissible limit set by the South Africa regulating body. Using the EU standard for urban wastewater which requires a 70-90% reduction at a concentration of 25 mg/l O₂ (Frost, 2009; CEC, 1991). The control sample using sterile distilled water as blanks has an average value of 0.6 mg/l. The measured BOD values exceeded the permissible values at the outflow from the WWTP. The total efficiency of the WWTP for BOD reduction was poor.

The measured BOD₅ at WW-Ama for the 12 months of sampling was above the limit of 2 mg/l expected for the maximum amount of oxygen required to be utilized. The month of December had the highest BOD level observed as shown in Table 4.2. At WW-Dim WWTP, BOD₅ was <2 mg/l which falls within the amount of oxygen expected to be utilized for the months of September 2012, February and May 2013 at the final effluent point and the discharge point. All other months had high BOD.

4.1.4 Chemical oxygen demand

The average value of COD during the monitored period was 36.8 and 35.7 mg/l at the final effluent point and discharge point outflow for WW-Dim WWTP and 92 mg/l for WW-Ama at the outflow. The measured values for WW-Dim did not exceed the permissible general limits of 75 mg/l but although they exceeded the special limit of 30 mg/l values for the outflow from a WWTP. The measured COD values for samples from WW-Ama central treatment works exceed both the general and special COD limits. The range of value for WW-Ama was 4.67 mg/l to 211 mg/l, while it was 7 mg/l to 339.33 mg/l for WW-Dim discharge point and 10.33 mg/l to 88.33 mg/l, at the WW-Dim Final Effluent point (Table 4.2 and 4.3). WW-Ama had 33% of the samples analyzed exceeding the recommended limit while the rest of the samples were slight high in COD levels. The highest COD concentrations were observed in the months of May through August. In contrast, WW-Dim had 83% of samples analyzed within the recommended limit with the months of September 2012 and April 2013 having the highest COD level recorded. The mean COD values as recorded for the final effluent and the discharge point were within the recommended general COD limit value of 75

mg/l, with a statistical significant difference for the final effluent (FE= -38.20, $p < 0.05$). The COD of the WW-Dim discharge point was slightly below the set limit of 75 mg/l with no statistical significant difference (DP= -11.66, $p > 0.05$). At COD special limit of 30 mg/l, both the WW-Dim effluent points were statistically insignificant ($p > 0.05$; FE=6.80, DP=33.33). The WW-Ama Treatment Plant has its mean COD value far exceeding the recommended limits both for the general and special limits of 75 mg/l and 30 mg/l respectively. It has statistical insignificance difference of $p > 0.05$ and mean difference of 62.05.

4.1.5 Total Dissolved Solids

The average value of total dissolved solids (TDS) during the monitored period was 102.03 mg/l at the final effluent spot of the WWTP and 99.04 mg/l at the discharge point outflow for WW-Dim Sewage Treatment Works while WW-Ama was 283.4 mg/l. The TDS value for WW-Ama ranged between 253 mg/l and 336.3 mg/l (Table 4.2). TDS ranged from 86.50 mg/l to 111.73 mg/l for WW-Dim discharge point and 86.83 mg/l to 127.47 mg/l at the WW-Dim Final Effluent point (Table 4.3). The WW-Ama Plant was inefficient in removing TDS showing a rather poor removal of TDS. The WW-Dim Sewage Treatment Works was more efficient in handling the TDS concentration level in the treated effluent. The mean TDS values for the WW-Dim final effluent and at the discharge point were within the recommended TDS value of 450 mg/l, with a statistically significant difference of $p < 0.05$; FE=-348, DP=-351. The mean TDS recorded for WW-Ama WWTP was also within the set standard, with a statistically significant difference at FE=-166, $p < 0.05$.

4.1.6 Nitrite nitrogen

The average value of nitrite nitrogen during the monitored period was 0.17 mg/l at both the WW-Dim final effluent point and discharge point. WW-Ama was 0.34 mg/l at the outflow from the WWTP. The nitrite level for WW-Ama ranged between 0.19 mg/l and 0.60 mg/l; while it ranged between 0.07 mg/l and 0.22 mg/l for WW-Dim discharge point and 0.01 mg/l and 0.43 mg/l at the WW-Dim Final Effluent point (Tables 4.2 and 4.3). The measured values for both treatment plants were in compliance with the permissible general limits of 15 mg/l and 1.5 mg/l values at the outflow from the WWTP. The nitrite values were statistically significant for both point at the set limits of 1.5 mg/l and 15 mg/l for WW-Dim ($p < 0.05$; -1.3 and -14) and WW-Ama ($p < 0.05$; -1.2 and -14)

4.1.7 Nitrate Nitrogen

The average value of nitrate nitrogen during the monitored period was 13.75 mg/l at the final effluent point and 14.64 mg/l at the discharge point of the WW-Dim WWTP, while it was 5.13 mg/l for the WW-Ama Central Treatment Works. The measured values for WW-Ama Treatment Plants did not exceed the permissible general limits of 15 mg/l, though they did exceed the special limits of 1.5 mg/l at the WWTP. Likewise, the WW-Dim plant was within the permissible limit except in few instances where the nitrate level was above the 15mg/l limit. This was observed at both the discharge point for the months of September 2012, March, April, July and August 2013 and at the final effluent for the months of September, November 2012, March and April 2013. The nitrate level for WW-Ama ranged between 0.00 mg/l and 6.13 mg/l, while it ranged

from 10.30 mg/l to 21.73 mg/l for WW-Dim discharge point and 8.03 mg/l to 18.70 mg/l at the WW-Dim Final Effluent point (Tables 4.2 and 4.3). The efficiency of the WWTP for nitrate nitrogen reduction was poor for WW-Dim. Their means difference was $FE=-1.2$ and $DP=-0.3$ with statistical insignificant difference of $p>0.05$ while the nitrate level was good for WW-Ama Treatment Plant with statistical significance difference of $p<0.05$, $FE=-9.9$. The WW-Ama WWTP was more efficient in removing nitrate than the WW-Dim WWTP.

4.1.8 Orthophosphate

The average value of the orthophosphate during the monitored period was 3.94 mg/l at the final effluent point and 4.27 mg/l at the discharge point of the WW-Dim WWTP, while it was 8.30 mg/l for WW-Ama Central Works. The recommended permissible level of orthophosphate is 10 mg/l. The phosphate level ranged from 3.89 mg/l to 20.57 mg/l for WW-Ama, while it ranged between 1.54 mg/l and 11.50 mg/l for WW-Dim discharge point and 1.29 mg/l to 11.37 mg/l at the WW-Dim Final Effluent point (Table 4.2 and 4.3). The WW-Dim WWTP was statistically significant at $p<0.05$ ($FE=-6.1$, $DP=-5.7$), it was not for the WW-Ama plant ($p>0.05$; $FE=-1.7$). The efficiency of the WWTP for the orthophosphate reduction was effective at the WW-Dim plant. The concentration level of the orthophosphate drops as the effluent leaves the plant to the discharge. The orthophosphate level was really poor at WW-Ama WWTP.

4.1.9 Free Chlorine

The average value of free chlorine during the monitored period was 0.94 mg/l at the final effluent point and 0.38 mg/l at the discharge point of the WW-Dim WWTP, while

it was 0.17 mg/l for WW-Ama Central Works. The free chlorine for WW-Ama ranged between 0.05 mg/l and 0.71 mg/l, while it ranged between 0.06 mg/l and 2.11 mg/l for WW-Dim discharge point and 0.06 mg/l to 7.18 mg/l at the WW-Dim Final Effluent point (Tables 4.2 and 4.3). A general limit of 0.25 mg/l and special limit of 0 mg/l was set for free chlorine. The chlorine values at the two WWTPs were statistically insignificant ($p>0.05$). About 67% of the WW-Ama samples had low free chlorine concentration below the recommended set limit while WW-Dim WWTP samples had good level of free chlorine concentration. There were instances where there high chlorine overdosing was recorded at both sites.

4.1.10 Temperature

The average value of temperature during the monitored period was 20 °C at both the final effluent and discharge points of WW-Dim WWTP, while it was 22 °C for WW-Ama Central Works. The range of value for WW-Ama was 17 °C to 28 °C, 14 °C to 29 °C for WW-Dim discharge point and 13 °C to 25 °C at the WW-Dim Final Effluent point. The regulation requires that discharge up to 2 000 cubic meters of wastewater on any given day into a listed and not listed water resource set out in the South Africa Water Acts, does not alter the natural ambient water temperature of the receiving water resource by more than 2 - 3 °C. The measured temperatures are within moderate levels. The temperature was high during the summer and drops during the winter period.

4.1.11 Turbidity

The average value of turbidity during the monitored period was 10.5 NTU at the final effluent point and 10.9 NTU at the discharge point of the WW-Dim WWTP, while it

was 17.4 NTU for WW-Ama Central Works. The turbidity for WW-Ama ranged between 4.76 NTU and 43.20 NTU, while it ranged between 4.17 NTU to 27.33 NTU for WW-Dim discharge point and 4.02 NTU to 24.33 NTU at the WW-Dim Final Effluent point (Tables 4.2 and 4.3). The DWAF regulation does not state any value to be use as quality guideline for turbidity for effluent except only for domestic use where a set value of 0 – 1 NTU was specified, 3 NTU was specified for recreational water and 25 NTU for aquaculture. A lower band limit of <5 NTU was set as the limit using the WHO standard for drinking water quality (Gorchev and Ozolins, 2008). About 83.3% of the WW-Ama samples analysed exceeded that limits while 41.7% of WW-Dim samples (final effluent) exceeded the set limit. There was an increase in turbidity level of the effluent at the WW-Dim discharge point as it leaves the plants.

4.1.12 Electrical conductivity

Electrical conductivity ranged from 39.43 mS/m to 52.50 mS/m for WW-Ama, (Table 4.3). It was 13.51 mS/m to 17.60 mS/m for WW-Dim discharge point and 13.57 mS/m to 19.90 mS/m at the WW-Dim Final Effluent point (Table 4.3). The mean value of electrical conductivity during the monitored period was 15.95 mS/m at the final effluent point and 15.48 mS/m at the discharge point of the WW-Dim WWTP, while it was 44.25 mS/m for WW-Ama Central Works. The measured values for both treatment plants did not exceed the permissible general limits of 70 mS/m but at the WW-Ama Plant, it exceeded the special limit of 50 mS/m for the months of September 2012 and August 2013 and generally the measured values are relatively high for the electrical conductivity at this plant. The EC values were statistically significant for both ($p =$

0.00) Treatment Plants for the regulatory set limits (general limits and special limits) at $p < 0.05$.

4.2 Bacteriological analysis

4.2.1 Coliform bacteria distributions

Faecal coliforms, *E. coli* and *Vibrio* counts from each of the WWTPs are shown in Table 4.4 below. The faecal coliform counts for WW-Ama Centre Treatment Work ranged between 0 and 2.92×10^4 CFU/100 ml with the highest being in the month of June 2013. The *E. coli* counts ranged between 0 and 1.85×10^5 CFU/100 ml with the month of June 2013 also having the highest counts. The *Vibrio* counts ranged between 0 and 9.93×10^3 CFU/100 ml. For all classes of bacteria assessed, the average bacteria counts for the plants exceeded the recommended limit of 1000 CFU/100 ml for effluent discharged.

WW-Dim Sewage Treatment Works had a faecal coliform count ranged between 0 and 1.88×10^2 CFU/100 ml at the final effluent point and 0 to 2.04×10^2 CFU/100 ml at the discharge point. The *E. coli* counts ranged between 0 and 1.86×10^4 CFU/100 ml at the final effluent and 0 to 2.16×10^4 CFU/100 ml with both points having the highest bacterial counts in August 2013. It is however observed that the *E. coli* counts were higher at the discharge point when compared to the final effluent point. The *Vibrio* counts range between 0 and 1.44×10^3 CFU/100 ml at the final effluent and 0 to 1.28×10^3 CFU/100 ml at the discharge point. For all classes of bacteria assessed, average counts were in compliance with the recommended limit of 1000 CFU/100 ml for effluent discharged.

Table 4.4:-Bacterial average counts for the wastewater Treatment Plant

Target Bacteria	Sampling site	Sep-12	Oct-12	Nov-12	Dec-12	Jan-13	Feb-13	Mar-13	Apr-13	May-13	Jun-13	Jul-13	Aug-13
Feecal coliform (cfu/100 ml)	WW-Ama	0	4.00 × 10 ³	9.30 × 10 ³	0	0	0	5.40 × 10 ²	1.00 × 10 ²	1.66 × 10 ⁴	2.92 × 10 ⁴	2.05 × 10 ⁴	6.13 × 10 ³
	WW-Dim final effluent	0	1	0	1.00 × 10 ²	0	0	71	26	0	0	6.67 × 10 ¹	1.88 × 10 ⁴
	WW-Dim discharge point	0	0	0	ND	0	0	38	18	0	0	1.33 × 10 ²	2.04 × 10 ⁴
<i>E. coli</i> (cfu/100 ml)	WW-Ama	0	2.80 × 10 ⁴	1.30 × 10 ²	1.10 × 10 ³	3.33 × 10 ²	0	1.60 × 10 ⁴	2.10 × 10 ²	2.09 × 10 ⁴	1.85 × 10 ⁵	6.00 × 10 ³	1.29 × 10 ⁴
	WW-Dim final effluent	1	0	0	1.31 × 10 ³	1.0	2	27	36	0	7	1.67 × 10 ²	1.86 × 10 ⁴
	WW-Dim discharge point	0	0	0	ND	8.3	30	66	40	1	2	2.67 × 10 ²	2.16 × 10 ⁴
<i>Vibrio</i> (cfu/100 ml)	WW-Ama	0	7.7 × 10 ³	4.6 × 10 ³	4.10 × 10 ³	6.00 × 10 ³	0	65	7.83 × 10 ³	4.30 × 10 ³	9.93 × 10 ³	8.40 × 10 ³	0
	WW-Dim final effluent	1	1	1.8 × 10 ¹	7.00 × 10 ²	1	1	2	118	0	59	3.67 × 10 ²	1.44 × 10 ³
	WW-Dim discharge point	1	3	1.5 × 10 ¹	ND	2	1	2	121	4	68	1.00 × 10 ²	1.28 × 10 ³

4.2.2 Confirmation Testing

E. coli confirmation

The presumptive isolates from WW-Ama Central Treatment Works and WW-Dim Sewage Treatment Works were confirmed using the PCR targeting the *uidA* gene, which encodes the beta-glucuronidase enzyme in *E. coli* with a band size of 147bp (Figure 4.1). A total of 843 presumptive isolates were tested and 476 were confirmed to be *E. coli*. The breakdowns of the isolates tested are shown in Table 4.5.

Table 4.5:-E. coli confirmation of the presumptive isolates

Site	Number of Isolates	Number of positive isolates (PCR)
WW-Ama	406	270
WW-Dim	437	206

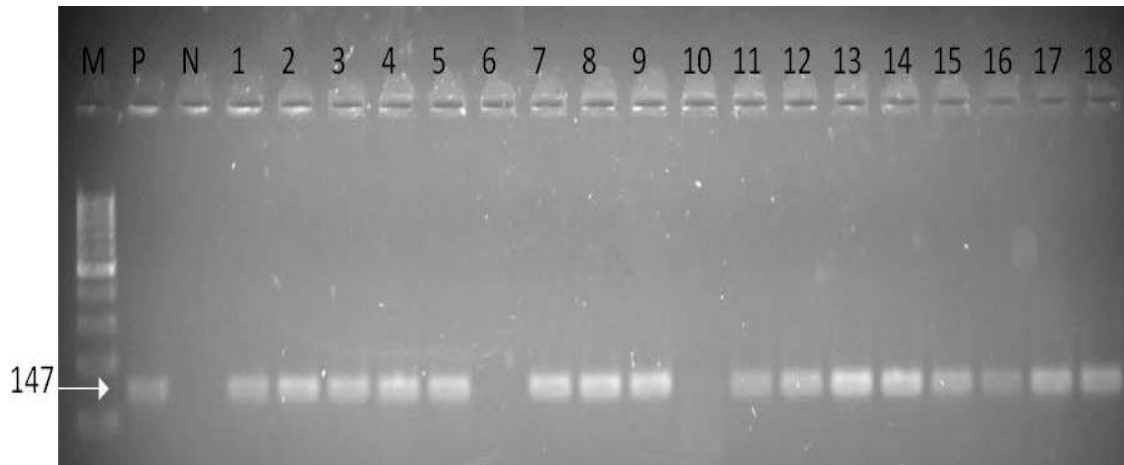


Figure 4.1: Agarose gel electrophoresis of uidA gene amplification products of *E. coli*

M: Molecular weight marker (100bp)

P: *Escherichia coli* ATCC 29522 (Positive control)

N: Negative control;

Lanes 1-18: *E. coli* isolates

4.2.3 Pathotyping

Latex agglutination

The presumptive isolates from the *E. coli* O157:H7 selective media were further tested to confirm them using the anti-sera method. A total of 540 isolates were tested and none was positive for the agglutinating test for the identification of *E. coli* O157:H7 (Table 4.6).

Table 4.6:-Result of agglutinating test for *E. coli* O157:H7

Site	Number of Isolates	Number of positive isolates on Sorbitol Mac-conkey	Number of positive isolates (Latex Agglutination)
WW-Ama Central Treatment Works	233	67	0
WW-Dim Sewage Treatment Works	307	82	0

Molecular confirmation of *E. coli* pathotypes

Three hundred confirmed *E. coli* isolates were randomly selected to cover both the treatment plants and the months sampled. With the specificity of the target pathotype genes assayed, each of the *E. coli* pathotypes were tested against the 300 selected confirmed isolates. Four (4) *E. coli* pathotypes were detected out of the 7 pathotypes tested for. The pathotypes detected were Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli*, Neonatal *E. coli* (NMEC) and Uropathogenic *E. coli* (UPEC). Two isolates were confirmed as EPEC, 5 isolates as NMEC, 11 isolates as EAEC and 27 isolates as UPEC, (Table 4.7).

Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC) and Diffuse-adhering *E. coli* (DAEC) were found to be negative. Some of the confirmed *E. coli* pathotype gave the expected band sizes. Figure 4.2 below shows the confirmation of EAEC targeting the EA_g gene with a band size of 194 bp.

Table 4.7:-Result of *E. coli* pathotyping

Pathotypes	Total number (n = 300)
Enteropathogenic <i>E. coli</i> (EPEC)	2
Enterotoxigenic <i>E. coli</i> (ETEC)	0
Enteroinvasive <i>E. coli</i> (EIEC)	0
Enteraggregative <i>E. coli</i> (EAEC)	11
Neo natal <i>E. coli</i> (NMEC)	5
Uropathogenic <i>E. coli</i> (UPEC)	27
Diffuse-adhering <i>Escherichia coli</i>	0

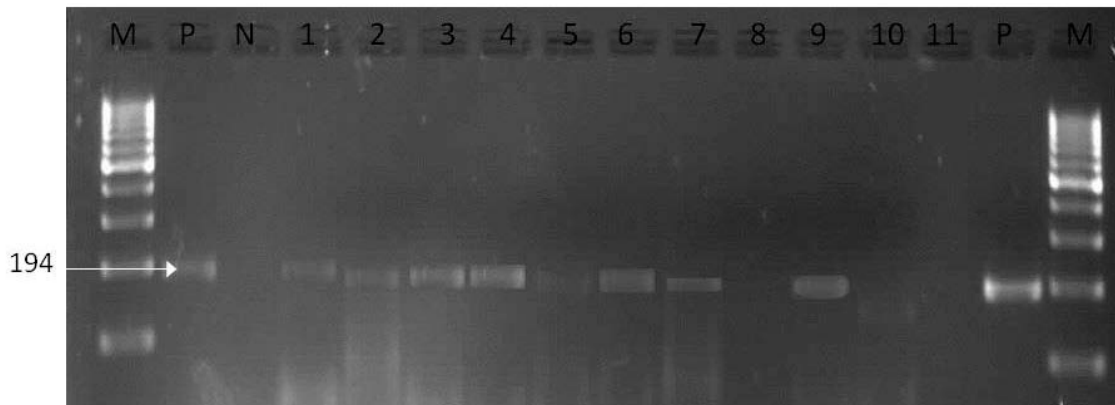


Figure 4.2: Agarose gel electrophoresis of EAgg gene amplification products of EAEC

M: Molecular weight marker (100bp)

P: *Escherichia coli* (EAEC) DSM 10974 (Positive control)

N: Negative control;

Lanes 1-5: *E. coli* isolates

Figure 4.3 below shows the confirmation of NMEC targeting the *ibe* gene with a band size of 171 bp.

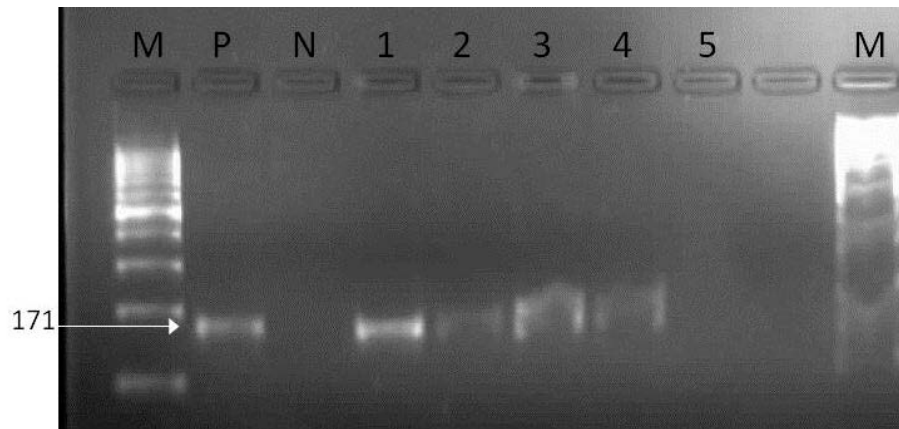


Figure 4.3: Agarose gel electrophoresis of *ibe* gene amplification products of NMEC

M: Molecular weight marker (100bp)

P: *Escherichia coli* (NMEC) DSM 10819 (Positive control)

N: Negative control;

Lanes 1-5: *E. coli* isolates

Figure 4.4 below shows the confirmation of UPEC targeting the pap gene with a band size of 328 bp.

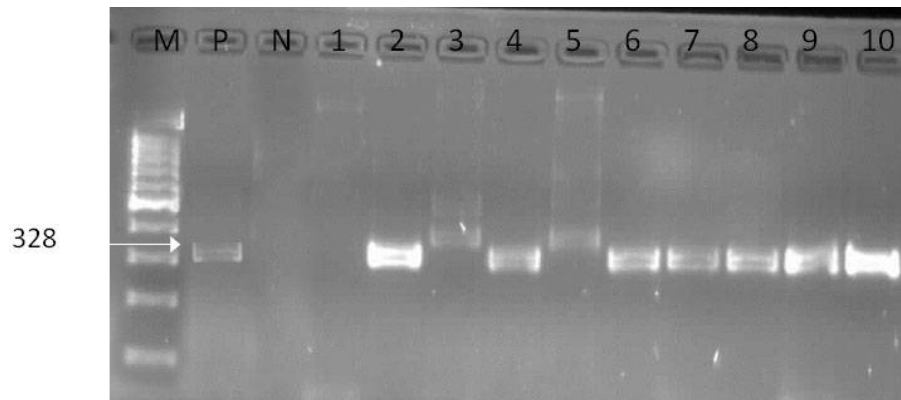


Figure 4.4: Agarose gel electrophoresis of pap gene amplification products of UPEC

M: Molecular weight marker (100bp)

P: *Escherichia coli* (UPEC) DSM 4618 (Positive control)

N: Negative control;

Lanes 1-10: *E. coli* isolates

***Vibrio* confirmation**

The molecular confirmation of the presumptive *Vibrio* isolates were done targeting 16S rRNA intergenic spacers (IGS) gene peculiar to the *Vibrio* species with a band size of 663 bp (Figure 4.5). A total of 786 presumptive isolates were tested and 368 isolates were confirmed. The breakdown of the isolates confirmed is shown in Table 4.8.

Table 4.8:-*Vibrio* confirmation of the presumptive isolates

Site	Number of isolates	Number of positive isolates
WW-Ama	340	206
WW-Dim	446	162

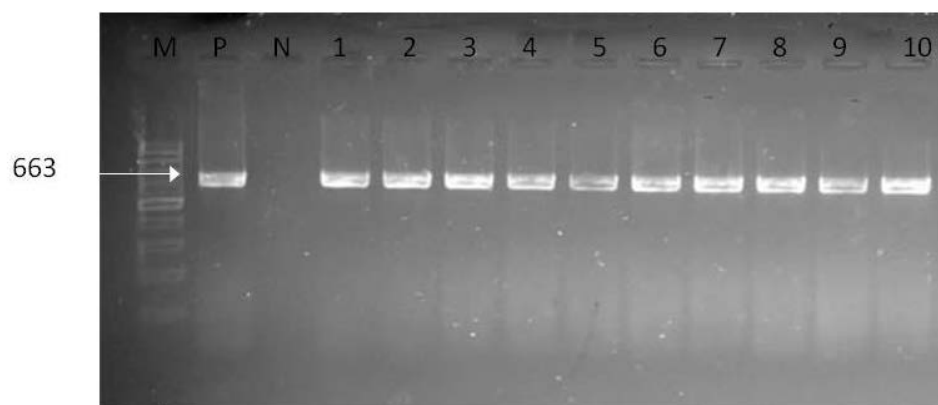


Figure 4.5: Agarose gel electrophoresis of 16s rRNA gene amplification products of *Vibrio*.

M: Molecular weight marker (100bp)

P: Positive control

N: Negative control;

Lanes 1-10: *Vibrio* spp. isolates

Molecular confirmation of *Vibrio* pathotypes

Three hundred confirmed *Vibrio* isolates were randomly selected to cover both the treatment plants and the months of samples. Each *Vibrio* pathotypes were ran against the 300 selected confirmed isolates. None of the three *Vibrio* pathotypes were detected.

4.3 Antimicrobial susceptibility test

A total of 107 *E. coli* isolates and 100 *Vibrio* isolates, were used for antimicrobial susceptibility testing against the selected panels of antibiotics disc. In all, the isolates tested displayed resistance to at least one or more antibiotics as shown in Table 4.9. The *E. coli* isolates showed resistance to 5 antibiotics except for meropenem. Overall, the results of antibiotic sensitivity test revealed that 63.6% of the *E. coli* isolates were resistance to ampicillin while 49.5% were resistant to tetracycline and cephalothin. The least resistances were observed against gentamicin (3.7%) and cefotaxime (1.9%). No resistance was observed against meropenem. For the *Vibrio* spp, resistance was most frequently observed against tetracycline (38%) ampicillin (26%), chloramphenicol (16%), cefotaxime (14%), trimethoprim-sulfamethoxazole (13%) and the least resistance observed was again floxacin (1%).

Table 4.9:-Antimicrobial resistant of *E. coli* and *Vibrio* spp isolates from effluents

Percentage (%) of isolates resistance to antibiotics		
Antimicrobial agent (µg)	<i>E. coli</i> n = 107	<i>Vibrio</i> spp n = 100
Tetracycline (30)	49.5	38
Ampicillin (10)	63.6	26
Cefotaxime (30)	1.9	14
Cephalothin (30)	49.5	N/T
Gentamicin (10)	3.7	N/T
Meropenem (10)	0	N/T
Trimethoprim-sulfamethoxazole (1.25/23.75)	N/T	13
Chloramphenicol (30)	N/T	16
Ciprofloxacin (5)	N/T	1

N/T = not tested.

4.4 Virology

4.4.1 PCR specificity, sensitivity, standard curve and detection limits

Adenovirus, hepatitis A, rotavirus and coxsackie A viruses were examined by real-time PCR assays. Reactivity of the primers and probes were observed with DNA and RNA viral standards used as a template. Real-time PCR did detect the adenovirus, hepatitis A, rotavirus and coxsackie A virus. The resulting standard curves (adenovirus 41, slope: -3.53 and Y- intercept: 28.34; Hepatitis A, slope: -3.22 and Y- intercept: 36.64; Rotavirus, slope: -3.36 and Y- intercept: 36.64; Enterovirus, slope: -3.81 and Y- intercept: 33.26), with strong correlation coefficients (r^2) of 0.99 and 0.98 respectively. PCR amplification efficiency for the reactions was over 92%. A detection limit of 10 copies of target DNA per reaction was set for all PCR assay. Prevention of PCR carryover contamination was confirmed as no amplification was observed in the negative controls demonstrating that there were no contaminations.

4.4.2 Detection of Adenovirus

Over the 12-months of study, 35 samples were collected from two WWTPs. WW-Ama WWTP had the highest prevalence of the virus which varied between 1.0×10^1 and 6.75×10^2 genome copy/litre. The virus was detected in 67% of the samples analysed, while WW-Dim also recorded a detection rate of 17% which ranged between 3.9×10^1 and 7.9×10^1 gc/l. The results of the samples for the 2 treatment plants are presented in Table 4.10. The table shows the range of genome copy per litre of adenovirus detected, frequency and quantity of HAdv detected from the real time PCR for each of the 2 WWTPs for the 12 months covered by the study.

Table 4.10:-Detection frequency and range of HAdv in effluent samples of the 2 WWTPs over a 12-month period.

WWTP	Detection frequency (%)	Detection range (gc/l)
WW-Ama	67	1.0×10^1 to 6.75×10^2
WW-Dim	17	3.9×10^1 to 7.9×10^1

4.4.3 Adenovirus Species and Serotyping

Of the 35 samples tested, 2 species of adenovirus out of the 4 species tested were detected. The samples analyzed tested positive for Adenovirus species C and F. Adenovirus species C was positive for adenovirus 2 serotypes and negative for human adenovirus 1, 5 and 6 serotypes. Adenovirus 41 serotypes were detected for Adenovirus F species and absent for Adenovirus 40 serotypes. The species and serotypes are shown in the Table 4.11 below. Five (5) Adenovirus 41 serotypes were detected and it was the most detected of the species F, this is followed by Adenovirus 2 serotypes with one serotype detected.

Table 4.11:-Characterization of HAdv detected in the effluent samples of the WWTPs

Species	Serotypes	No of positives
B	HAdv3	0
	HAdv7	0
	HAdv 21	0
C	HAdv 1	0
	HAdv 2	1
	HAdv 5	0
	HAdv 6	0
E	HAdv 4	0
F	HAdv 40	0
	HAdv 41	5

4.4.4 Detection of Rotavirus

Same samples from above were tested for rotavirus. WW-Ama WWTP had the highest prevalence of the virus which varied between 16 and 5.2×10^3 genome copy/litre. The virus was detected in 42% of the samples analysed, while WW-Dim also recorded a detection rate of 17% which ranged between 1 and 5 gc/l. The results of the samples for the 2 Treatment Plants are presented in Table 4.12. The result shows the frequency and quantity of rotavirus detected from the real time PCR for each of the 2 WWTPs for the 12 months covered by the study as well as the range of genome copy per litre of rotavirus detected and frequency of detection.

Table 4.12:-Detection frequency and range of rotavirus in effluent samples of the 2 WWTPs over a 12-months period.

WWTP	Detection frequency (%)	Detection range (gc/l)
WW-Ama	42	16 - 5.2×10^3
WW-Dim	17	1 - 5

4.4.5 Detection of Hepatitis A

Over the 12 months of study, 35 samples were collected from the two wastewater treatment plant(s). The outcome of the real time PCR reaction was negative for the virus.

4.4.6 Detection of Enteroviruses and Norovirus

Over the 12 months covered by the study, 35 samples in total were collected from the 2 WWTPs. and using the conventional PCR; the reaction was negative for both viruses.

CHAPTER FIVE

DISCUSSION

This study is on the evaluation of the final effluent of two WWTPs in the Buffalo City Municipality in the Eastern Cape of South Africa. The results of the physicochemical parameters and microbiological (bacteriology and virology) analyses are fully discussed.

5.1 Physicochemical qualities

5.1.1 pH

The South Africa (SA) guidelines for pH for final effluents allowed to be discharged into a river are in the range of 5.5 to 9.5 for general limit and 5.5 to 7.5 for special limit (DWAF, 2013). The pH for WW-Dim was found to be within the lower pH limit of 5.5 for the months of September 2012 and March 2013. Subsequent months also remain within the set upper limits of 7.5 and 9.5. The treatment plant's ability to treat wastewater was revealed through the measured pH values which were relatively stable at the recommended pH set limits by DWAF. This is also evident in the level of significance of the mean pH as against the set limit. The two extreme cases of low pH levels below the 5.5 pH limit were directly attributed to chlorine overdosing. The effluents' pH at the WW-Ama WWTP was above the 5.5 lower pH limit but ranged between the 7 and 9.5 upper pH limit which still complied with the set regulatory limit. In corroboration of previous reports on the pH of the effluents from the Eastern Cape plants they were found to be within the recommended limit (Osode, 2007; Morrison et

al., 2001a; Odjadjare and Okoh, 2010; Igbinosa, 2010) which also suggests their suitability for such end uses as domestic, fishery, and recreational purposes (Odjadjare and Okoh, 2010). Wide variation in the pH values of effluents can affect the rate of biological reaction and survival of various microorganisms (Ramteke, Awasthi, Srinath and Joseph, 2010). pH is a primary factor governing the chemistry of natural water systems. The pH of water directly affects physiological functions of plants and animals and is, therefore, an important indicator of the health of a water system (Wilde and Ed, 2008b). pH plays important roles in maintaining conducive conditions for biochemical and metabolic reactions to take place (Zuma, 2010). As an indicator of the acidity or basicity of water, it is seldom a problem by itself. The normal pH range for irrigation water is from 6.5 to 8.4; pH values outside this range are a good warning that the water is abnormal in quality. For water or effluent water considered for irrigation, pH is a routine parameter measure in irrigation water quality assessment (Pescod, 1992). pH affects the activity of microbial enzymes. It affects the ionization of chemicals and thus plays a role in the transport of nutrients and toxic chemicals into the cell (Bitton, 2005). High or low effluent pH problems can occur for different reasons. Low effluent pH (<7.0) may be due to both organic overloading and low oxygen conditions or due to nitrification when the treatment alkalinity (buffer capacity) is low. High effluent pH is always due to extensive algae growth. Algae consume alkalinity (inorganic carbon) for growth, and the more alkalinity consumed, i.e. in the order of carbon dioxide, bicarbonate and carbonate, the more there is an increase in the pH (Richard and Bowman, 1998). It is therefore deduced that the effect of algae growth observed at the effluent point of the WW-Ama Treatment Plant probably accounts for the relatively

high level of pH (>7.0) measured at the plant even though the mean pH was below the 9.5 upper pH limit. It has been reported there is a diurnal pH change caused by algal and macrophyte photosynthesis which are expected to raise pH values between 8.4 and 9.0 in the period around midday each day (Morrison et al., 2001a).

Also, pH changes are also controlled by temperature, the organic and inorganic ions and biological activity. The pH plays crucial roles in toxicity and availability of metals and non-metallic ions e.g. ammonium. Industrial effluents and increased biological reaction activities due to sewage treatment work effluents can lead to pH changes. If not buffered properly, low pH levels can allow for the formation of toxic substances, leading to species diversity, impaired recreational uses of water and structure alterations (Zuma, 2010). As well, this can correspond with increased photosynthesis in a stream. Pollution may cause a long-term increase in pH (Michaud, 1991).

A change in pH from that normally encountered in unpolluted streams affects the biota while high pH values has the possibility of altering the toxicity of other pollutants in the river. At pH value > 8.5, ammonia is considered to be much more toxic in alkaline water than acid because free ammonia (NH_3) at high pH values (pH > 8.5) is more toxic to aquatic biota than when it is in the oxidized form (NH_4^+). It also “strips” into the atmosphere and it is lost to the water. A low pH could also reduce the solubility of certain essential elements such as selenium and increases the solubility of many other elements (Morrison et al., 2001a). The interactions of various environment components in respect to pH showed that this physicochemical component is important. Faecal coliforms are reported to be more sensitive to lowered pH. However, most faecal

coliforms and their potentially associated pathogens are generally pH-tolerant, as demonstrated by their survival and proliferation in gastrointestinal systems of host organisms (Macmaster, 2008).

5.1.2 Dissolved oxygen (DO)

No standard is established by DWAF for DO for effluent discharge. Concentration of DO varied at all sampling sites and has mean values of 8.07 ± 0.8 mg/l (FE) and 9.07 ± 0.7 mg/l (DP) for the WW-Dim Sewage Treatment Works and the WW-Ama plant recorded 5.21 ± 1.5 mg/l. Typically, DO concentrations remained relatively constant at the WW-Dim Sewage Treatment Works with $DO > 6.0$ mg/l as dissolved oxygen levels in excess of 7.0 mg/l are desired to maintain aquatic ecosystem health (KRIS, 2011). The DO concentration at the WW-Dim WWTP is therefore well saturated with oxygen. The ranges of values recorded for DO at the WW-Ama treatment plant are relatively below what is suitable for the receiving water body. This low DO signifies potential danger to the receiving surface water. A study done by Odjadjare (2010) found the DO at the WW-Dim plant to be of unacceptable concentration. Likewise, Momba, Osode and Sibewu (2009) reported on some treatment plants in the Eastern Cape to produce effluent with low DO concentration. Concentrations below 5 mg/l may adversely affect the function and survival of biological communities, and below 2 mg/l can lead to the death of most fishes (Pearce, Chaudhry and Ghulam, 1998).

The dissolved oxygen content of water is influenced by the source, raw water temperature, treatment and chemical or biological processes taking place in the

distribution system (WHO, 2008). The solubility of oxygen can be enhanced by salinity and atmospheric pressure; it decreases with rising temperature and salinity, and increases with rising atmospheric pressure. Freshwater at sea level has a saturation DO concentration of about 14.6 mg/l at 0 °C (32F) and 8.2 mg/l at 25 °C (77F) (KRIS, 2011; Kiepper, 2010). For maintenance of aquatic health, dissolved oxygen concentrations should approach saturation – that concentration which is in equilibrium with the partial pressure of atmospheric oxygen (KRIS, 2011). Depletion of dissolved oxygen in water can encourage the microbial reduction of nitrate to nitrite and sulphate to sulphide. It can also cause an increase in the concentration of ferrous iron in solution. No health-based guideline value is recommended by the WHO (WHO, 2011). The European Union has a baseline of 5.0-9.0 mg/l, Russia, 4.0-6.0 mg/l and Canada 5.0-9.5 mg/l for concentration of water for aquatic life (Pearce, Chaudhry and Ghulam, 1998). Low concentrations of dissolved oxygen, when combined with the presence of toxic substances may lead to stress responses in aquatic ecosystems because the toxicity of certain elements, such as zinc, lead and copper, is increased by low concentrations of dissolved oxygen (Helmer and Hespagnol, 1997). Low DO content of treated effluent suggested an increase in the organic matter content (Verma, Ramteke and Garg, 2008). This was also indicated by the elevated BOD value as in the case of the WW-Ama Treatment Plant; the BOD is an important indication of organic pollution. As the number of organisms increases, the demand for oxygen increases proportionately (Verma, Ramteke and Garg, 2008). In light of this, the discharging of industrial and domestic wastewater generates serious organic pollution in rivers, since the decrease of

DO was mainly caused by the decomposition of organic compounds. Extremely low DO content ($\text{DO} < 2 \text{ mg/l}$) usually indicates the degradation of an aquatic system.

5.1.3 Biological Oxygen Demand

The result of this study showed that 17% of the samples from WW-Dim Treatment Sewage Works have low BOD levels for the period of study and the remaining 83% had high BOD levels. The mean BOD values for the final effluent was $4.36 \pm 2.5 \text{ mg/l}$ and the discharge point was $5.89 \pm 2.3 \text{ mg/l}$ were within the recommended BOD value of 3 – 6 mg/l limit standard set for the protection of fish life/fisheries by the European Union (CEC, 1978). Another EU standard for urban wastewater which requires a 70-90% (2.5 – 7.5 mg/l) reduction at a concentration of 25 O₂ mg/l was used as the set limit (Frost, 2009; CEC, 1991). While there is no recommended set limit by South Africa's DWAF for BOD, the EU standard for urban wastewater was used. The WW-Ama Treatment Plant has BOD within the EU set standard. The mean BOD value for the final effluent of $4.29 \pm 1.6 \text{ mg/l}$ was within the recommended EU set limit. There was high consumption of oxygen relative to the initial values of dissolved oxygen observed for the two treatment plants. Sometimes by the end of the 5-day incubation period the dissolved oxygen level is $< 1 \text{ mg/l}$. This shows the effluent has a lot of organic pollution (US EPA, OW, 2012). Similar work by Momba, Osode and Sibewu (2009) also reported high BOD values at WW-Dim Sewage Treatment Works. The WW-Ama Treatment Plant had a general poor BOD level for the period of study. Reported high BOD levels from both the industrial effluent and the receiving surface water confirm that poorly treated effluent with high BOD influences BOD level of the

receiving surface water with its consequent negative impact on the environment (Phiri et al., 2005). BOD is the traditional, most widely used test to establish concentration of organic matter in wastewater samples. It is based on the principle that if sufficient oxygen is available, aerobic biological decomposition (i.e. stabilization of organic waste) by microorganisms will continue until all waste is consumed (Kiepper, 2010; Licis, 1995). BOD refers to the quantity of oxygen required by bacteria and other micro-organisms in the biochemical degradation and transformation of organic matter under aerobic conditions. The basic principle underlying the BOD determination is the measurement of the dissolved oxygen content of the sample before and after 5 days' incubation at 20 °C (Yadav and Dagar, 2012; Khaleeq, 2011). The BOD₅ test is widely used to determine the degree to which a waste stream will contribute to pollution of receiving waters by depriving organisms in those waters (fish) of their source of oxygen. The BOD₅ test is of prime importance in regulatory programmes and in determining the overall health of receiving waters (Dugan, 1999). With the poor quality of BOD from the two plants studied, judging from the high oxygen consumption, there is no doubt that the high oxygen demand will put pressure on any receiving surface water as well as the aquatic life present in such an environment as a result of the effluents from these plants. BOD values can be used to make suppositions regarding the attributes of a water body, as well as the biological activity of the incubated microflora. The introduction of effluents with a high level of oxygen consumption (high BOD value) can lead to an oxygen starvation of the body thus killing the aquatic animals. In another case, the performance of a treatment plant can be checked by comparing the

effluent BOD levels before and after an effluent treatment. In general, the following conclusions may be drawn:

- that effluent with high BOD value signifies an excessive content of biodegradable organic materials which cause stress on the oxygen level of the receiving water body;
- that a low BOD reading in the effluent signifies either a low content of organic materials (that means, low stress on the oxygen level of a water course), or substances which are difficult to break down, or other various functional problems.

The effluent may contain poisons or inhibiting substances, or have an extremely high pH, etc (Lovibond, n.d.). It is important here to note that low BOD content is an indication of good quality water, while a high BOD indicates polluted water. The greater the BOD, the more rapidly oxygen is depleted in the water. This means less oxygen is available to higher forms of aquatic life. The consequences of high BOD are the same as those for low dissolved oxygen (DO) because under this state both have a negative impact on aquatic organisms making them to become stressed, suffocate, and hence die (Lokhande, Singare and Pimple, 2012).

Treatment technology also plays an effective role in the reduction of BOD and trickling filters have a somewhat lower BOD removal (less than 85 percent, compared with 90 percent for activated sludge) (Licis, 1995; Babu, 2007). This as well could play a role in the quality of BOD observed in WW-Dim and WW-Ama. WW-Ama uses the trickling filter technology as compared to the activated sludge method in WW-Dim. It

was observed that in comparing the two plants, the WW-Dim Sewage Treatment Works fare better in BOD quality than the WW-Ama Treatment Plant. This thus emphasizes the importance of technology/ies employed in the treatment of wastewater. Other reasons contributing to poor BOD effluent can be attributed to inappropriate organic loading on the system, cross sectional discharge of wastewater to the system which creates organic shock due to the negative effects of raw wastewater on floc formation of microorganisms in aeration tanks, improper performance of secondary sedimentation, micro-organisms' death resulting from drugs and antibiotics in raw wastewater, improper return of sludge into aeration tanks and thermal shock to the system of laundry (Mahvi et al., 2009). The synergistic effect of heavy metals also increases BOD in effluent water (Hromada and Zhang, 2006).

5.1.4 Chemical oxygen demand

Chemical oxygen demand (COD) measures non-biodegradable as well as biodegradable organics (Licis, 1995). Most of the COD comes from household waste with majority of the load coming from the toilet while the rest comes from greywater sources. Traditional sewage plants are able to effectively remove the COD with only 4% of the house load ending up as effluent. The stormwater and runoffs also contribute to the COD load of treatment plants (Gray and Becker, 2002). Biodegradable organics are principally composed of proteins, carbohydrates and fats, and are commonly measured in terms of Chemical Oxygen Demand (COD). If discharged untreated into the environment, their biological stabilization can lead to the depletion of the oxygen level and the development of unhygienic septic conditions. If the COD exceeds the

required limits, intervention is required. That is, there will be the need to optimize operation at the treatment plant to rectify the situation (Emanti Management, 2011). The efficiency of the wastewater treatment plant for COD is good for WW-Dim while it was poor for WW-Ama. Findings by Osode (2007) and Momba, Osode and Sibewu (2009) support the observed COD level for WW-Dim to be within the recommended limit. WW-Ama, however, displayed high COD levels from the month of May 2013 to August 2013 in an incremental fashion, but subsequently other months fared better with the level of COD level measured. Odjadjare and Okoh (2010) observed the COD of a treatment plant in the Eastern Cape fell short of the recommended standard. Another study by Momba, Osode and Sibewu (2009) evaluating four WWTPs in Buffalo City and Nkokonbe municipalities in the Eastern Cape found the COD level to be within the recommended limit with no risk to the receiving surface water. High COD level is a demonstration of carbonaceous contamination which also shows how much oxygen is needed to oxidize all the carbonaceous contaminants to carbon dioxide. Of great importance is the technology employed in the treatment of the wastewater; in this case, COD removal would be achieved as aerobic decomposition of organic matter is enhanced through aeration (Caballero, 2011). An activated sludge system offers more aeration capability than the biofilter treatment method. This is particularly observed in the measured COD values for the two treatment plants. The WW-Dim Sewage Treatment Works using the activated sludge technology had a better COD level when compared to the WW-Ama Treatment Plant which uses the Biofilter/trickling filter technology (see tables 4.2 and 4.3). It is also worth noting that temperature also has great influence on the COD level in the final effluent. At low temperatures of 11 °C

COD, BOD, and TSS removal rates obtained were reported to be high (Oh, 2012). High temperature decreases the solubility of gases in water which is ultimately expressed as high BOD/COD (Prasad and Rao, 2011). Different temperatures reduce different COD removal rates (Caballero, 2011). Also, the high COD may be attributed to a large amount of inorganic compounds present, which are not affected by bacteria, and hence results in higher COD (Verma, Ramteke and Garg, 2008).

5.1.5 Total dissolved solid

TDS is a measurement of the concentration of particulate solids that can dissolve or be suspended in wastewater (Kiepper, 2010). TDS is made up of inorganic salts, as well as a small amount of organic matter. Common inorganic salts that can be found in water include calcium, magnesium, potassium and sodium and iron, manganese, etc which are all cations, and carbonates, nitrates, bicarbonates, chlorides and sulphates, which are all anions. Cations are positively charged ions and anions are negatively charged ions (Safe Drinking Water Foundation, 2009; Health Canada, 1991; Lokhande, Singare and Pimple, 2012). The DWAF regulation does not state any value to be used as quality guideline for total dissolved solids for effluent except for domestic, industrial and agricultural uses. A 0-450 mg/l limit was set for domestic water use, 0 – 1600 mg/l for industrial use but with category of use and 0 – 3000 mg/l for livestock watering in agricultural use (DWAF, 1996). For this study, the 450 mg/l limit is used because of the effect it can have on human use and other activities requiring use of water (DWAF, 1996). This study showed that both treatment plants at WW-Dim and WW-Ama had their TDS within the recommended limit. However, the WW-Dim Treatment Plant

shows a higher quality level of TDS in its effluent than the WW-Ama plant. Odjadjare et al. (2012) is a similar work which reported TDS for WW-Dim to be within the recommended limit. The same results were found for some plants in the Eastern Cape whose TDS measurements were reported to be within the recommended limit (Osode, 2010; Odjadjare et al., 2012). This, however, is in contrast to another study done for another treatment plant in the Eastern Cape which found the TDS not to be within the acceptable limit (Mazibuko, 2012). In the rural area of the Eastern Cape, the TDS levels for treated effluent were also within the set limit (Odjadjare, 2010). Osode (2010) and Odjadjare and Okoh (2010) also highlighted the influence of seasonal change on the TDS of the final effluent in the Eastern Cape. As for the process of removing of TDS, Health Canada (1991) noted that Total Dissolved Solids are not substantially removed using conventional water treatment processes and the addition of chemicals during conventional water treatment generally increases the TDS concentration. The use of lime-soda ash softening and sodium exchange zeolite softening in certain treatment process can either slightly decrease or increase the TDS concentration, respectively. Demineralization processes are required for significant TDS removal (Gray and Becker, 2002; Health Canada, 1991). Morrison et al. (2001) reported that high salt concentration in wastewater can result in adverse ecological effects on aquatic biota. A high content of dissolved solid elements affects the density of water, influences osmoregulation of freshwater organisms, reduces solubility of gases (like oxygen) and utility of water for drinking, irrigation and for industrial purposes (Lokhande, Singare and Pimple, 2012; Igbiosa and Okoh, 2009). Salinity is also a measure of TDS and a high level of salinity in water may have an adverse effect

on fresh water flora and fauna, which are not salt tolerant. High levels of salinity also have implications when using water for livestock consumption (Hunter-Central Rivers Waterwatch, n.d.). When using effluent water for irrigation one must consider its level of salinity either by measuring of the electrical conductivity or TDS (Osode, 2010). While an appropriate concentration of salts is vital for aquatic plants and animals, salinity that is beyond the normal range for any species of organism will cause stress or even death to that organism. Salinity also affects the availability of nutrients to plant roots. Water containing a TDS level of over 500 mg/L is unsuitable for irrigation of many plants and tastes unpleasant when taken as drink (Hunter-Central Rivers Waterwatch, n.d.). Odjadjare (2010) reported that at TDS concentration of <500 mg/l, there is no noticeable effect on soil or crops, implying that the quality of the wastewater under study is generally good for agriculture. Other noted noticeable impacts' affect on aquatic organism depend on adaptations of individual species; community structure; and on microbial and ecological processes such as rates of metabolism and nutrient cycling (DWAF, 1996a). The General Standards for the discharge of effluents do not specify any limit for TDS, but there have been target limits set by others for reclaimed wastewater. Total dissolved solids (TDS) target limit in reclaimed wastewater for agriculture range from <500-2000 mg/l. This, however, depends on the sensitivity of the crop to salinity. Although there are no recommended limits for TDS concentration for water use in aquaculture, recreation use and aquatic ecosystems, however for domestic use the set limit recommended is 0-450mg/l. The recommended limit for industrial use is 0 - 1600 mg/l though this recommended limit still depends on categories and range of use. For livestock, 0- 3000 mg/l is recommended with specific

categories and range of use (DWAF, 1996c). Despite effluent quality regulations, salinisation is an important problem threatening water supplies in South Africa (Slabbert, 2007). The synergy between TDS and pH of water is a helpful indicator of the level of water pollution. When a water source has a high level of TDS or a low pH, it is likely that there are other harmful contaminants in the water. Both TDS and pH are also easy to measure and if something is happening to the water, such as pollution, chances are that both TDS and pH levels will change and, therefore, keeping track of those changes can act as an early warning signal that something is happening to the water. For these reasons it is important to monitor the TDS and pH levels, so that action can be taken immediately whenever change is witnessed between both TDS and pH levels (SDWF, 2009).

5.1.6 Electrical conductivity

Electrical conductivity (EC) is an indicator of total dissolved salts (TDS) (DWAF, DHE and WRC, 2001). The electrical conductivity measured (mS/m) at the two treatment plants were within the general and special set limits (Table 4.1). For the WW-Dim final effluent, the measured conductivity was 15.9 ± 1.9 mS/m, the discharge point was 15.5 ± 1.4 mS/m and WW-Ama was 44.3 ± 4.0 mS/m. The WW-Ama Treatment Plant had the highest values for E.C. as compared to the WW-Dim Sewage Treatment Works. This is attributed to the poorly treated effluent at the WW-Ama WWTP. Electrical conductivity in conjunction with total dissolved solids are monitored for effluent used in domestic, recreation, industrial, agricultural and the aquatic ecosystem (DWAF, 1996a; b; c; d). This serves as a checkmate to the build-up of salinity and

nutrients which can adversely affect surface water and land application (Shabalala, Combrinck and McCrindle, 2013). Conductivity is also affected by temperature: the warmer the water, the higher the conductivity. Hence, conductivity is reported as conductivity at 25 degrees Celsius (25 °C). Discharges to streams are capable of altering the conductivity depending on their composition. A failing sewage system being discharged would raise the conductivity because of the presence of chloride, phosphate, and nitrate (US EPA, 2012b). The electrical conductivity measured for both plants were found to be within the permissible limits of DWAF guidelines. This was in contrast to what was reported by Igbiosa (2010) and Osode (2010), where the EC measure exceeded the regulatory limit.

5.1.7 Nitrates and nitrites

In a wastewater treatment plant, ammonia is normally oxidized to nitrites and then to nitrates. In situations where the nitrate/nitrite concentration exceeds the required limits, intervention is vital to remedy the situation (e.g. makes sure of source protection, ensures that the treatment plant can effectively remove nitrate/nitrite, and optimizes the operation at the treatment plant) (Emanti Management, 2011). The nitrogenous contaminants are mainly in the form of amines, nitrites and nitrates. In the laboratory these are determined either as the individual compounds or as the total nitrogen or the Kjeldahl nitrogen. The nitrogenous contaminants are best removed by first oxidizing the amines and the nitrites to nitrates.

Eutrophication results from nutrients entering surface water. It is, therefore, important to measure variables such as the nitrate, nitrite, ammonia, total phosphorus (filtered and unfiltered) in water (UNEP/WHO, 1996).

5.1.7.1 Nitrates

Though the nitrate measured was within the recommended general limit of 15 mg/l for some of the samples analyzed, none met the special limit of 1.5 mg/l. It was observed that WW-Dim generally has poor nitrate levels. Mean nitrate measured at the final effluent (14.27 ± 2.8 mg/l) and at the discharge point (14.64 ± 3.4 mg/l) was high and close to the set general limit of 15 mg/l. The concentration levels were high showing poor performance of the plant in reducing nitrate. Quite the opposite was the WW-Ama treatment plant; it recorded an excellent performance in reducing nitrate (5.13 ± 2.8 mg/l) and good quality nitrate level within the regulatory set limit. Previous works on WW-Dim by Odjadjare et al. (2012) and Osode (2007) reported that nitrate quality for WW-Dim met the recommended standard. While some other plants in the Eastern Cape were reported to fall short of the set limit in terms of the concentration of the nitrate in the final effluent discharged onto surface water (Morrison, Fatoki, Zinn and Jacobsson, 2001b; Igbinsosa, 2010; Osode, 2007; Odjadjare et al., 2012). And the high level nitrate level from effluent in the Eastern Cape is reported to promote eutrophication in receiving water body (Mazibuko, 2012). Mahvi et al. (2009) identified weak denitrification, otherwise referred to as hydraulic shock, to be responsible for the presence of nitrate in the wastewater plant and also improper return of sludge into aeration tanks from the secondary sedimentation tank and because of the frequent

effluent point discharge, run-off from agriculture and from oxidation of nitrogenous waste products in human and animal excreta, including septic tanks, have been identified as significant sources of nitrogenous compounds (Plessis, 2008; WHO, 2008; Hunter-Central Rivers Waterwatch, n.d.). In natural waters nitrite is normally present only in low concentrations (a few tenths of a milligram per litre). Higher concentrations may be present in sewage and industrial wastes, in treated sewage effluents and in polluted waters (UNEP/WHO, 1996; Ministry of Environment, Lands and Parks and Geographic Data BC, 1998). Excessive concentration of nitrate in freshwater supplies is highly toxic and their removal is difficult and expensive (Plessis, 2008). High nitrate levels in waste effluents could also contribute to the nutrient load of the receiving water and so contribute to eutrophication effects (Osode, 2007). If other necessary nutrients are present, algal blooms can occur in surface water with as little as 0.50 mg/L NO₃-N (Westminster College, 1993),

Nitrate does not cause direct toxic effects, but its reduced form, nitrite, does and is 10 - 15 times more toxic than nitrate (DWAF, 1996b). The nitrate toxicity to aquatic organisms is due to nitrate ions, which lead to the conversion of oxygen carrying pigments to the forms that are incapable of carrying oxygen. Nitrate toxicity in aquatic ecosystems particularly affects fish and crayfish (Zuma, 2010). In animals, nitrite is formed through the biological reduction of nitrate in the rumen, and ruminants are therefore susceptible to nitrite poisoning (DWAF, 1996b). The presence of nitrate and nitrite in water has been associated with methaemoglobinaemia, especially in bottle-fed infants.

However, due to the low permeability of nitrate ions to most aquatic organisms, its toxicity levels are limited. A maximum level of 2 mg NO₃-N/L has been proposed to protect sensitive aquatic animals (Zuma, 2010). In another report nitrate level greater than > 3 mg/l in waterways is not the best. A much lower concentration of less than 0.5 mg/l is allowed (Hunter-Central Rivers Waterwatch, n.d.; DWAF, 1996c). Also it has been shown that the addition of an exogenous nitrogen source increased survival of *E. coli* bacteria in aquatic environments, even in the presence of competing microorganisms. Thus, sewage outflow and surface run-off not only serve as sources of pathogens and their indicators, but also of nutrients that promote the survival of those microbes (Macmaster, 2008).

5.1.7.2 Nitrite

The nitrite level measured at the two treatment plants were within the general and special set limit for the WW-Dim final effluent (0.15±0.1 mg/l), the discharge point (0.16±0.0 mg/l) and WW-Ama (0.34±0.2). Reports by Osode (2010), Odjadjare et al. (2012) and Mazibuko (2012) found the nitrite level of the final effluents in their various studied plants in the Eastern Cape to be within acceptable limits. Another report on effluent from a wastewater plant in the Eastern Cape by Igbinosa (2010) and Igbinosa and Okoh (2009) revealed high nitrite concentrate with a negative impact on the receiving surface water body.

It is also worth noting that the pH of the wastewater during nitrification impacts the final outcome of the process. At a temperatures of 25-30 °C and a pH of 7 – 8.5 has been optimized as the optimum pH for a good nitrification (Lenntech, 2014). The

reported pH and temperature for the two plants studied are within this range which could have aided the nitrification processes in minimizing the amount of nitrite in the final effluents. As ammonia is converted to nitrite and nitrate, alkalinity decreases and the pH of the wastewater may drop. Nitrite levels should be very low throughout the entire treatment process. High levels of nitrite (NO_2) in the system indicate there may be a problem with the nitrification cycle. An effluent with high nitrite (NO_2) concentrations will be difficult to disinfect because of the tremendous chlorine demand it poses and very high nitrite levels are usually associated with water of unsatisfactory microbiological activity (WDNR, 2010; Fayyad and Al-Sheikh, 2001; Rich, 2003; CPCB, n.d.). To demonstrate the importance of the treatment process in the removal of nitrite, Howard et al. (2004) reported an increase in the level of nitrite in the final effluent from a low level concentration measured for influent showing that the treatment process before final discharge must be effective and efficient.

5.1.8 Ortho-Phosphate

The mean value concentrations of the ortho-phosphate measured for both the WW-Dim final effluent (4.05 ± 3.3 mg/l), the discharge point (4.27 ± 3.5 mg/l) and at the WW-Ama treatment plant (8.30 ± 6.0 mg/l) satisfied the recommended limit set for it. The mean value for the WW-Ama treatment plant was relatively high in relation to the set standard. In few instances was the concentration of phosphate recorded at the plants found to be above the recommended limit of 10 mg/l set by DWAF; WW-Dim in the months of March and April and WW-Ama in the months of February, March and August recorded a high level concentration of phosphate. The orthophosphate level

reported by Igbiosa (2010), Gusha (2012) and Osode (2010) found effluents from WWTPs exceeded the South African target limit for phosphate level discharged into water systems that can induce the growth of algae and other plants and this implies more degradation of the receiving water body.

High phosphate levels could lead to similar problems described above for nitrate. The presence of nitrogen and phosphorus in fresh water can create environmental conditions that favour the growth of toxin-producing cyanobacteria and algae (Akpor, 2011). Some of the main sources of phosphorus in the environment and surface water have been attributed to effluent from WWTPs and onsite sewage disposal units; detergents and fertilizers that have been washed down drains or that have run off from properties due to poor land management practices and storm water pollution and decaying organic matter (Hunter-Central Rivers Waterwatch, n.d.). It therefore important that the levels of phosphates and nitrates in waterways should ideally be below the low detectable limit (Hunter-Central Rivers Waterwatch, n.d.).

5.1.9 Free chlorine

The regularity standard for free chlorine is 0.25 mg/l for the general limit and a special limit of 0 mg/l for effluent discharge. Residual chlorine levels varied at the two treatment sampling sites. The statistical mean of free chlorine measured exceeded the regulatory limit. At the WW-Ama treatment plant the measured chlorine value was below the required limit. Chlorine overdosing has been previously reported at the WW-Dim Sewage Treatment Works a couple of times (Momba, Osode and Sibewu, 2009). The over chlorination was attributed to poor operation and lack of skilled personnel

operators (Mema, 2004). This study suggests a similar trend was observed as high residual chlorine was recorded, which was due to the chlorine overdose. The effectiveness of the WW-Dim Sewage Treatment Works was able to reduce chlorine demand on the effluent in lieu of the other measured physicochemical parameters, which were in compliance. Subsequently, the disinfection process was effective in eliminating faecal coliform. But the effects of the excess free chlorine above the set limit negatively impact the surface water into which it is being discharged. In a similar study, Momba, Osode and Sibewu (2009) and Mazibuko (2012) reported chlorine overdosing at the WW-Dim Treatment Plant; likewise, low chlorine concentration in the effluent was reported by Gussha (2012). The free chlorine level at the WW-Ama plant was generally poor. The treated effluent quality was poor and this accounted for the inefficient and ineffective disinfection stage and there was also the possibility of no chlorine disinfection. The high BOD measured in the effluent shows the presence of excessive organic matter and inorganic matter exerts a high chlorine demand which reduces available chlorine for disinfection. Good contact time, pH, temperature and low turbidity are factors that are important in providing good disinfection when using chlorine. Effectiveness of chlorine decreases occur during disinfection in source water with excessive turbidity. High turbidity causes increased chlorine demand; this may occur or be caused by the inadequate treatment of influent (TLC, 2007). The total organic carbon (TOC) associated with turbidity exerts a chlorine demand and thus hinders with the preservative effect of chlorine residual in water. It was also shown that the protective effect of particulates in water and wastewater depends on the nature and the size of the particles. As it was shown that solid-associated viruses are more resistant

to chlorination than are “free” viruses (Bitton, 2005). In a study of the relationship between coliforms and *E. coli* with suspended inorganic particles in a drinking water reservoir, a strong correlation was found and it was noted that greater than 80% of the bacteria were physically associated with particles, and particle association would alter bacterial sedimentation rates in surface water (Stedtfeld et al., 2007). Provided there are efficient treatment processes for effluents, free chlorine can inactivate some enteric viruses (Thurston-Enriquez, Haas et al., 2003; Kahler et al., 2010; Thurston-Enriquez et al., 2005). High residual chlorine concentrations in treated effluents with high levels of turbidity were found not to be effective against the faecal coliform and pathogenic bacteria isolated in the final effluent (Dungeni, van Der Merwe and Momba, 2010; Odjadjare, 2010; Igbinosa, 2010; Odjadjare et al., 2012). This suggests that the two treatment plants do not comply with the regulatory set limit. The WW-Dim Sewage Treatment Works was overdosing with chlorine. In contrast to this, Osode (2010) reported compliance with the set residual chlorine limit at the same treatment plant. The WW-Ama plant was considered to be under chlorinated as any effective chlorination was shadowed by the low quality effluent being produced.

5.1.10 Turbidity

No standard is given by DWAF for turbidity for effluent discharge. Turbidity of the effluent varies at all sampling sites and mean values of 10.29 ± 7.3 NTU (FE) and 10.91 ± 8.1 NTU (DP) were recorded for the WW-Dim Sewage Treatment Works and the WW-Ama plant recorded 17.40 ± 13.1 NTU. Turbidity in water is composed of inorganic (e.g., silt, clay, iron oxides) and organic matter as well as microbial cells. It is

measured by determining light scattering by particulates present in water (Bitton, 2005). Turbidity reported by Mazibuko (2012) from the Eastern Cape wastewater treatment plants was found to be slightly above the WHO suggested limit of <5 mg/l while Igbiosa (2010), Odjadjare and Okoh (2010) and Odjadjare (2010) reported turbidity of effluent within the <5 NTU recommended by WHO for drinking water quality in treatment plants in the Eastern Cape. In the Gauteng provinces, 4 WWTPs tested were reported to have poor turbidity for the effluent (Dungeni, van Der Merwe and Momba, 2010). Suspended solids contribute to turbidity and silt load and generally require sedimentation or filtration for removal (Licis, 1995). Turbidity of the final effluent can be used as a measure of treatment efficiency (Barth and Ettinger, 1965; Hussain, 2010). It indicates problems with treatment processes, particularly coagulation/sedimentation and filtration. No health-based guideline value for turbidity has been proposed; ideally, however, median turbidity should be low for effective disinfection, and changes in turbidity are an important process control parameter (WHO, 2008).

For an efficient disinfection process to result in the removal of pathogenic bacteria and coliphages, low turbidity should be ensured (Dungeni, van Der Merwe and Momba, 2010). Increasing turbidity was associated with increasing *E. coli* abundance. Reagent disinfection or UV irradiation requires a pre-treatment stage to eliminate suspended solids or decrease turbidity as increases in any of these factors decreases the disinfection efficiency (Osode, 2007, 2010).

The UV radiation is found to be effective in destroying organisms within its reach; the water must be relatively free of turbidity, however. The effectiveness of UV disinfection is dependent upon UV power, contact time, liquid film thickness, wastewater absorbance, wastewater turbidity, system configuration, and temperature. It is important that pre-treatment provide a high degree of suspended and colloidal solids removal (U S EPA, 2002). Microbiological quality can be significantly improved at turbidity below 0.2 NTU (Xagorarakis et al., 2004).

Turbidity in water is composed of inorganic and organic substances as well as microbial cells. It interferes with the detection of coliforms in water but it can also reduce the disinfection efficiency of chlorine and other disinfectants. The need to remove turbidity is based on the fact that particle-associated micro-organisms are more resistant to disinfection than freely suspended micro-organisms. Reducing turbidity to less than 0.1 NTU could be a preventive measure for counteracting the protective effect of particulate matter during disinfection (Bitton, 2005). High turbidity causing increased chlorine demand may occur or be caused by the inadequate treatment of water (TLC, 2007). Turbidity has been linked to incidence of waterborne diseases since the presence of particulate matter can stimulate the growth of micro-organisms, including pathogenic ones (Mazibuko, 2012; DWAF, 1996d).

Listeria prevalence was attributed to the turbidity of the final treated effluent of a wastewater treatment plant (Odjadjare, 2010). Some other studies reported that increasing turbidity was associated with increasing *Vibrio* abundance found in the final effluent of a wastewater treatment plant (Igbinsola, 2010; Johnson et al., 2010; Igbinsola,

Obi and Okoh, 2009). Viral concentrations have also been reported to be influenced by the turbidity of the water (Venter et al., 2007; DWAF, 1996d; Hernandez-Morga et al., 2009). In another study by Samie et al. (2009), they found sewage plants with reduce turbidity at different microbial indicators counts to still contain several pathogenic bacteria organisms detected in the final effluent.

High turbidity of final effluent is also inherently dangerous to the health of the aquatic life and humans due to its potential in the formation of a carcinogenic by-product called trihalomethane during chlorination (Mazibuko, 2012). In addition, an increase in turbidity impacts on feeding patterns of filter feeders, causes physiological damage and limits habitat for certain invertebrate species (Slabbert, 2007). Visual predators display a reduction in feeding efficiency due to decreased reactive distances and may cease feeding if turbidity levels are too high. Breeding behaviour may also be altered by the presence of turbid water; this is especially true for salmon and species which require gravel beds or a clean substratum for spawning (DWAF, 1996c). Turbidity also influences the chemical composition of natural waters because the particles are generally charged, thus forming adsorption and desorption surfaces (Slabbert, 2007).

5.1.11 Temperature

The DWAF recommended effluent discharge into a water body should not alter the natural ambient water temperature of the receiving resource by more than 2 to 3 degrees Celsius (DWAF, 2013). The average water temperature measured at the WW-Dim Sewage Treatment Works was 20 ± 4.7 °C and 22 ± 3.2 °C for the WW-Ama Treatment Plant. Water temperature is one of the most important characteristics of an

aquatic system affecting dissolved oxygen levels, chemical processes, biological processes, species composition of the aquatic ecosystem and water density and stratification (RAMP, 2014).

Temperature as well as contact time, turbidity and pH influence the efficiency of disinfectants such as chlorination and thus require monitoring (NHMRC, 2011). Temperature, a physical factor, can affect the metabolism of aquatic organisms, a biological process (World Water Assessment Programme, 2009). It can directly affect the growth and survival of microorganisms (NHMRC, 2011). It is important in the nitrification and denitrification process as it is reported that a drop in temperature affects the bacteria involved in the treatment process (Ilies and Mavinic, 2001) and high temperature caused a more negatively charged sludge, a shift in filamentous organisms, and a reduction in protozoan/metazoan concentrations and diversity (Morgan-Sagastume and Allen, 2003). Igbiosa (2010) also reported the correlation between temperature and *Vibrio* abundance in effluent. Effluent discharge with high temperature has been reported to cause thermal shock to the aquatic biota (Oliver and Fidler, 2001), while the survival of some aquatic invertebrates are shown not to be significantly affected by water temperature (3-20 °C) (Alberta Research Council Project, 2008).

5.2 Microbiological quality

5.2.1 Faecal Coliform (FC)

The count of faecal coliform bacteria in the final effluent at the WW-Dim Sewage Treatment Works, which was being discharged into the Mdenzi stream, was relatively low. The FC of the final effluent point ranged from 0 and 1.9×10^4 CFU/100 ml while at the discharge point, it ranged between 0 and 2.0×10^4 CFU/100 ml. The recommended faecal coliform count for effluent by DWAF is < 1000 CFU/100 ml (DWAF, 2013). However, there were low counts of viable faecal coliform which accounted for 91.7% of the samples analysed for 11 months for the WW-Dim effluent points. About 58% of the samples analysed complied with the DWAF special limit of 0 CFU/100 ml (DWAF, 2013). Only in the month of August was a high faecal coliform (FC) count experienced. When compared to the level of faecal coliform bacteria for effluent discharge recommended in the guideline set by DWAF, the mean faecal coliform counts exceeded the set limits of 0 and 1000 CFU/100 ml. The reason for the high count is explained under the *E. coli* section. Previous report by Lehr et al. (2005) showed that the WW-Dim Sewage Treatment Works effluent complied with the set limit while in another study by Momba, Osode and Sibewu (2009) they reported non-compliance.

The observed count for faecal coliform at the WW-Ama Treatment Plant was very poor. The average count was nine times the set limit at 9.2×10^3 CFU/100 ml. The bacterial counts range between 0 and 2.9×10^4 CFU/100 ml. Most of the samples (60%) analysed do not comply with the set limit. About 33.3% of the tested samples

complied with the special limit of 0CFU/100 ml while the remaining 6.7% were less than 1000 CFU/100 ml.

Faecal coliform is one of the most commonly used indicators for microbial water quality and human health risk assessment (Jiang, 2006) because it is correlated with the presence of several organisms that cause waterborne diseases (Myers, 2003; Myers et al., 2007). This study shows that the two treatment plants are sources of faecal coliform in the environment. However, comparison of both plants demonstrates that the WW-Ama Central Works releases more faecal bacteria into the environment than the WW-Dim Sewage Treatment Works. The monthly effluent samples collected from WW-Ama revealed the presence of higher amounts of coliform bacteria than the standard limit. The presence of faecal coliform in the downstream river of WWTPs in the Eastern Cape has also been reported (Sibanda, Chigor and Okoh, 2013; Chigor, Sibanda and Okoh, 2013); likewise, in another study effluent which emanated from Gauteng Province was reported as sources of faecal pollution to the downstream river into which it is being discharged (Dungeni, van Der Merwe and Momba, 2010). The faecal pollution reported for the rivers was very high (Chigor, Sibanda and Okoh, 2013). The high counts of coliform was an indication of the presence of microbial contaminants in the effluents discharged into the surface water (Mazibuko, 2012). The failure of the South African WWTPs to produce effluents of a high microbiological quality is responsible for the contamination and pollution of water resources (Dungeni, van Der Merwe and Momba, 2010).

5.2.2 *Escherichia coli* (*E. coli*)

The presence of *Escherichia coli* (*E. coli*) in water is an indication of faecal contamination from warm-blooded animals and this point to a feasible presence of pathogens. A few strains of *E. coli* are pathogenic, such as *E. coli* O157:H7, but most strains are not (Myers, 2003; Myers et al., 2007).

The average count of *E. coli* (CFU/100 ml) in the treated effluent samples for WW-Dim's final effluent was 2.7×10^3 and 2.0×10^3 at the discharge point. This is above the regulatory limit for faecal coliform. Furthermore, the count of *E. coli* ranged between 0 - 1.86×10^4 CFU/100 ml for the final effluent and 0 – 2.16×10^4 CFU/100 ml for the discharge point. The DWAF has no set guideline for effluent discharge on *E. coli* except for faecal coliforms which was used as the baseline for the study. About 83.3% of the analysed samples complied with the effluent discharge limit using the faecal coliform set limit. Two of the months (December 2012 and August 2013) failed to meet the set limit. One of the months (December 2012) was as a result of inadequate treatment due to rain run-off into the treatment plant which affected the treatment process. To minimize pressure overload of the plant, the wastewater was discharged without adequate treatment thus contributing to the high counts of *E. coli*. A possible cause of the high counts of *E. coli* observed in August 2013 was suspected to be due to lack of disinfectant during this month as the plant was found to have run out of chlorine gas used for the disinfectant.

At WW-Ama Central Works, the *E. coli* counts of the samples (58%) were > 1000 CFU/100 ml more than the set guideline and the *E. coli* counts ranged from 0 – $1.85 \times$

10^5 CFU/100 ml. The average *E. coli* count was 2.21×10^4 CFU/100 ml. The abundance of *E. coli* in the effluent presents a gloomy picture of the state of this wastewater treatment plant as the discharge of effluent from wastewater treatment plant(s) (WWTPs) could have major damaging effects on the health of aquatic ecosystems (Wright-Walters and Volz, 2009). Inadequate disinfection processes or a poorly operated treatment plant can result in multiplication or survival of various micro-organisms in already treated wastewater effluent, as they make their way into the environment (Anastasi et al., 2012). Previous studies have reported the isolation of *E. coli* from treated effluent in the Eastern Cape (Dungeni, van Der Merwe and Momba, 2010). River water receiving effluent discharges have also been reported to harbor *E. coli* (Sibanda, Chigor and Okoh, 2013; Chigor, Sibanda and Okoh, 2013) and, as a result of this, dangerously high levels of *E. coli* have been recorded in many of the Eastern Cape's rivers. *E. coli* and faecal coliforms have been reported to exceed two million per 100 ml at a river in Port Elizabeth used for recreational activities. This is in contrast to the acceptable standard for recreational use at 130 CFU/100 ml (Straton, 2013; Gyedu-Ababio, 2011).

The public health consequence of the presence of *E. coli* in the environment was reported by Bateman (2008), where eighty babies were reported dead in the Eastern Cape from diarrhoea-related diseases. A recent *E. coli* outbreak in Bloemhof in North West, which killed two babies with many others reporting to the clinic (Stone, 2014), confirms the dangerous effects of uncontrolled and poor monitoring of pollution in the environment and surface water.

Studies conducted by EPA to determine the link between faecal bacteria indicators and the incidence of digestive system infection recommended that the best indicators of health risk from recreational water contact in fresh water are *E. coli* and enterococci. Faecal coliforms as a group were established to be a poor indicator of the risk of digestive system infection (US EPA, 2012a). This study showed an important finding on the incidence of pathogenic *E. coli* strains in the final effluent discharge into the surface water. Their presence in the environment calls for concern because of their public health consequences (Clements et al., 2012). About eight pathogenic *E. coli* pathotypes investigated by this study were identified. Five of the pathotypes can cause invasive intestinal infections, watery diarrhoea and dysentery in humans and animals while the remaining three cause extra-intestinal infections caused by extra-intestinal pathogenic *E. coli* (ExPEC) (Bekal et al., 2003). Four out of eight pathotypes identified and tested in this study are shown in Table 4.7. Both the invasive and extra-intestinal pathotypes were identified. A previous study done by Osode (2010) identified two *E. coli* pathotypes at the WW-Dim Sewerage Works; EHEC and EAEC were identified while EIEC was confirmed from another treatment plant. This was in contrast to the outcome of this study for WW-Dim where none of the pathotypes were identified. However, the efficiency of the treatment plant could be one of the reasons why no pathogenic *E. coli* were detected at these sites. Alternatively, it could be that *E. coli* strains found in these sites did not carry any virulent genes. This is evidenced by the absence of *E. coli* pathotypes from the *E. coli* isolated and confirmed. This similar situation was reported by Masters et al., (2011). However, the four pathotypes identified in this study were found at the WW-Ama Treatment Works Centre. The identified

pathotypes (Table 4.7) are of great public health importance. Apart from the EAEC previously identified by Osode (2010) which was also identified in this study, EPEC, NMEC and UPEC make up the major findings at the WW-Ama Treatment Plant. Of the 300 confirmed *E. coli* isolates tested, UPEC was about 9% followed by EAEC at 3.7%, NMEC at 1.7% and EPEC at 0.7%. In a similar study by Verma, Ramteke and Garg (2008) in India, they reported high incidence of UPEC in the treated final effluent as well as EPEC but at a lower concentration. Anastasi et al. (2012, 2010) demonstrated that some *E. coli* strains with uropathogenic properties survived treatment stages of sewage treatment plants and are released into the environment. The presences of EPEC in another study were found to be more prevalent in city wastewater compared to slaughterhouse wastewater where the prevalence of ExPEC was not affected by the wastewater treatment process and the prevalence of a typical EPEC was found to be very low in the final effluents (Diallo et al., 2013). The occurrence of EAEC in water was reported by Masters et al. (2011). They investigated for the presence of the virulence genes attribute to EAEC. This strain was identified in conjunction with EPEC pointing to a possible source of faecal contamination. Hamelin et al. (2007) reported the presence of EAEC, EPEC, UPEC and NMEC in river water receiving urban municipal wastewater. Also Koba (2013), in a study of the water from two rivers in the Eastern Cape, identified the presence of ETEC, EIEC and EPEC in one of the studied rivers and EAEC in both of the rivers studied. One of the studied sites, WW-Ama Treatment Plant, also demonstrated a large diversity of *E. coli* pathotypes. The presence of this pathogenic organism group has also been observed in previous studies where these strains were associated with both human and non-human extra-intestinal

infections (Bekal et al., 2003). Agricultural products and other aquaculture productions have been reported to have a high risk of diarrhoea as well as people who were in direct contact with wastewater had a higher risk of infection than those who were not (Trang et al., 2007). In the Eastern Cape and Limpopo Provinces of South Africa, the presence of these pathogenic *E. coli* with the exception of NMEC and UPEC have been isolated from diarrhoea patients with EAEC being the predominant cause of infection (Bisi-Johnson et al., 2011; Samie, Obi and Dillingham, 2007).

5.2.3 *Vibrio*

The presumptive *Vibrio* species counts range from 0 to 1.4×10^3 CFU/100 ml for the WW-Dim final effluent point and 1 to 1.3×10^3 CFU/100 ml for the discharge point. The average was 2.2×10^2 CFU/100 ml for the final effluent and 1.4×10^2 CFU/100 ml at the discharge point. The DWAF has no set limit for *Vibrio* spp. Therefore, the faecal coliforms guideline is used as the base limit for the evaluation of the *Vibrio* spp. Based on the calculated average, the WW-Dim Sewage Treatment Works complied with the general limit for the permissible amount of faecal coliform allowed for effluent to be discharged but failed for the special limit. Also, in all the samples analysed for this plant, 91.7% of the *Vibrio* counts were < 1000 CFU/100 ml allowed for effluent discharged for the 11 months sampled. However, one of the months failed to comply with the 1000 CFU/100 ml set limit and the reason was as a result of no chlorine disinfection. The WW-Ama Treatment Plant *Vibrio* counts range from 0 to 9.9×10^3 CFU/100 ml with an average count of 3.8×10^3 CFU/100 ml. The average count for this plant exceeded the required limit of 1000 CFU/ml for faecal coliforms as set by DWAF (DWAF, 2013). Many of the samples (58.3%) analysed failed to comply with the set limit; 25% of the samples were found to comply with the special limit of 0 CFU/100 ml while 16.7% of samples were < 1000 CFU/100 ml. In this study, it was observed that the WW-Ama Treatment Plant had a high prevalence of *Vibrio* in contrast to the WW-Dim Treatment Plant which had a very low prevalence of the organism in all the samples analysed. The existing report on WW-Dim by Igbinsosa (2010) isolated *Vibrio* spp. from the final effluent of the WW-Dim Wastewater Treatment Plant. In another area of South Africa, in Gauteng, *Vibrio* was found in the

final effluent of the wastewater plant (Dungeni, van Der Merwe and Momba, 2010). In a more recent work by Ye and Zhang (2013) in Hong Kong, high prevalence of *Vibrio* was also reported in the effluent of the studied treatment plant. Rojas and Hazen (1989) demonstrated that *Vibrio cholera* could survive in effluent provided that the right optimum conditions suitable for growth exist. A similar study by Wennberg et al. (2013) compared the survival of *Vibrio cholera* and *Vibrio parahaemolyticus* in treated and untreated water and found the bacteria proliferate in the treated water. The ability of *Vibrio cholera* to persist in an aquatic environment under limiting conditions is attributed to the RNA polymerase sigma factor (rpoS sigma) which aids survival during starvation and stressed periods (Yildiz and Schoolnik, 1998). *V. cholerae* is considered an autochthonous member of the estuarine microbial community which can grow under conditions of organic nutrient concentration and salinity typical of estuaries (Singleton et al., 1982; Thomas et al., 2006). Evaluating the treatment technologies used showed that the activated sludge system was far more effective in reducing the *Vibrio* pathogen than the biofilter/trickling filter system. The results coincide with the report of Okemo, Kiplagat and Ngari (2011), which had effluent from trickling filters having a low removal rate of pathogens. In contrast, Ramteke et al. (2010) found the activating sludge system to have a high removal rate of *Vibrio*. A high level of chlorination (high free chlorine) was observed for some periods of samplings in both the plants studied. High free chlorine was more frequent in the WW-Dim Wastewater Treatment Plant than in the WW-Ama Wastewater Plant. *Vibrio* was persistently isolated from the high chlorinated effluent in WW-Dim, though at low counts as compared to WW-Ama with high counts. Also the organism was more frequently isolated at the WW-Dim discharge

point than at the final effluent point suggesting the possibility of recontamination as the effluent leaves the treatment plant. Even at the recommended free chlorine level, *Vibrio* was isolated. A similar situation was recorded for effluents in some of the treatment plants in the Eastern Cape which also include the study of other pathogens apart from *Vibrio* which found the presence of other target organisms in disinfected treated effluent (Igbinsosa, 2010; Odjadjare, 2010; Igbinsosa, Obi and Okoh, 2009; Momba, Osode and Sibewu, 2009). The ability of some *Vibrio* strains to survive chlorination have been attributed to its ability to shift to a phenotype having a rugose colony morphology associated with the excretion of slime that can easily respond and adapt to starvation (Sousa et al., 2001; Mizunoe et al., 1999; Morris et al., 1996). Other factors such as contact time, temperature and pH may aid the survival of *Vibrio* spp. if disinfectant efficiency is poor (Odjadjare, 2010) due to the presence of organic compounds and ammonia in the effluent (Shang, Qi and Lo, 2005; DEFRA, 1988). It is therefore important that the effluent should be of a high quality for the maximum effect of disinfectant (Sibanda, Chigor and Okoh, 2013). The presence of the pathogens also signified that there are carriers of this form of the organism from the community from where the wastewater is received. Examining the frequency from the areas sampled, it was observed that the WW-Ama community had the higher detection rate than WW-Dim. It can be said that the *Vibrio* is more prevalent in WW-Ama. Coupled with the under performance of the WW-Ama Treatment Plant in eliminating the pathogen, the organism is being re-introduced back into the environment and this can create a vicious cycle of outbreak of infections from the organism. The Green Drop status, which implies excellent wastewater management and a respect for the environment and the

health of the community at large, is given to municipalities that comply with good wastewater discharge standards for 90 percent of the time (Henning, 2010). The present Green Drop status of WW-Ama Central Treatment Works was awarded a medium risk plant between 2010-11 and 2012 (DWAF, 2012, 2011). The outcome of this current study on the plant showed it is a high risk plant with potential danger to the environment, therefore, the plant needs urgent attention. The Green Drop of 2012 also identified some of the challenges facing the WW-Ama Plant which included effluent non-compliance and operating capacity that exceeds design capacity (DWAF, 2012). In contrast, the green status for WW-Dim went from a medium risk rating to a low risk rating (DWAF, 2012, 2011). The effluent quality of the plant also showed it as a low risk wastewater plant. However, the detection of *Vibrio* spp., though at a very minimal level, is of concern judging from the nature of the organism as one having the potential to initiate epidemic infection.

The samples that were positive for *Vibrio* spp were further screened for the *Vibrio* pathotypes using the PCR, which are *V. parahaemolyticus*, *V. vulnificus* and *V. fluvialis*. There was the limitation of not having the positive control for *V. cholera* for testing as part of the pathotyping. All the screened isolates were negative for the tested *Vibrio* pathotypes. The genes (Table 3.4) specific for the identification of these pathotypes were not detected in the tested isolates. The absence of the targeted pathotypes could be due to the fact that the tested isolates are not carriers of the genes specific to the identification of the strains or the pathotypes are totally absent. With the exception of *V. cholera* which could not be tested the tested strains are not ubiquitous

to the natural fresh or salt aquatic environment as are the *V. cholera* (Eddabra et al., 2010). *V. fluvialis*, *V. parahaemolyticus* and *V. vulnificus* are reported as the most frequently encountered pathogenic *Vibrios* in marine environments, coastal, estuaries and brackish waters as well as seafood, which is considered a natural habitat for this strains of *Vibrio* spp. (Wong, You and Chen, 2012; Ramamurthy et al., 2014). The study done by Igbiosa (2010) reported the presence of the *V. fluvialis*, *V. parahaemolyticus* and *V. vulnificus* in the final effluent of a wastewater treatment plant. The prevalence of this pathogenic *Vibrio* in the environment was reported to be influenced by temperature and salinity. The concentration of salinity differs for each *Vibrio* spp. at which they can survive (Amin and Salem, 2012; Maugeri, Caccamo and Gugliandolo, 2000). The public health consequences of this pathogenic spp. cannot be over emphasized as all these strains have been part of human diseases (Tarr et al., 2007) and are known to cause gastrointestinal disease syndrome (Drake, 2008).

5.2.4 Virus

The two WWTPs were evaluated for enteric viruses. The real time PCR method was used to determine and quantify the occurrence of enteric viruses in effluent samples collected from the plant and the results are discussed below.

5.2.4.1 *Adenovirus*

Adenovirus was detected in the two plants. The WW-Ama Central Treatment Works had the highest prevalence of the virus in the final effluent. About 67% of the samples were positive for adenovirus at concentrations ranging between 1.0×10^1 gc/l and 6.8×10^2 gc/l. The virus was also detected at the WW-Dim discharge point and final effluent samples at concentrations ranging 3.9×10^1 gc/l and 7.9×10^1 gc/l. The WW-Ama WWTP had the highest occurrence and concentration of the virus in their final effluent with WW-Dim having the lowest concentration.

At the present, there are no regulatory guidelines on the concentration of human enteric viruses that are allowed for effluent discharged into surface water in South Africa. The Canadian Water Authority has a guideline of a minimum of 4-log reduction/inactivation for domestic water use (Health Canada, 2004). The outcomes of this study provide conclusive evidence of the levels of infectious viruses with regard to HAdv, genomic copies that are being released in the environment. During the current study, the presence of HAdv was detected in 31.4% of the treated effluent wastewater samples analysed with real time PCR. In a previous study done on WW-Dim, the presence of other enteric viruses like Hepatitis A virus and Coxsackie A virus were found in the effluents of these plants (Gusha, 2012). The presence of HAdv in river

water which receives effluents from the wastewater treatment plant in the Eastern Cape was reported in an earlier study by Chigor and Okoh (2012a) and Sibanda and Okoh (2012). Likewise, the virus was reportedly found in river water and treated drinking water in South Africa (Van Heerden et al., 2005). The presence of polio virus was also reported in wastewater (Grabow et al., 1999) in Gauteng Province of South Africa. Studies on the prevalence of HAdv in the environment are limited. Much of the reported cases of HAdv in South Africa have been on clinical patients (Taylor, Marx and Grabow, 1997; Moore, Steele and Alexander, 2000). The results of this study are consistent with past studies which found HAdv in treated effluent released to the environment (Carducci et al., 2009; Kuo et al., 2010; Kokkinos et al., 2011).

Simmons and Xagorarakis (2011) and Water Research Foundation (2010) reported in their work that, given the right treatment system and correct configuration of the treatment process, the chlorine disinfection will be able to inactivate HAdv in effluent. It was observed in this study, that one of the treatment plants, WW-Dim, which recorded a high level of chlorine in most of the samples analysed, could have explained the possible absence of the HAdv through inactivating it. Interestingly, the virus was detected in one of the unchlorinated effluent samples. Studies done by Thurston-Enriquez et al. (2003, 2005) were able to demonstrate inactivation of HAdv 40 with chlorine and chlorine dioxide. They found that the disinfection process is effective at a pH of 5 to 8, a temperature of 5 °C to 15 °C and < 30 mins contact time was enough to inactivate the HAdv 40. They were, however, quick to point out that there is need for more studies to be carried out to reduce viruses in an aggregated state, associated with

particular matter, and in natural water. The treatment technology employed in treating wastewater to reduce the virus particles in treatment plant is important. It has shown the effective reduction of HAdv in membrane bioreactor as compared to the conventional wastewater treatment plant (Simmons, 2010; Simmons and Xagorarakis, 2011; Simmons, Kuo and Xagorarakis, 2011). Berg (1973) claimed that activated sludge is the best biological method for removing virus particles from sewage while trickling filters and oxidation ponds are erratic, probably because of frequent short-circuiting that characterizes the latter. This is supported by the work done by Dahling, Safferman and Wright (1989) and Belguith et al. (2007), which found trickling filter plants discharge high levels of virus particles into receiving water. A similar trend was observed for the two treatment plants: WW-Ama uses a trickling filter as part of the wastewater treatment process while WW-Dim uses the activated sludge system. It is interesting to know that high prevalence of HAdv was found in WW-Ama which uses the trickling filter technology. The WW-Dim Treatment Plant which uses the activated sludge system had a lower HAdv detection. Other factors such coagulation, flocculation, sedimentation and filtration remove virus particles onto particulate matter. The efficiency of removal varies depending on the adsorptive affinities of the virus particles and the absorbents (Okoh, Sibanda and Gusha, 2010).

Adenovirus serotyping

The samples that were positive for adenovirus were further screened for the adenovirus serotypes including HAdv Species B (HAdv 3, 7, 21), Species C (HAdv 1, 2, 5, 6), Species E (HAdv 4) and Species F (HAdv 40, 41). Only Species C (HAdv 2) and

Species F (HAdv 41) were detected at rates of 2.9% and 14.3% respectively. Sibanda and Okoh (2012b) reported the detection of Species C and F adenoviruses in river water in the Eastern Cape. The incidence of these two types of species in wastewater effluent was also reported by Fong et al. (2010) and Kuo et al. (2010). They were able to detect more of the HAdv 41 than HAdv 2 which was in agreement with this study's findings. A study of the HAdv in sewages of Taiwan area reported the presence of both the Species C and F adenoviruse with more of HAdv 41 in their findings (Shih, 2013). But in contrast to the report of Sibanda and Okoh (2012a), more of the Species C adenovirus were found than the Species F in the river water. Species C adenovirus have been attached to respiratory illness (WRF, 2010) while Species F is considered one of the major causes of viral gastroenteritis infections (Kuo et al., 2010). Species F has been reported to cause serious infections in immunocompromised individuals (Jong, 2003) and it was found to be a co-infection in HIV patients (Kolawole, Oladosu, Abdulkarim and Okoh, 2013).

5.2.4.2 *Rotavirus, Hepatitis A, Enterovirus and Norovirus*

This study has shown that 42% of the samples tested positive to rotavirus at concentrations between 0 to 5.2×10^3 gc/l at WW-Ama and 17% at WW-Dim plant at concentrations between 0 to 5 gc/l. Hepatitis A, Enterovirus and Norovirus were not detected in any of the plants even though their presence in wastewater and faecal pollution studies have been documented in other countries (Flannery et al., 2012; Nordgren et al., 2009; Prado et al., 2012; Rigotto et al., 2010; Battistone et al., 2014). Norovirus has been reported in sewage-polluted river water and among hospitalized

paediatric patients in South Africa (Mans et al., 2010, 2012; Ramudingana, 2010). In a similar study done on rivers receiving treated effluent, Norovirus was detected in the river water (Sibanda and Okoh, 2013). There is limited literature on the study of Norovirus including South Africa (Murray, Mans and Taylor, 2013). Work done by Murray, Mans and Taylor (2013) could not detect Norovirus in wastewater treatment plants in the five provinces tested in South Africa.

The presence of Hepatitis A, Rotavirus and Enterovirus in the Eastern Cape rivers of South Africa was reported by Chigor and Okoh (2012b). A similar study by Sibanda and Okoh (2013) could only detect the presence of Hepatitis A and Rotavirus while enteroviruses were absent in the analysed river samples. The prevalence of Hepatitis A in the treated effluent in the Eastern Cape was reported for the WWTPs studied suggesting that treatment plants are potential sources of the virus in the environment. Also Prado et al. (2012) detected Hepatitis A virus in the raw effluent and none for the treated effluent, suggesting that the activated sludge technology could have aided the removal of the virus from raw sewage. The effectiveness of activated sludge to remove hepatitis A has been reported to minimize the presence of the virus in the environment (Arraj et al., 2005; Prado, Gaspar and Miagostovich, 2014; Anastasi et al., 2008). With reference to the studied plants which use the activated sludge systems, the absence of the virus in the final effluent could have been due to the same reason.

The prevalence of rotavirus as one of the viral gastroenteritis is understudied in the South Africa aquatic environment. However, clinical infections have been reported most specially among infants (Taylor, Marx and Grabow, 1997; Steele et al., 2002).

Since these enteric viruses were detected in various concentrations of final effluent samples, subsequent studies will have to ascertain how the presence of these viruses correlates with human pathogens. Enteroviruses, hepatitis A and noroviruses may not have been detected in the effluent samples, but their absence does not mean they are none existence. Therefore, it is good to note that the use of these viruses as indicators of faecal pollution could lead to wrong conclusions on the extent of faecal contamination. The results of this study support prior findings regarding the prevalence of adenoviruses and rotavirus in final effluents (Fong et al., 2010; He et al., 2008; Symonds, Griffin and Breitbart, 2009).

The prospect of using adenoviruses, enteroviruses, noroviruses, and rotavirus as indicators of water quality

These viruses are suitable emerging pathogens because of their adaptability and their ability to infect new hosts and to adjust to new environments. These waterborne pathogens called enteric viruses are among the commonest and most hazardous in causing both sporadic and outbreak-related illnesses. The main health effect associated with enteric viruses is gastrointestinal illness; however, they are also implicated in hepatitis, conjunctivitis, central nervous system infections, respiratory symptoms and chronic diseases (La Rosa et al., 2012).

To protect public health, finding a practical method for assessing faecal pollution in water used for recreation becomes imperative. This is often accomplished through the detection of indicators, which are used to approximate the presence of pathogens. The bacterial indicators of faecal contamination currently in use are not good indicators of

wastewater pollution or human health risk in the marine environment (US EPA, 2012a; Savichtcheva and Okabe, 2006; Ahmed, Goonetilleke and Gardner, 2008; Woods, 2010). With the variance of detection as reported by this study and others in the literature, adenoviruses are suggested to be the most promising viral indicators of faecal pollution. Studies have shown the use of adenoviruses to monitor water quality (Hartmann, Dartscht, Szewzyk and Selinka, 2013) because the DNA viruses exist within the population and are generally considered extremely stable (Fong and Lipp, 2005). To assess the potential of these enteric viruses as indicators to monitor water quality, future studies will need to determine how the presence, abundance, and stability of these viruses correlate with relevant pathogens in the wastewater treatment process and in contaminated coastal environments. It will also be necessary to determine if these viruses occur naturally in the environment in the absence of faecal pollution. However, further research is needed. Nonetheless, limitation of the molecular method include their potential incompatibility with concentration methods, quantity of the water sample volume to assay for viruses, and the inability to discern between infectious and non-infectious material have been identified as a setback in exploring this medium (Bosch, 1998). Testing is complicated, expensive, not available for all viruses, and beyond the capabilities of most laboratories involved in routine water quality monitoring. The best means of safeguarding against the presence of human enteric viruses are based upon the application of adequate treatment and the absence of faecal indicator organisms such as *Escherichia coli* (Health Canada, 2004).

Since three of the five viruses under studying were not detected in this research, it is imperative to recognize the boundaries of this research. It is anticipated that the viral load in the human population varies with each virus with daily and seasonal changes in their concentration in raw sewage. The effluent samples analysed in this study represent only a year's study at each treatment facility and, as a result, it is likely that the categories of viruses found could vary if samples were collected to cover more sampling periods at different times of the year. Besides, the occurrence of these enteric viruses in the final effluent will also vary in response to variability in treatment efficiency. Also, the outcomes of this study may have been influenced by the degree of differences in recovery efficiency of the concentration and nucleic acid isolation methods for different viral groups. For example, viruses adhering to particles may have been lost during the filtration process. It is also possible that inefficient reverse transcription or the degradation of RNA could have skewed the results. In the same vein, it is possible that undetected viruses were present at concentrations below the assay detection limits on the day of sampling. Further studies need to be carried out to verify the absence of undetected or under detected viruses in WWTPs in the Eastern Cape.

5.3 Antimicrobial susceptibility test

5.3.1 Antimicrobial susceptibility testing for *E. coli*

It has been shown in a number of studies the presence of antibiotic-resistant bacteria or genes conferring resistance in the aquatic environment. These genes are transferred into the normal flora of humans and animals, where they exert a strong selective pressure for the emergence and spread of resistance in both pathogenic and commensal bacteria and ultimately they find their way into the environment via wastewater, manure and sewage sludge (Amaya et al., 2012; Kinge, Ateba and Kawadza, 2010). *E. coli* have been reported to be resistant to several antibiotics such as: ampicillin, cefalothin (cephalothin), tetracycline, cefotaxime and gentamicin (Kümmerer, 2004; Galvin et al., 2010; Jakobsen et al., 2008; Amaya et al., 2012). The result of this current study revealed that *E. coli* isolated from the wastewater treatment plants effluent had higher antibiotic resistance levels to ampicillin (63.9%). This form of ampicillin resistance has been reported for *E. coli* isolates from effluents (Kinge, Ateba and Kawadza, 2010; Amaya et al., 2012). *E. coli* isolates resistance to tetracycline and cephalotin (which are the next most resistant drugs in this study) have also been reported in the works of Picão et al. 2013 and Galvin et al. 2010. The increasing observance of resistance to cephalosporins among members of enterobacteriaceae has been mainly attributed to the spreading of Extended-spectrum β -Lactamases (Rasheed et al., 2014). Levy (2002) reported that the prolong use of tetracycline as growth promoter in animals conferred resistance on *E. coli* which was isolated from the animals as against the isolates from the control test animals. In their submission, Harris et al. (2014) asserted that the

resistance to tetracycline due to long term and widespread use has significantly impacted the development of resistance in the microorganism. Medicine, animal husbandry, aquaculture, agriculture and food technology are known to have widespread use of antimicrobial drugs in human and animal. These industries are possible vehicles for transmission of resistant bacteria (Kinge, Ateba and Kawadza, 2010). Enitan, Swalaha and Adeyemo (2012) concluded in their research that antibiotic abuse and overuse can make bacterial communities acquire resistant genes which could lead to multi-resistance to different classes of antibiotics. A similar resistant pattern of *E. coli* to ampicillin and tetracycline (as observed in this present study) were reported by Chigor et al. (2010) and Koba (2013). The low resistance reported for cefotaxime and gentamicin in this study has also been reported for *E. coli* isolates tested against these antibiotics (Rasheed et al., 2014; Jafar, Shakibaie and Poormasoomi, 2013). Amaya et al. (2012) reported high susceptibility of *E. coli* to the two antibiotics. In a study by Jakobsen et al. (2008), it was demonstrated that gentamicin resistant gene can be acquired through horizontal transfer to other organisms. They also found high prevalence of gentamicin resistant *E. coli* in hospital wastewater plant as against the residential wastewater plant. Hospital wastewater was shown to be reservoirs of antimicrobial resistance as well as virulence genes, with the possibility that the resistance and virulence genes from the hospital will spread to the environment (Jakobsen et al., 2008). The emergence of Carbapenem Resistance (CRE) in Enterobacteriaceae has become an important threat to global health (Van Duin, Kaye, Neuner and Bonomo, 2013). Infections caused by CRE have limited treatment options and have been associated with high mortality rates (Gupta, Limbago, Patel and Kallen,

2011). This study also aims to identify the possible presence of CRE in *E. coli* isolates from the final effluents. The results showed the tested isolates are susceptible to meropenem. *E. coli* isolated from hospital wastewater and food sources were found to be susceptible to meropenem (Jafar, Shakibaie and Poormasoomi, 2013; Hleba, 2013). High levels of antibiotic resistance have been reportedly found in *E. coli* and among Enterobacteriaceae isolates in sewage water (Amaya et al., 2012; Picão et al., 2013). This corroborate with this study where the isolates show resistances to more than one antibiotic. The presence of multi-drugs antibiotic resistance *E. coli* have been reported to be found in rivers and effluent discharge in some provinces of South Africa (Olaniran, Naicker and Pillay, 2009; Osode, 2010; Koba, 2013).

5.3.2 Antimicrobial susceptibility testing for *Vibrio* spp.

Vibrio spp. are considered to be significant infectious pathogens which are the causative agents for vibriosis (Vaseeharan, Ramasamy, Murugan and Chen, 2005; Lee, Najiah, Wendy and Nadirah, 2009). They are characterized by diarrhea, wound infections, primary septicemia, and gastroenteritis or other extra-intestinal infections related to exposure to contaminated sources (CDC, 2013). Most isolates tested in this study were susceptible to the antimicrobial agents recommended for primary testing by CLSI (CLSI, 2010). Treatment recommendations for *Vibrio* infections include: tetracyclines (doxycycline, tetracycline), flouroquinolones (ciprofloxacin, levofloxacin), third-generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone), aminoglycosides (amikacin, apramycin, gentamicin, streptomycin) and folate pathway inhibitors (trimethoprim-sulfamethoxazole) (Shaw et al., 2014; Han et al., 2007).

All *Vibrio* spp. studied here showed some degree of resistance to all the antibiotics used for testing. In the present study, data on antibiotic resistant zones indicate that all the 100 isolates of *Vibrio* spp. were 38% resistant to tetracycline, 26% to ampicillin, 16% to chloramphenicol, 14% to cefotaxime, 13% to trimethoprim-sulfamethoxazole and 1% to ciprofloxacin. In a similar study, Okoh and Igbinosa (2010) reported resistances to this antibiotics except for cefotaxime and ciprofloxacin which were not included in their study. In contrast to this study, Han et al. (2007) reported high susceptibility of *Vibrio* spp. to tetracycline, cefotaxime and ciprofloxacin but with high resistance to ampicillin. The high ampicillin resistance of *Vibrio* spp. have also been reported by Raissy, Moumeni, Ansari and Rahimi (2012). Similar report by Shaw et al. (2014) showed high susceptibility to trimethoprim-sulfamethoxazole in addition to the aforementioned antibiotics which was against the low resistance recorded for trimethoprim-sulfamethoxazole in this study. Particularly interesting is the relative high resistance of the *Vibrio* spp. to cefotaxime as compared to other studies. Liang et al. (2013) reported a single isolate resistance to cefotaxime while three others showed intermediate reactions. Similarly, Shaw et al. (2014) also reported intermediate resistance to cefotaxime in their study. Most studies have shown high susceptibility of *Vibrio* spp. to cefotaxime as reported in the work done by Han et al. (2007) and Zanetti et al. (2001). For the treatment of *V. vulnificus* infection, cefotaxime and minocycline were found to act synergistically in inhibiting the organism (Chiang and Chuang, 2003). On the basis of our results, ciprofloxacin was being the most effective antibiotic as shown in this study. Only one isolate was resistant to the antibiotic. The high susceptibility of *Vibrio* spp. to ciprofloxacin was reported by Han et al. (2007) and Shaw et al. (2014), while

Ahmad (2011) reported low resistance to the antibiotic which was in agreement with this study.

Therefore, continued monitoring of both the prevalence and the antimicrobial susceptibility profile is important to better ensure environmental safety; particularly single resistance to ciprofloxacin observed against *Vibrio* also limit treatment effectiveness and should be monitored. As most of the antimicrobial agents recommended for treatment of *E. coli* and *Vibrio* illnesses by CLSI showed some form of resistances is likely to be problematic. Based on our data, treatment of illnesses may benefit from the use of meropenem that was 100% effective against *E. coli* and ciprofloxacin which was the only antibiotics that was 99% effective against *Vibrio* spp. in this study.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

This thesis focuses on the evaluation of the quality indices of the final effluents of two WWTPs in Buffalo City Metropolitan Municipality in the Eastern Cape Province. This first section of this chapter synthesizes the empirical findings in order to answer the study's hypothesis and research questions. The rest of the chapter is structured to answer the research questions raised in chapter 1 under the Introduction, drawing conclusions on the basis of the findings of the study. This is followed by proposed topics for further research based on the limitations of the dissertation's results.

It is hypothesized that the final effluents of the two selected Wastewater Treatment Works in Buffalo City Local Municipality are contaminated with human microbial and viral pathogens emanating from the treated effluent wastewater. The presence of human faecal contamination and viral pathogen was found in the final effluent from the two treatment plants. Prevalence of the enteric pathogens studied like the faecal coliform, *E. coli*, *Vibrio*. spp., adenoviruses and rotaviruses was high in the final effluent of the WW-Ama Treatment Plant. The presence of the enteric pathogens was also found in the WW-Dim Sewage Works though at a very minimal level. Adenovirus and rotavirus were the only detected enteric viruses in the final effluent with low viral concentrations. The presence of Hepatitis A and Coxsackie viruses were previously reported for WW-Dim (Gusha, 2012). This study is the first report demonstrating the presence of

adenovirus and rotavirus in the final effluent of the two studied treatment plants. To the best of our knowledge, no previous study has been carried out on the WW-Ama Treatment Plant. An extensive literature assessment also revealed a wide research gap on the study of physicochemical and microbiological qualities of WWTPs in the Eastern Cape of South Africa. The findings of this research have shown that enteric pathogens were present in the final treated effluent.

The second hypothesis looked for a relationship between the physicochemical indicators of the final effluent of the treated wastewater and its microbiological indicators. Treated effluent with high organic carbon measured as BOD and COD were found to have high presence of faecal bacteria at the WW-Ama Treatment Plant as compared to the WW-Dim Treatment Plant whose treated effluent has high BOD and less prevalence of faecal bacteria. The level of dissolved oxygen was found to be < 5 mg/l for most of the effluent samples from the WW-Ama plant but when compared to the WW-Dim Treatment Plant, the dissolved oxygen was very high for the samples analysed. Low dissolved oxygen (DO) primarily results from excessive algae growth caused by phosphorus. As the algae die and decompose, the process consumes dissolved oxygen. The presence of algae growth was observed at the WW-Ama plants and algae grows in the presence of organic matter. The level of organic matter also impacts on the turbidity of the effluent. High turbidity can confer protection to pathogenic organisms by shielding the effect of chlorine disinfection. This is evident at the WW-Ama plant where the turbidity was high compared to the WW-Dim plant with

relative low turbidity hence the high prevalence of faecal bacteria. The interaction of these physicochemicals can be seen to influence the quality of the effluent.

Both the research questions 1 and 3 will be answered together.

- Is the faecal bacteria load of the selected final effluent within the acceptable limits as indicated by the Department of Water Affairs and Forestry (DWAF)?
- What is the prevalence of the enteric bacteria and viruses in the final effluent wastewater treatment?

The use of bacterial indicator organism to assess the microbiological quality of water is well established with the main aim of using indicator organisms and analysis frequently related to their examination to indicate the degree of water contamination by faecal wastes. Faecal coliforms indicate the possible presence of pathogens responsible for the gastrointestinal diseases like salmonellosis, dysentery, cholera and typhoid fever. The effluents were evaluated against their compliance with the set limit of 1000 CFU/100 ml for faecal coliform. The presence of enteric bacteria, which includes faecal coliform, *E. coli* and *Vibrio*, were investigated. The WW-Ama plant failed to comply with the set limits for all the enteric bacteria tested for. High counts were recorded for most of the samples tested and analysed. This treatment plant is therefore a potential source of enteric bacteria pollutants coming into the environment. Pathogenic strains of *E. coli* were also found at this plant. Likewise the presences of enteric viruses (adenovirus and rotavirus) were found to be in high concentration. Though there is no set limit for enteric viruses, their presence in effluent discharge is a sign that the environment could be polluted with viruses.

The WW-Dim Treatment Plant recorded a low level of faecal bacteria in the final effluent. Most of the samples were in compliance with the special limit of 0 CFU/100 ml as against the general limit of 1000 CFU/100 ml for faecal coliform discharged. There were few instances where colonies of < 100 CFU were recorded. There were cases where high colonies were recorded (> 1000 CFU) and this was due to lack of chlorine disinfection. No pathogenic strains of *E. coli* were detected at the plant. However, enteric viruses (Adenovirus and Rotavirus) were detected twice at the treatment plant. The plant somehow still shows high efficiency in producing good quality effluents notwithstanding instances of poor quality effluent discharge as observed through the physicochemical and microbiological testing. The WW-Dim plant effluent is in compliance with the regulatory guidelines.

Do the physiochemical parameters of the final effluents meet the set standards for wastewater quality?

The Government Gazette Regulation (S A. DWAF, 2013) stipulates the limits for treated effluent discharge. The indicators and values of permissible level of pollution for discharge of wastewater into surface waters for irrigation use, sewerage systems and in other such sensitive areas are clearly spelt out in the government amended gazette 2013. Proper tracking and monitoring of the efficiency of the WWTPs are needed to help determine the cause of pollution in the outflow of treated wastewater from WWTP. The goal of our work was therefore designed to obtain data from the selected WWTP for this purpose. Samples were collected for a period of 12 months and this stage was followed by the determination of values for the parameters for waste water

pollution (BOD, COD, total dissolved solids, pH, dissolved oxygen, NO₂-N, NO₃-N, temperature, turbidity, EC, free chlorine, orthophosphate) and calculation of the efficiency of the removal process of the various organic and inorganic materials. This is according to the DWAF General/Special effluent discharge standards (S.A. DWAF, 2013). No guideline was set for BOD, TDS, turbidity and dissolved oxygen. Other guidelines used were:

- water quality assessments: a guide to the use of biota, sediments and water in the environment, monitoring (Chapman, WHO and CRC Press, 1996) for dissolved oxygen,
- Council Directive of 21 May 1991 concerning urban waste water treatment (91/271/EEC) (CEC, 1991) for BOD
- South Africa water quality guideline for domestic use, (DWAF, 1996f) for total dissolved solids and
- guidelines for drinking-water quality (WHO, 2008) for turbidity.

In response to the Research question 2, the level of efficiency of the waste water treatment plant in WW-Ama did not meet minimum acceptable treatment requirements of the discharged wastewater effluent. Five out of the twelve parameters examined did not comply with the set limits. WW-Ama had a poor record for BOD, COD, free chlorine, turbidity and phosphate. WW-Dim had a poor parameters record for BOD, free chlorine, turbidity and nitrate. The minimum acceptable treatment efficiency of the discharged wastewater was not met for these noted parameters. The plants are obliged to comply with the permissible value as stipulated in the guideline. The average

measured values of some of the parameters exceeded the limits while some did not meet the set limits. The evaluation of the efficiency of the treatment plants found that the reduction of pollutants into receiving surface water was very high for the WW-Ama Central Treatment Plant and low for the WW-Dim Treatment Plant. This was due to hydraulic overloading of the waste water treatment plant and a number of the machineries for the treatment process had been broken down due to poor maintenance allowing very high metabolic loads of wastewater to enter the WWTP. In contrast to this, WW-Dim has been effective and efficient in the treatment of wastewater. This is evident in the quality of effluent produced. But it was observed that there is usually high chlorine dosing in their effluent. A major contribution to the improved quality of effluent produced in WW-Dim plant was the shutdown of the industries that dump their influent into the treatment plant in the area. But for the shutdown of these industries, this would have been a major challenge on the quality of the effluents produced by the WW-Dim Treatment Plant.

This research also made a comparison of the efficiency of the cleaning process of the two plants. Both plants are located in a peri-urban environment but they are different in their treatment technologies. The difference in their treatment controlling process is manifested in the quality of the effluent being discharged into the environment. A poorly managed treatment process negatively impacts the effluent quality and the receiving environment. Also as reported by Mema (2004), poor operation at treatment plants was attributed to lack of skilled personnel and use of untrained operators at the

treatment plants. The lack of optimum time for the treatment processes for each of the stages in treatment can adversely affect the final quality of the effluent.

6.2 Observations and recommendations

The problems of poor operation and maintenance of wastewater and sewage treatment plants are well known and are still observed to be common practice in the treatment plants. The plants lack skilled personnel and have inadequate trained plant operators to bridge the knowledge gaps on how to effectively manage plant operations. The lack of adequate maintenance and absence of on-site maintenance/technicians contribute to poor quality effluent. Obsolete equipment and their frequent breakdown cause the release of poor effluent into the environment. Above all, the problem of human attitude is exhibited in the operations in the two studied plants whose effluent discharge falls below the required standard. It is a common knowledge that the quality of our world is indeed a direct product of the quality of our minds. Just as the age long saying also confirms that 'all is well that ends well' it is also true that all must produce good result that begins with correct intention. For instance that the two studied treatment plants lack a functional laboratory, equipment for basic testing like pH, dissolve oxygen and temperature shows that all did not begin well and it will not be too adequate to explain this technical inadequacy away as lack of means alone but a tendency on the part of management that may have decided to allow some extraneous factors like profit-making to influence their thinking over and above the need for environmental safety. Most of the laboratory work is outsourced to an outside private laboratory. Though this approach is no doubt in line with the world best practices, incidences of sharp practices

cannot be entirely ruled out if the approach is weighed against the backdrop that very many business concerns are set up with profit-making as their driving philosophy. Our above observations do not however, mean that it is already sunset for these treatment plants and others like them that may be operating at the same low level of efficiency across the country. The current shortfalls as we have aptly identified should rather be accepted as a new dawn, that is, a sunrise of a new beginning and a new strategy towards a renewed commitment on the part of the management of these treatment plants under study to pursue the ideals of clean environment to which the national government of South Africa and indeed the world over are equally committed.

It is therefore concluded that since this poor quality effluent from the two plants studied is expected to be discharged into the environment, the bacteria, viruses and other organic and inorganic nutrients we have already reported in this project, will increase the pollution load and other health hazards. Furthermore, the disposal of this poor quality effluent may also contaminate water often used for irrigation and potable purposes in the society. Thus, as stated above, the two plants need to step up their efforts to develop adequate and efficient technology for effluent treatment. There is also the need for the development of positive attitude on the part of the management realizing that improved technical efficiency of their plants and a correct attitude in their usage will lead to quality effluent to be discharged into the environment. In so doing, they will not only be contributing to the quality of the environment, by extension they will also be improving quality of health of the community. It is expected that our investigations would be of utmost use to regulatory agencies and public health

departments for water quality, safety management and for the clinical management of diseases among people caused by constant contact with contaminated surface water and effluents.

6.2.1 Recommendations

In views of the above, I wish to recommend as follows

- The various treatment plants in operation in South Africa, particularly the two treatment plants under study to set up on-site technicians to repair and troubleshoot problems that might arise while waste water treatment is on.
- The Green Drop programme should further promote best practices and healthy competition among treatment facilities.
- The Management of WW-Ama Treatment Plant to upgrade its existing obsolete systems with modern technologies in order to achieve the desired technical efficiency.
- The national government to evolve and enforce policies governing the safety of the environment and ensure strict compliance.
- The national government through its regulatory institution to partner with the private sector and academic institutions for effective monitoring and evaluation of the state of WWTPs.
- The two studied treatment plants and any other treatment plant in the country to have well equipped and functional laboratories.

6.3 Future research

- Investigation into the performance of Eastern Cape wastewater systems requires further research.
- A comparative study on the prevalence of enteric bacteria and viruses across the WWTPs in the Eastern Cape is needed and should be encouraged.
- There should be an investigation into the presence of pharmaceuticals and personal care products in WWTPs in the Eastern Cape.
- Other developed countries have researched the removal efficiency of activated sludge systems and biofilter systems. The removal efficiency of these technologies needs to be assessed to determine the effectiveness of the current sewage treatment plants and processes in controlling the concentrations of contaminants discharged and evaluate whether these new processes offer the possibility of improved waste control.

REFERENCES

- 25 Degrees in Africa, 2010. *25 Degrees in Africa - Efficiency the only way to secure water supply in SA*. [online] Available at: <http://www.25degrees.net/oldsitebackup/index.php/component/option,com_zine/Itemid,146/id,1125/view,article/> [Accessed 16 Jun. 2014].
- Abe, C.M., Salvador, F.A., Falsetti, I.N., Vieira, M.A.M., Blanco, J., Blanco, J.E., Blanco, M., Machado, A.M.O., Elias, W.P., Hernandez, R.T. and Gomes, T.A.T., 2008. Uropathogenic *Escherichia coli* (UPEC) strains may carry virulence properties of diarrhoeagenic *E. coli*. *FEMS immunology and medical microbiology*, 52(3), pp.397–406.
- Abrantes, N., Pereira, R., Figueiredo, D.R. De, Marques, C.R., Pereira, M.J. and Gonc, F., 2009. A whole sample toxicity assessment to evaluate the sub-lethal toxicity of water and sediment elutriates from a lake exposed to diffuse pollution. *Environmental Toxicology*, pp.259–270.
- Agency, U.S.E.P., 1999. EPA Wastewater Technology Fact Sheet: Chlorine Disinfection. *EPA 832-F-99-062*.
- Ahmad, N., 2011. Antibiotic resistance and biosafety of *Vibrio cholerae* and *Vibrio parahaemolyticus* from freshwater fish at retail level. *International Food Research Journal*.
- Ahmed, W., Goonetilleke, A. and Gardner, T., 2008. Alternative indicators for detection and quantification of faecal pollution. *Water: Journal of the Australian Water Association*, 39, pp.46–49.
- Akpor, O., 2011. Wastewater effluent discharge: Effects and treatment processes. *3rd International Conference on Chemical, Biological and Environmental Engineering*, 20, pp.85–91.
- Akpor, O. and Muchie, B., 2011. Environmental and public health implications of wastewater quality. *African Journal of Biotechnology*, 10(13), pp.2379–2387.
- Alberta Environment, 2000. *Guidelines for Municipal Wastewater Irrigation*.
- Alberta Research Council Project, 2008. Effects of water temperature and treated pulp mill effluent on survival and growth of *Daphnia magna* (Cladocera: Daphnidae) and *Taenionema* (Plecoptera: Taeniopterygidae). *Centers for Medicare &*

Amaya, E., Reyes, D., Paniagua, M., Calderón, S., Rashid, M.-U., Colque, P., Kühn, I., Möllby, R., Weintraub, a and Nord, C.E., 2012. Antibiotic resistance patterns of *Escherichia coli* isolates from different aquatic environmental sources in León, Nicaragua. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, 18, pp.E347–54.

Amin, R. and Salem, A., 2012. Specific detection of pathogenic *Vibrio* species in shellfish by using multiplex polymerase chain reaction. *Global Veterinaria*, 8(5), pp.525–531.

Amiri, F., Mesquita, M.M.F. and Andrews, S.A., 2010. Disinfection effectiveness of organic chloramines, investigating the effect of pH. *Water Research*, 44, pp.845–853.

Anastasi, E.M., Matthews, B., Gundogdu, A., Vollmerhausen, T.L., Ramos, N.L., Stratton, H., Ahmed, W. and Katouli, M., 2010. Prevalence and persistence of *Escherichia coli* strains with uropathogenic virulence characteristics in sewage treatment plants. *Applied and environmental microbiology*, 76, pp.5882–5886.

Anastasi, E.M., Matthews, B., Stratton, H.M. and Katouli, M., 2012. Pathogenic *Escherichia coli* found in sewage treatment plants and environmental waters. *Applied and environmental microbiology*, 78(16), pp.5536–41.

Anastasi, P., Bonanni, E., Cecchini, G., Divizia, M., Donia, D., Di Gianfilippo, F., Gabrieli, R., Petrinca, A.R. and Zanobini, A., 2008. Virus removal in conventional wastewater treatment process. *Igiene e sanità pubblica*, 64(3), pp.313–30.

APHA, A.P.H.A., AWWA, A.W.W.A. and WEF, W.E.F., 2012. *Standard methods for the examination of water and wastewater*. 22nd ed. p.1368.

Arp, D.J. and Stein, L.Y., 2003. Metabolism of inorganic N compounds by ammonia-oxidizing bacteria. *Critical reviews in biochemistry and molecular biology*, 38, pp.471–495.

Arraj, A., Bohatier, J., Laveran, H. and Traore, O., 2005. Comparison of bacteriophage and enteric virus removal in pilot scale activated sludge plants. *Journal of applied microbiology*, 98(2), pp.516–24.

Ashbolt, N., Grabow, W. and Snozzi, M., 2001. Indicators of microbial water quality. *IWA Publishing*, (Grabow 1996).

Ayyildiz, O., Ileri, B. and Sanik, S., 2009. Impacts of water organic load on chlorine dioxide disinfection efficacy. *Journal of Hazardous Materials*, 168, pp.1092–1097.

Babu, B., 2007. Effluent Treatment: Basics and A Case Study. *Water Digest*.

Ballance, A. and King, N., 1999. The State of the Environment South Africa—An Overview.

Barth, E. and Ettinger, M., 1965. Summary report on the effects of heavy metals on the biological treatment processes. *Journal (Water Pollution Control Federation)*, 37(1), pp.86–96.

Bateman, C., 2008. Incompetent maintenance/ inept response – 80 more E-Cape babies die. *South African Medical Journal; Vol 98, No 6 (2008)*.

Battistone, A., Buttinelli, G., Bonomo, P., Fiore, S., Amato, C., Mercurio, P., Cicala, A., Simeoni, J., Foppa, A., Triassi, M., Pennino, F. and Fiore, L., 2014. Detection of Enteroviruses in Influent and Effluent Flow Samples from Wastewater Treatment Plants in Italy. *Food and Environmental Virology*, 6, pp.13–22.

Beddow, V., 2010. *Coagulation and Flocculation in Water and Wastewater Treatment*. [online] Available at: <<http://www.iwawaterwiki.org/xwiki/bin/view/Articles/CoagulationandFlocculationinWaterandWastewaterTreatment>> [Accessed 18 Jun. 2014].

Bekal, S., Brousseau, R., Masson, L., Prefontaine, G., Fairbrother, J. and Harel, J., 2003. Rapid identification of Escherichia coli pathotypes by virulence gene detection with DNA microarrays. *Journal of clinical microbiology*, 41, pp.2113–2125.

Belguith, K., Hassen, A., Bouzlama, L., Khira, S. and Aouni, M., 2007. Enterovirus circulation in wastewater and behavior of some serotypes during sewage treatment in Monastir, Tunisia. *Journal of environmental health*, 69(10), pp.52–6.

Bellos, D., Sawidis, T. and Tsekos, I., 2004. Nutrient chemistry of River Pinios (Thessalia, Greece). *Environment International*, 30, pp.105–115.

Berg, G., 1973. Removal of viruses from sewage, effluents, and waters. I. A review. *Bulletin of the World Health Organization*, 49(5), pp.451–60.

Bisi-Johnson, M.A., Obi, C.L., Vasaikar, S.D., Baba, K.A. and Hattori, T., 2011. Molecular basis of virulence in clinical isolates of Escherichia coli and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa. *Gut pathogens*, 3, p.9.

Bitton, G., 2005. *Wastewater microbiology*. Third Edit ed. John Wiley & Sons, Inc., Hoboken, New Jersey.

Bopp, C., Ries, A. and Wells, J., 1999. Laboratory methods for the diagnosis of epidemic dysentery and cholera.

- Bosch, a, 1998. Human enteric viruses in the water environment: a minireview. *International microbiology: the official journal of the Spanish Society for Microbiology*, 1(3), pp.191–6.
- Boyd, L. and Mbelu, A., 2009. *Guideline for the Inspection of Wastewater Treatment Works*.
- Caballero, A.R., 2011. *Study of bacterial communities– A wastewater treatment perspective*. Mälardalen University.
- Cañigral, I., Moreno, Y., Alonso, J.L., González, A., Ferrús, M.A. and Ferrús, M. a, 2010. Detection of *Vibrio vulnificus* in seafood, seawater and wastewater samples from a Mediterranean coastal area. *Microbiological research*, 165(8), pp.657–64.
- Carducci, A., Battistini, R., Rovini, E. and Verani, M., 2009. Viral Removal by Wastewater Treatment: Monitoring of Indicators and Pathogens. *Food and Environmental Virology*, 1(2), pp.85–91.
- Catterall, K., Morris, K., Gladman, C., Zhao, H., Pasco, N. and John, R., 2001. The use of microorganisms with broad range substrate utilisation for the ferricyanide-mediated rapid determination of biochemical oxygen demand. *Talanta*, 55, pp.1187–1194.
- CDC, C. for D.C. and P., 2013. *Vibrio Illness (Vibriosis)*. [online] Available at: <<http://www.cdc.gov/vibrio/>> [Accessed 2 Oct. 2014].
- CEC, C. of the E.U., 1991. Council Directive of 21 May 1991 concerning Urban Waste Water Treatment (91/271/EEC). *Official J. Eur. Commun. L*, 34(May 1991), pp.1–16.
- Chakraborty, R., Sinha, S., Mukhopadhyay, A.K., Asakura, M., Yamasaki, S., Bhattacharya, S.K., Nair, G.B. and Ramamurthy, T., 2006. Species-specific identification of *Vibrio fluvialis* by PCR targeted to the conserved transcriptional activation and variable membrane tether regions of the *toxR* gene. *Journal of medical microbiology*, 55(Pt 6), pp.805–8.
- Chan, Y.J., Chong, M.F., Law, C.L. and Hassell, D.G., 2009. *A review on anaerobic-aerobic treatment of industrial and municipal wastewater*. *Chemical Engineering Journal*, .
- Chapman, D., WHO, W.H.O. and CRC Press, 1996. *Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring*. Second ed. UNESCO/WHO/UNEP.
- Chen, J.-S., Zhang, L.-S. and Wang, J.-L., 2007. A novel biosensor for the rapid determination of biochemical oxygen demand. *Biomedical and environmental sciences : BES*, 20, pp.78–83.

Chiang, S.-R. and Chuang, Y.-C., 2003. *Vibrio vulnificus* infection: clinical manifestations, pathogenesis, and antimicrobial therapy. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi*, 36(2), pp.81–8.

Chigor, V.N. and Okoh, A.I., 2012a. Quantitative detection and characterization of human adenoviruses in the Buffalo River in the Eastern Cape Province of South Africa. *Food and environmental virology*, 4(4), pp.198–208.

Chigor, V.N. and Okoh, A.I., 2012b. Quantitative RT-PCR detection of hepatitis A virus, rotaviruses and enteroviruses in the Buffalo River and source water dams in the Eastern Cape Province of South Africa. *International journal of environmental research and public health*, 9(11), pp.4017–32.

Chigor, V.N., Sibanda, T. and Okoh, A.I., 2013. Studies on the bacteriological qualities of the Buffalo River and three source water dams along its course in the Eastern Cape Province of South Africa. *Environmental science and pollution research international*, 20(6), pp.4125–36.

Chigor, V.N., Umoh, V.J., Smith, S.I., Igbinosa, E.O. and Okoh, A.I., 2010. Multidrug resistance and plasmid patterns of *Escherichia coli* O157 and other *E. coli* Isolated from diarrhoeal stools and surface waters from some selected sources in Zaria, Nigeria. *International journal of environmental research and public health*, 7(10), pp.3831–41.

Civil-Guy, 2010. *Three Important Parameters of Water Quality*. [online] Available at: <<http://amudu-gowripalan.blogspot.com/2011/09/three-important-parameters-of-water.html#.U6DcGfmSySo>> [Accessed 18 Jun. 2014].

Clarke, N.A., Stevenson, R.E., Chang, S.L. and Kabler, P.W., 1961. Removal of enteric viruses from sewage by activated sludge treatment. *American journal of public health and the nation's health*, 51, pp.1118–29.

Clean Water Team (CWT), 2004. Dissolved Oxygen Fact, SheetFS-3.1.1.0(DO). In: *The Clean Water Team Guidance Compendium for Watershed Monitoring and Assessment*. Sacramento, CA: Division of Water Quality, California State Water Resources Control Board (SWRCB), pp.1–5.

Clements, A., Young, J., Constantinou, N. and Frankel, G., 2012. *Infection strategies of enteric pathogenic E. coli*. *Gut Microbes*, .

CLSI, 2010. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria: Approved Guideline.

CLSI, 2012. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement*. Clinical and Laboratory Standards Institute.

Coeurdassier, M., de Vaufleury, A., Crini, N., Scheifler, R. and Badot, P.-M., 2005. Assessment of whole effluent toxicity on aquatic snails: bioaccumulation of Cr, Zn, and Fe, and individual effects in bioassays. *Environmental toxicology and chemistry / SETAC*, 24(1), pp.198–204.

Conroy, O., 2006. *Estrogenic and anti-estrogenic activity present in wastewater effluent and reclaimed water*. University of Arizona.

Conservation Ontario, 2001. *The importance of watershed management in protecting Ontario's drinking water supplies*.

Costafreda, M.I., Bosch, A. and Pintó, R.M., 2006. Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. *Applied and environmental microbiology*, 72(6), pp.3846–55.

CPCB, n.d. *Guide manual: Water and Wastewater analysis*.

Crockett, A., 1997. Water and wastewater quality monitoring, McMurdo Station, Antarctica. *Environmental monitoring and assessment*, pp.39–57.

CSIR, 2011. *The River Health Programme goal and objectives*. [online] Available at: <<http://www.dwaf.gov.za/iwqs/rhp/goal.html>> [Accessed 16 Jun. 2014].

Dahling, D.R., Safferman, R.S. and Wright, B.A., 1989. Isolation of enterovirus and reovirus from sewage and treated effluents in selected Puerto Rican communities. *Applied and environmental microbiology*, 55(2), pp.503–6.

Dalton, B., 2012. *Measuring Nitrite in Wastewater Treatment - C.C. Lynch Blog*. [online] Available at: <<http://cclynchblog.com/measuring-nitrite-in-wastewater-treatment/>> [Accessed 14 Jun. 2014].

Deborde, M. and von Gunten, U., 2008. *Reactions of chlorine with inorganic and organic compounds during water treatment-Kinetics and mechanisms: A critical review*. *Water Research*, .

DEFRA, 1988. *Review of Operational and Experimental Techniques for the Removal of Bacteria, Viruses and Pathogens from Sewage Effluents*. London.

Department of Environmental Affairs and Tourism, 2006. *South Africa Environment Outlook: A report on the state of environment of South Africa*. p.371.

Diallo, A.A., Brugère, H., Kérourédan, M., Dupouy, V., Toutain, P.L., Bousquet-Mélou, A., Oswald, E. and Bibbal, D., 2013. Persistence and prevalence of pathogenic and extended-spectrum beta-lactamase-producing *Escherichia coli* in municipal

wastewater treatment plant receiving slaughterhouse wastewater. *Water Research*, 47, pp.4719–4729.

Donnermair, M.M. and Blatchley, E.R., 2003. Disinfection efficacy of organic chloramines. *Water Research*, 37, pp.1557–1570.

Drake, S.L., 2008. *The Ecology Vibrio vulnificus and Vibrio parahaemolyticus from Oyster Harvest Sites in the Gulf of Mexico*.

Dugan, M., 1999. BOD Biochemical Oxygen Demand. In: *Field Training Manual for Laboratory Analysts*. Fort Gaines.

Van Duin, D., Kaye, K.S., Neuner, E.A. and Bonomo, R.A., 2013. *Carbapenem-resistant Enterobacteriaceae: A review of treatment and outcomes*. *Diagnostic Microbiology and Infectious Disease*, .

Dungeni, M., van Der Merwe, R.R. and Momba, M., 2010. Abundance of pathogenic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in the Gauteng Province, South. *Water SA*, 36(5), pp.607–614.

DWAF, 2008. *Water for Growth and Development in South Africa*.

DWAF, D. of W.A., 1984. *General and Special Standards*. pp.1–6.

DWAF, D. of W.A., 1996a. *South African Water Quality Guidelines - Agricultural Water Use: Irrigation*. Second ed. Department of Water Affairs and Forestry.

DWAF, D. of W.A., 1996b. *South African Water Quality Guidelines Agricultural Water Use: Livestock Watering*. Second ed. Pretoria: Department of Water Affairs and Forestry.

DWAF, D. of W.A., 1996c. *South African Water Quality Guidelines: Agricultural Water Use: Aquaculture*. Second ed. Pretoria.

DWAF, D. of W.A., 1996d. *South African Water Quality Guidelines: Aquatic Ecosystems*. Second ed. Pretoria.

DWAF, D. of W.A., 1996e. *South African Water Quality Guidelines: Domestic Use*. Second ed. Pretoria: Department of Water Affairs and Forestry.

DWAF, D. of W.A., 1996f. *South African Water Quality Guidelines: Field Guide*. Department of Water Affairs and Forestry.

DWAF, D. of W.A., 2004. *Mzimvubu to Keiskamma Water Management Area: Amatole – Kei Internal Strategic Perspective*.

DWAF, D. of W.A., 2011. *Green Drop Report*.

DWAF, D. of W.A., 2012. Chapter 5 – Eastern Cape Province. In: *Green Drop Report*. pp.102–149.

DWAF, D. of W.A. & F., 2013. Revision of General Authorisations in Terms of Section 39 of The National Water Act, 1998 (ACT NO. 36 OF 1998) (THE ACT). *South African Government Gazette*, (36820), pp.3–32.

DWAF, DHE and WRC, 2000. *Quality of Domestic Water Supplies Volume 2: Sampling Guide*.

DWAF, DHE and WRC, 2001. *Quality of Domestic Water Supplies Volume 3: Analysis Guide*.

Eddabra, R., Moussaoui, W., Prévost, G., Delalande, F., Dorsselaer, A., Meunier, O., Scheftel, J.-M. and Mimouni, R., 2010. Occurrence of *Vibrio cholerae* non-O1 in three wastewater treatment plants in Agadir (Morocco). *World Journal of Microbiology and Biotechnology*, 27(5), pp.1099–1108.

Eddy, L.J., 2003. *Sewage wastewater management in South Africa*. University of Johannesburg.

El-Bestawy, E., Hussein, H., Baghdadi, H.H. and El-Saka, M.F., 2005. Comparison between biological and chemical treatment of wastewater containing nitrogen and phosphorus. *Journal of industrial microbiology & biotechnology*, 32(5), pp.195–203.

Ellis, T., 2004. Chemistry of wastewater. *Encyclopedia of Life Support System (EOLSS)*, II.

Emanti Management, 2011. *eWQMS*. [online] Available at: <<https://www.wqms.co.za/infopages/230>> [Accessed 14 Jun. 2014].

ENBITEC Environmental Solution, n.d. *Waste Water Treatment Biological: Technical Report*.

Enitan, A., Swalaha, F. and Adeyemo, J., 2012. Incidence of high-level antimicrobial resistance in bacteria of animal, human and water origin. In: *WISA 2012 BIENNIAL CONFERENCE PROCEEDINGS*.

European Parliament, C. of the E.U., 2006. Directive 2006/44/EC of the European Parliament and of the Council of 6 September 2006 on the quality of fresh waters needing protection or improvement in order to support fish life. *Official Journal of the European Union*.

FAO, 1993. *The state of food and agriculture*. Italy.

Fayyad, M.K. and Al-Sheikh, A.M., 2001. Determination of N-chloramines in As-samra chlorinated wastewater and their effect on the disinfection process. *Water research*, 35(5), pp.1304–10.

Field, K., Bernhard, A. and Brodeur, T., 2003. Molecular approaches to microbiological monitoring: fecal source detection. *Coastal Monitoring through ...*, pp.313–326.

Flannery, J., Keaveney, S., Rajko-Nenow, P., O’Flaherty, V. and Dore, W., 2012. *Concentration of Norovirus during Wastewater Treatment and Its Impact on Oyster Contamination*. *Applied and Environmental Microbiology*, .

Fong, T. and Lipp, E., 2005. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiology and Molecular Biology Reviews*.

Fong, T.-T., Phanikumar, M.S., Xagorarakis, I. and Rose, J.B., 2010. Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river. *Applied and environmental microbiology*, 76, pp.715–723.

Frost, R., 2009. EU practice in setting wastewater emission limit values. (March), pp.1–29.

Fulhage, C.D., Sievers, D. and Porter, J., 1993. *Collecting and Preserving Waste and Wastewater Samples for Analysis / University of Missouri Extension*. [online] Available at: <<http://extension.missouri.edu/p/G1895>> [Accessed 3 Jun. 2014].

Gagnon, G.A., O’Leary, K.C., Volk, C.J., Chauret, C., Stover, L. and Andrews, R.C., 2004. *Comparative Analysis of Chlorine Dioxide, Free Chlorine and Chloramines on Bacterial Water Quality in Model Distribution Systems*. *Journal of Environmental Engineering*, .

Galvin, S., Boyle, F., Hickey, P., Vellinga, A., Morris, D. and Cormican, M., 2010. Enumeration and characterization of antimicrobial-resistant *Escherichia coli* bacteria in effluent from municipal, hospital, and secondary treatment facility sources. *Applied and environmental microbiology*, 76(14), pp.4772–9.

GE Power & Water, 2012. *Handbook of Industrial Water Treatment*. [online] Available at: <<http://www.gewater.com/handbook/index.jsp>> [Accessed 15 Jun. 2014].

Gedalanga, P., 2010. *Molecular-based analysis and monitoring of microbial groups in activated sludge to advance the knowledge of biological processes in wastewater treatment*. University of California.

Gee, C.S., Pfeffer, J.T. and Suidan, M.T., 1990. Nitrosomonas and Nitrobacter Interactions in Biological Nitrification. *Journal of Environmental Engineering*, 116, pp.4–17.

Godshall, F., 1996. Dissolved oxygen management for improved wastewater treatment. *Defense Technical Information Center Compilation Part Notice ADP022456*.

Gorchev, H.G. and Ozolins, G., 2008. WHO guidelines for drinking-water quality. *WHO chronicle*, 38(3), pp.104–8.

Grabow, W.O., Botma, K.L., de Villiers, J.C., Clay, C.G. and Erasmus, B., 1999. Assessment of cell culture and polymerase chain reaction procedures for the detection of polioviruses in wastewater. *Bulletin of the World Health Organization*, 77(12), pp.973–80.

Gray, S.R. and Becker, N.S.C., 2002. Contaminant flows in urban residential water systems. *Urban Water*, 4(4), pp.331–346.

Gregory, J.B., Litaker, R.W. and Noble, R.T., 2006. Rapid one-step quantitative reverse transcriptase PCR assay with competitive internal positive control for detection of enteroviruses in environmental samples. *Applied and environmental microbiology*, 72(6), pp.3960–7.

Gupta, N., Limbago, B.M., Patel, J.B. and Kallen, A.J., 2011. *Carbapenem-resistant enterobacteriaceae: Epidemiology and prevention*. *Clinical Infectious Diseases*, .

Gusha, S., 2012. *Production of high quality wastewater final effluents remain a challenge in the Eastern Cape Province of South Africa*. Vasa, University of Fort Hare, pp.1–100.

Gyedu-Ababio, T., 2011. Pollution Status of Two River Estuaries in the Eastern Cape, South Africa, based on Benthic Meiofauna Analyses. *Journal of Water Resource and Protection*, 03(07), pp.473–486.

Hamelin, K., Bruant, G., El-Shaarawi, A., Hill, S., Edge, T.A., Fairbrother, J., Harel, J., Maynard, C., Masson, L. and Brousseau, R., 2007. Occurrence of virulence and antimicrobial resistance genes in Escherichia coli isolates from different aquatic ecosystems within the St. Clair River and Detroit River areas. *Applied and environmental microbiology*, 73, pp.477–484.

- Han, F., Walker, R.D., Janes, M.E., Prinyawiwatkul, W. and Ge, B., 2007. Antimicrobial susceptibilities of *Vibrio parahaemolyticus* and *Vibrio vulnificus* isolates from Louisiana Gulf and retail raw oysters. *Applied and environmental microbiology*, 73(21), pp.7096–8.
- Hanaki, K., Wantawin, C. and Ohgaki, S., 1990. Nitrification at low levels of dissolved oxygen with and without organic loading in a suspended-growth reactor. *Water Research*, 24, pp.297–302.
- Haramoto, E., Katayama, H., Phanuwat, C. and Ohgaki, S., 2008. Quantitative detection of sapoviruses in wastewater and river water in Japan. *Letters in applied microbiology*, 46(3), pp.408–13.
- Haramoto, E., Katayama, H., Utagawa, E. and Ohgaki, S., 2009. Recovery of human norovirus from water by virus concentration methods. *Journal of virological methods*, 160(1-2), pp.206–9.
- Harris, S., Morris, C., Morris, D., Cormican, M. and Cummins, E., 2014. Antimicrobial resistant *Escherichia coli* in the municipal wastewater system: effect of hospital effluent and environmental fate. *The Science of the total environment*, 468-469, pp.1078–85.
- Hartmann, N.M., Dartscht, M., Szewzyk, R. and Selinka, H.-C., 2013. Monitoring of adenovirus serotypes in environmental samples by combined PCR and melting point analyses. *Virology journal*, 10(1), p.190.
- Hazen, T.C., 1988. Fecal coliforms as indicators in tropical waters: A review. *Toxicity Assessment*, 3, pp.461–477.
- He, X.Q., Cheng, L., Li, W., Xie, X.M., Ma, M. and Wang, Z.J., 2008. Detection and distribution of rotavirus in municipal sewage treatment plants (STPs) and surface water in Beijing. *Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering*, 43, pp.424–429.
- Health Canada, 1991. Total Dissolved Solids (TDS). In: *Guidelines for Canadian Drinking Water Quality - Technical Documents*. pp.22–24.
- Health Canada, 2004. *Guidelines for Canadian Drinking Water Quality: Guideline Technical Document: Enteric Viruses*.
- Van Heerden, J., Ehlers, M.M., Heim, A. and Grabow, W.O.K., 2005. Prevalence, quantification and typing of adenoviruses detected in river and treated drinking water in South Africa. *Journal of applied microbiology*, 99(2), pp.234–42.
- Helmer, R. and Hespánhol, I., 1997. *Water pollution control: a guide to the use of water quality management principles*.

- Henning, W., 2010. *Green Drop*. [online] Available at: <<http://www.erwat.com/page.php?pageID=7>> [Accessed 17 Jun. 2014].
- Henze, M., 2008. *Biological Wastewater Treatment: Principles, Modelling and Design*. IWA Publishing, p.511.
- Hernandez-Morga, J., Leon-Felix, J., Peraza-Garay, F., Gil-Salas, B.G. and Chaidez, C., 2009. Detection and characterization of hepatitis A virus and Norovirus in estuarine water samples using ultrafiltration--RT-PCR integrated methods. *Journal of applied microbiology*, 106(5), pp.1579–90.
- Hleba, L., 2013. Antibiotic resistance of *Escherichia coli* isolated from intestinal tract of *Cyprinus carpio*. *Scientific Papers Animal Science and Biotechnologies*.
- Höglund, C., 2001. *Evaluation of microbial health risks associated with the reuse of source-separated human urine*. Swedish Institute for Infectious Disease Control (SMI).
- Holenda, B., Domokos, E., Rédey, Á. and Fazakas, J., 2008. Dissolved oxygen control of the activated sludge wastewater treatment process using model predictive control. *Computers & Chemical Engineering*, 32, pp.1270–1278.
- Howard, I., Espigares, E., Lardelli, P., Martín, J.L. and Espigares, M., 2004. Evaluation of microbiological and physicochemical indicators for wastewater treatment. *Environmental toxicology*, 19(3), pp.241–9.
- Hromada, T. and Zhang, X., 2006. Investigation of high BOD in final plant effluent. In: *Proceedings of the Water Environment Federation 2006*. pp.2004–2007.
- Hua, G. and Reckhow, D.A., 2007. Comparison of disinfection byproduct formation from chlorine and alternative disinfectants. *Water Research*, 41, pp.1667–1678.
- Huang, M., Ph, D., Nguy, L., Ferrenberg, J., Boeckh, M., Cent, A. and Corey, L., 2009. Development of Multiplexed Real-Time Quantitative PCR Assay for Detecting Human Adenoviruses. *Diagn Microbiol Infect Dis.*, 62(206), pp.263–271.
- Hudzicki, J., 2013. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. (December 2009), pp.1–24.
- Hunter-Central Rivers Waterwatch, n.d. *Water quality parameters & indicators*.
- Hussain, S.A., 2010. *Isolation And Identification Of Pathogenic Bacteria From Drinking Water Of Khairpur, Sukkur And Rohri*. Shah Abdul Latif University, Khairpur, pp.1–252.

Igbinosa, E., 2010. *Surveillance of invasive vibrio species in discharged aqueous effluents of wastewater treatment plants in the Eastern Cape province of South Africa*. University of Fort Hare, p.309.

Igbinosa, E.O., Obi, L.C. and Okoh, A.I., 2009. Occurrence of potentially pathogenic vibrios in final effluents of a wastewater treatment facility in a rural community of the Eastern Cape Province of South Africa. *Research in microbiology*, 160(8), pp.531–7.

Igbinosa, E.O. and Okoh, a. I., 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 & Technology*, 6(2), pp.175–182.

Igbinosa, E.O. and Okoh, A.I., 2010. *Vibrio fluvialis*: an unusual enteric pathogen of increasing public health concern. *International journal of environmental research and public health*, 7(10), pp.3628–43.

Ilemobade, A., Adewumi, J. and Van Zyl, J., 2009. Framework for assessing the viability of implementing dual water reticulation systems in South Africa. *Water SA*, 35(2), pp.216–227.

Ilies, P. and Mavinic, D., 2001. The effect of decreased ambient temperature on the biological nitrification and denitrification of a high ammonia landfill leachate. *Water Research*, 35(8), pp.2065–2072.

Jackson, S.A., Patel, I.R., Barnaba, T., LeClerc, J.E. and Cebula, T.A., 2011. Investigating the global genomic diversity of *Escherichia coli* using a multi-genome DNA microarray platform with novel gene prediction strategies. *BMC genomics*, 12, p.349.

Jackson, S.A.F., Bailey, I., Britz, R., Fortune, A., Johnston_Robertson, A.J., Naidoo, K.M. and Sundram, A., 2002. Report on the South African Colilert™ ring trial. In: *Biennial Conference of the Water Institute of Southern Africa (WISA) 19*.

Jafar, E., Shakibaie, M.R. and Poormasoomi, L., 2013. Isolation of a Novel Antibiotic Resistance Plasmid DNA from Hospital Isolates of *Pseudomonas aeruginosa*. *Journal of Clinical & Experimental Pathology*, 3(2).

Jakobsen, L., Sandvang, D., Hansen, L.H., Bagger-Skjøt, L., Westh, H., Jørgensen, C., Hansen, D.S., Pedersen, B.M., Monnet, D.L., Frimodt-Møller, N., Sørensen, S.J. and Hammerum, A.M., 2008. Characterisation, dissemination and persistence of gentamicin resistant *Escherichia coli* from a Danish university hospital to the waste water environment. *Environment international*, 34(1), pp.108–15.

Jiang, S.C., 2006. Human adenoviruses in water: occurrence and health implications: a critical review. *Environmental science & technology*, 40(23), pp.7132–40.

Jimenez, B. and Asano, T., 2008. Water Reuse: An International Survey of current practice, issues and needs. *Water Intelligence Online*, p.628.

Johnson, C.N., Flowers, A.R., Noriega, N.F., Zimmerman, A.M., Bowers, J.C., DePaola, A. and Grimes, D.J., 2010. Relationships between environmental factors and pathogenic Vibrios in the Northern Gulf of Mexico. *Applied and environmental microbiology*, 76(21), pp.7076–84.

Johnson, T.L. and Durme, G.P. Van, 1987. Design treatment and evaluation systems. *Water Environment Federation*, 59(12), pp.1043–1049.

Jong, J. De, 2003. Epidemiology of enteric adenoviruses 40 and 41 and other adenoviruses in immunocompetent and immunodeficient individuals. *Perspectives in Medical Virology*, pp.407–445.

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. and Erdman, D.D., 2005. Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. *Applied and environmental microbiology*, 71(6), pp.3131–3136.

Jothikumar, N., Kang, G. and Hill, V.R., 2009. Broadly reactive TaqMan assay for real-time RT-PCR detection of rotavirus in clinical and environmental samples. JIN2@cdc.gov. *Journal of virological methods*, 155(2), pp.126–31.

Jouanneau, S., Recoules, L., Durand, M.J., Boukabache, A., Picot, V., Primault, Y., Lakel, A., Sengelin, M., Barillon, B. and Thouand, G., 2014. *Methods for assessing biochemical oxygen demand (BOD): A review. Water Research*, .

Junli, H., Li, W., Nanqi, R., Fang, M. and Juli, 1997a. Disinfection effect of chlorine dioxide on bacteria in water. *Water Research*, 31, pp.607–613.

Junli, H., Li, W., Nenqi, R., Li, L.X., Fun, S.R. and Guanle, Y., 1997b. Disinfection effect of chlorine dioxide on viruses, algae and animal planktons in water. *Water Research*, 31, pp.455–460.

Kahler, A.M., Cromeans, T.L., Roberts, J.M. and Hill, V.R., 2010. Effects of source water quality on chlorine inactivation of adenovirus, coxsackievirus, echovirus, and murine norovirus. *Applied and environmental microbiology*, 76(15), pp.5159–64.

Kale, M. and Mehrotra, I., 2009. Rapid Determination of Biochemical Oxygen Demand. *Proceedings of World Academy of Science: ...*, (09917720969), pp.318–325.

Karat, I., 2013. Advanced Oxidation Processes for Removal of COD from Pulp and Paper Mill Effluents. *diva-portal.org*.

Kaysner, C. and DePaola, A., 2004. *Vibrio*. In: *Bacteriological analytical manual*. FDA: Center for Food Safety and Applied Nutrition.

Keegan, A., Wati, S., Blackbeard, J. and Monis, P., 2011. Chlorine and monochloramine disinfection of human pathogenic viruses in recycled waters. In: *8th IWA International Conference on Water Reclamation and Reuse of Barcelona*.

Keirungi, J., 2007. *The design and assessment of an integrated municipal waste beneficiation facility: towards improved sewage sludge management in developing countries*. Rhode University.

Khaleeq, H. syed, 2011. *A thesis Doctor of Philosophy (Ph.D.) in Botany: 3. MATERIALS AND METHODS*. [online] Available at: <<http://athesisdoctorofphilosophyphdinbotany.blogspot.com/p/3-materials-and-methods.html>> [Accessed 18 Jun. 2014].

Khan, M.S., Knapp, J., Clemett, A., Chadwick, M., Mahmood, M. and Sharif, M.I., 2006. *Managing and Monitoring Effluent treatment plants. Managing Industrial ...*

Kiepper, B., 2010. Understanding Laboratory Wastewater Tests. *University of Georgia Engineering Outreach Service*, pp.1–8.

Kinge, C.N.W., Ateba, C.N. and Kawadza, D.T., 2010. Antibiotic resistance profiles of *Escherichia coli* isolated from different water sources in the Mmabatho locality, Northwest Province, South Africa. *South African Journal of Science*, 106(1/2), p.6 Pages.

Koba, S., 2013. *Assessment of the incidence of E. coli in Tyume and Bufallo rivers in the Eastern Cape Province of South Africa*. University of Fort Hare, p.123.

Koivunen, J., Siitonen, A. and Heinonen-Tanski, H., 2003. Elimination of enteric bacteria in biological-chemical wastewater treatment and tertiary filtration units. *Water research*, 37(3), pp.690–8.

Kokkinos, P., Filippidou, S., Karlou, K. and Vantarakis, A., 2010. Molecular Typing of Enteroviruses, Adenoviruses, and Hepatitis A Viruses in Untreated and Treated Sewage of a Biological Treatment Plant in Greece. *Food and Environmental Virology*, 2(2), pp.89–96.

Kokkinos, P.A., Ziros, P.G., Mpalasopoulou, A., Galanis, A. and Vantarakis, A., 2011. Molecular detection of multiple viral targets in untreated urban sewage from Greece. *Virology journal*, 8, p.195.

Kolawole, O., Oladosu, T., Abdulkarim, A. and Okoh, A., 2013. Adenovirus respiratory tract and HIV co-infections in patients attending the University Of Ilorin, Teaching Hospital, Ilorin. *sci-int.com*, 25(3), pp.501–508.

KRIS, 2011. *Dissolved Oxygen*. [online] Available at: <<http://www.krisweb.com/stream/do.htm>> [Accessed 18 Jun. 2014].

Kümmerer, K., 2004. *Resistance in the environment*. *Journal of Antimicrobial Chemotherapy*, .

Kuo, D.H.-W., Simmons, F.J., Blair, S., Hart, E., Rose, J.B. and Xagorarakis, I., 2010. Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater. *Water research*, 44(5), pp.1520–30.

Lai, H.-C., Lin, S.-J., Lin, H.-R., Ku, C.-S., Wang, L. and Yang, C.-C., 2005. Phylogenetic analyses of human rotavirus in central Taiwan in 1996, 2001 and 2002. *Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology*, 32(3), pp.199–217.

LeChevallier, M.W. and Au, K.-K., 2004. *Water treatment and pathogen control: Process efficiency in achieving safe drinking-water*.

Lee, S.H., Lee, B.C., Lee, K.W., Choi, Y.S., Park, K.Y. and Iwamoto, M., 2007. Phosphorus recovery by mesoporous structure material from wastewater. *Water science and technology: a journal of the International Association on Water Pollution Research*, 55, pp.169–176.

Lee, S.W., Najiah, M., Wendy, W. and Nadirah, M., 2009. Comparative study on antibiogram of *Vibrio* spp. isolated from diseased postlarval and marketable-sized white leg shrimp (*Litopenaeus vannamei*). *Frontiers of Agriculture in China*, 3, pp.446–451.

Lehr, J., Keeley, J., Lehr, J. and Kingery, T., 2005. *Domestic, Municipal, and Industrial Water Supply and Waste Disposal*.

Lenntech, 2014. *nitrate and nitrite*. [online] Available at: <<http://www.lenntech.com/hazardous-substances/nitrate-and-nitrite.htm>> [Accessed 18 Jun. 2014].

Levy, S.B., 2002. Factors impacting on the problem of antibiotic resistance. *Journal of Antimicrobial Chemotherapy*, 49(1), pp.25–30.

Li, D., Zeng, S., Gu, A.Z., He, M. and Shi, H., 2013. Inactivation, reactivation and regrowth of indigenous bacteria in reclaimed water after chlorine disinfection of a

municipal wastewater treatment plant. *Journal of Environmental Sciences (China)*, 25, pp.1319–1325.

Liang, P., Cui, X., Du, X., Kan, B. and Liang, W., 2013. The virulence phenotypes and molecular epidemiological characteristics of *Vibrio fluvialis* in China. *Gut pathogens*, 5(1), p.6.

Licis, I.J., 1995. Design of common effluent treatment plants. In: *Pollution prevention possibilities for small and medium-sized industries : results of the WRITE Project*. Washington DC: United States Environmental Protection Agency.

Lin, J. and Ganesh, A., 2013. Water quality indicators: bacteria, coliphages, enteric viruses. *International journal of environmental health research*, 23(6), pp.484–506.

Lin, S., 1974. *Evaluation of Methods for Detecting Coliforms and Fecal Streptococci in Chlorinated Sewage Effluents*. Urbana.

Liu, Y., Zhang, T. and Fang, H.H.P., 2005. Microbial community analysis and performance of a phosphate-removing activated sludge. *Bioresource Technology*, 96, pp.1205–1214.

Lokhande, R.S., Singare, P.U. and Pimple, D.S., 2012. Study on Physico-Chemical Parameters of Waste Water Effluents from Talaja Industrial Area of Mumbai, India. *International Journal of Ecosystem*, 1(1), pp.1–9.

Lovibond, n.d. *Determining Biochemical Oxygen Demand (BOD)*. pp.4–6.

Mackintosh, G. and Colvin, C., 2003. Failure of rural schemes in South Africa to provide potable water. *Environmental Geology*, 44(1), pp.101–105.

Macmaster, C., 2008. *Toward understanding fecal coliform and Escherichia coli distributions in the Annapolis River*. Dalhousie University, Halifax, Nova Scotia.

Mahananda, M., Mohanty, B. and Behera, N., 2010. Physico-chemical analysis of surface and ground water of Bargarh District, Orissa, India. *International Journal of Research and Reviews in Applied Sciences*, 2(March), pp.284–295.

Mahvi, A., Rajabizadeh, A., Fatehizadeh, A., Yousefi, N., Hosseini, H. and Ahmadian, M., 2009. Survey Wastewater Treatment Condition and Effluent Quality of Kerman Province Hospitals. 7(12), pp.1521–1525.

Mans, J., Netshikweta, R., Magwalivha, M., Van Zyl, W.B. and Taylor, M.B., 2012. *Diverse norovirus genotypes identified in sewage-polluted river water in South Africa*. *Epidemiology and Infection*, .

Mans, J., de Villiers, J.C., du Plessis, N.M., Avenant, T. and Taylor, M.B., 2010. Emerging norovirus GII.4 2008 variant detected in hospitalised paediatric patients in South Africa. *Journal of Clinical Virology*, 49, pp.258–264.

Mantech, n.d. Biochemical Oxygen Demand. (519), pp.1–8.

Mara, D. and Horan, N.J., 2003. *Handbook of Water and Wastewater Microbiology*. Elsevier Science, p.832.

Masters, N., Wiegand, A., Ahmed, W. and Katouli, M., 2011. Escherichia coli virulence genes profile of surface waters as an indicator of water quality. *Water Research*, 45, pp.6321–6333.

Maugeri, T.L., Caccamo, D. and Gugliandolo, C., 2000. Potentially pathogenic vibrios in brackish waters and mussels. *Journal of applied microbiology*, 89(2), pp.261–6.

Mazibuko, N.N., 2012. *Evaluation of the quality indices of the final effluents of an urban wastewater treatment plant in the Eastern Cape Province of South Africa*. University of Fort Hare.

McMahon, M., 2014. *What Is Enteric Bacteria?*. [online] Available at: <<http://www.wisegeek.com/what-is-enteric-bacteria.htm>> [Accessed 15 Jun. 2014].

Mema, V., 2004. Impact of poorly maintained waste water and sewage treatment plants: Lessons from South Africa. (3).

Metzgar, D., Osuna, M. and Yingst, S., 2005. PCR analysis of Egyptian respiratory adenovirus isolates, including identification of species, serotypes, and coinfections. *Journal of Clinical Microbiological*, 43(11), pp.5743–5752.

Michaud, J.P., 1991. *Stream pH | Chapter 3 | A Citizen's Guide to Understanding and Monitoring Lakes and Streams | Water Quality Program | Washington State Department of Ecology*. [online] Available at: <<http://www.ecy.wa.gov/programs/wq/plants/management/joysmanual/streamph.html>> [Accessed 11 Jun. 2014].

Ministry of Environment, Lands and Parks and Geographic Data BC, 1998. Guide to Interpreting Ambient Water and Effluent Variables. In: *Guidelines for Interpreting Water Quality Data*.

Mizunoe, Y., Wai, S.N., Takade, A. and Yoshida, S.-I., 1999. Isolation and Characterization of Rugose Form of *Vibrio cholerae* O139 Strain MO10. *Infect. Immun.*, 67(2), pp.958–963.

Mladenov, N., Strzepek, K. and Serumola, O.M., 2005. Water quality assessment and modeling of an effluent-dominated stream, the Notwane River, Botswana. *Environmental monitoring and assessment*, 109(1-3), pp.97–121.

Molahalli, P., 2011. Chemical Pre-Precipitation of Municipal Wastewater Treatment.

Momba, M., Osode, A. and Sibewu, M., 2009. The impact of inadequate wastewater treatment on the receiving water bodies – Case study: Buffalo City and Nkokonbe Municipalities of the Eastern Cape Province. *Water SA*, 32(5), pp.687–692.

Moore, P.L., Steele, A.D. and Alexander, J.J., 2000. Relevance of commercial diagnostic tests to detection of enteric adenovirus infections in South Africa. *Journal of clinical microbiology*, 38(4), pp.1661–1663.

Morace, G., Aulicino, F. a, Angelozzi, C., Costanzo, L., Donadio, F. and Rapicetta, M., 2002. Microbial quality of wastewater: detection of hepatitis A virus by reverse transcriptase-polymerase chain reaction. *Journal of applied microbiology*, 92(5), pp.828–36.

Morgan-Sagastume, F. and Allen, D.G., 2003. Effects of temperature transient conditions on aerobic biological treatment of wastewater. *Water research*, 37(15), pp.3590–601.

Morris, J.G., Sztein, M.B., Rice, E.W., Nataro, J.P., Losonsky, G.A., Panigrahi, P., Tacket, C.O. and Johnson, J.A., 1996. *Vibrio cholerae* O1 can assume a chlorine-resistant rugose survival form that is virulent for humans. *The Journal of infectious diseases*, 174(6), pp.1364–8.

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

Morrison, G., Fatoki, O.S., Linder, S. and Lundehn, C., 2004. Determination of Heavy Metal Concentrations and Metal Fingerprints of Sewage Sludge from Eastern Cape Province, South Africa by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) and Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP. *Water, Air, & Soil Pollution*, 152(1-4), pp.111–127.

Morrison, G., Fatoki, O.S., Zinn, E. and Jacobsson, D., 2001b. Sustainable development indicators for urban water systems: A case study evaluation of King William ' s Town , South Africa , and the applied indicators. 27(2), pp.219–232.

- Münch, E. V., Lant, P. and Keller, J., 1996. Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors. *Water Research*, 30, pp.277–284.
- Munir, M., Wong, K. and Xagorarakis, I., 2011. Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. *Water Research*, 45, pp.681–693.
- Murray, T.Y., Mans, J. and Taylor, M.B., 2013. *Human calicivirus diversity in wastewater in South Africa*.
- Myers, B.D.N., Stoeckel, D.M., Bushon, R.N., Francy, D.S. and Brady, A.M.G., 2007. Fecal indicator bacteria: U.S. Geological Survey Techniques of Water-Resources Investigations. In: *National Field Manual for the Collection of Water-Quality Data*.
- Myers, D.N., 2003. Fecal indicator bacteria: U.S. Geological Survey Techniques of Water-Resources Investigations. In: *National Field Manual for the Collection of Water-Quality Data*. pp.1–64.
- Narkis, N., Armon, R., Offer, R., Orshansky, F. and Friedland, E., 1995. Effect of suspended solids on wastewater disinfection efficiency by chlorine dioxide. *Water Research*, 29, pp.227–236.
- National Research Council, 1982. *Quality Criteria For Water Reuse*.
- National Research Council, 2012. *Water Reuse: Potential for Expanding the Nation's Water Supply Through Reuse of Municipal Wastewater*. The National Academies Press.
- Nelson, K., Sheikh, B. and Cooper, R., 2003. Efficacy of pathogen removal during full-scale operation of water reuse facilities in Monterey, California. *IWA 4th International Symposium on Wastewater Reclamation and Reuse*, p.8.
- Ngari, J., Kotut, K. and Okemo, P., 2011. Potential threat to wildlife posed by enteric pathogens from Nakuru sewage treatment plant. *African Journal of Health Sciences*, 18(1), pp.85–95.
- NHMRC, N., 2011. Australian drinking water guidelines paper 6 national water quality management strategy. ... *Ministerial Council, Commonwealth of Australia, ...*
- Noble, R.T., Griffith, J.F., Blackwood, A.D., Fuhrman, J.A., Gregory, J.B., Hernandez, X., Liang, X., Bera, A.A. and Schiff, K., 2006. Multitiered approach using quantitative PCR to track sources of fecal pollution affecting Santa Monica Bay, California. *Applied and environmental microbiology*, 72(2), pp.1604–12.

Nordgren, J., Matussek, A., Mattsson, A., Svensson, L. and Lindgren, P.-E.E., 2009. Prevalence of norovirus and factors influencing virus concentrations during one year in a full-scale wastewater treatment plant. *Water research*, 43(4), pp.1117–25.

Norström, A., 2005. *Treatment of domestic wastewater using microbiological processes and hydroponics in Sweden*. Royal Institute of Technology.

Odjadjare, E., 2010. *Prevalence of Listeria pathogens in effluents of some wastewater treatment facilities in the Eastern Cape Province of South Africa*. University of Fort Hare.

Odjadjare, E.E., Igbinosa, E.O., Mordi, R., Igere, B., Igeleke, C.L. and Okoh, A.I., 2012. Prevalence of multiple antibiotics resistant (MAR) Pseudomonas species in the final effluents of three municipal wastewater treatment facilities in South Africa. *International journal of environmental research and public health*, 9(6), pp.2092–107.

Odjadjare, E.E.O. and Okoh, A.I., 2010. Physicochemical quality of an urban municipal wastewater effluent and its impact on the receiving environment. *Environmental monitoring and assessment*, 170(1-4), pp.383–94.

Oh, J.H., 2012. *Performance evaluation of the pilot-scale static granular bed reactor (SGBR) for industrial wastewater treatment and biofilter treating septic tank effluent using recycled*. Iowa State University.

Okoh, A.I. and Igbinosa, E.O., 2010. Antibiotic susceptibility profiles of some Vibrio strains isolated from wastewater final effluents in a rural community of the Eastern Cape Province of South Africa. *BMC microbiology*, 10, p.143.

Okoh, A.I., Sibanda, T. and Gusha, S.S., 2010. Inadequately treated wastewater as a source of human enteric viruses in the environment. *International journal of environmental research and public health*, 7(6), pp.2620–37.

Olaniran, A.O., Naicker, K. and Pillay, B., 2009. Antibiotic resistance profiles of Escherichia coli isolates from river sources in Durban, South Africa. *World Journal of Microbiology & Biotechnology*, 25, pp.1743–1749.

Oliveira, S.C. and von Sperling, M., 2011. Performance evaluation of different wastewater treatment technologies operating in a developing country. *Journal of Water, Sanitation and Hygiene for Development*, 1(1), p.37.

Oliver, G.G. and Fidler, L.E., 2001. *Towards a Water Quality Guideline for Temperature in BC*. [online] Available at: <<http://www.env.gov.bc.ca/wat/wq/BCguidelines/temptech/index.html#TopOfPage>> [Accessed 19 Jun. 2014].

Omar, K. and Barnard, T., 2010. The occurrence of pathogenic *Escherichia coli* in South African wastewater treatment plants as detected by multiplex PCR. *Water SA*, 36(2), pp.172–176.

Osode, A., 2010. *Assessment of the prevalence of virulent Escherichia coli strains in the final effluents of wastewater treatment plants in the Eastern Cape province of South Africa*. University of Fort Hare, pp.1–158.

Osode, A.N., 2007. *The impact of wastewater quality on receiving water bodies and public health in Buffalo City and Nkonkobe Municipalities*. Vasa, University of Fort Hare, p.2007.

Paul, A.L., 2012. *The Effects Of Ozone And UV Radiation On The Molecular Weight And Optical Properties Of Natural Water And Wastewater Effluent Dissolved Organic Matter*. Florida State University College of Arts and Sciences.

Pearce, G., Chaudhry, M.R. and Ghulam, S., 1998. *A simple methodology for water quality monitoring*.

Pepper, Ian L., Gerba, C.P. and Gentry, T.J., 2014. *Environmental Microbiology*. Elsevier, p.728.

Pescod, M.B., 1992. *Wastewater treatment and use in agriculture*.

PG&E NEW CONSTRUCTION, 2003. Municipal wastewater treatment plant energy baseline study.

Phiri, O., Mumba, P., Moyo, B.H.Z. and Kadewa, W., 2005. Assessment of the impact of industrial effluents on water quality of receiving rivers in urban areas of Malawi. *International Journal of Environmental Science & Technology*, 2(3), pp.237–244.

Picão, R.C., Cardoso, J.P., Campana, E.H., Nicoletti, A.G., Petrolini, F.V.B., Assis, D.M., Juliano, L. and Gales, A.C., 2013. The route of antimicrobial resistance from the hospital effluent to the environment: focus on the occurrence of KPC-producing *Aeromonas* spp. and Enterobacteriaceae in sewage. *Diagnostic microbiology and infectious disease*, 76(1), pp.80–5.

Pintó, R.M., Costafreda, M.I. and Bosch, A., 2009. Risk assessment in shellfish-borne outbreaks of hepatitis A. *Applied and environmental microbiology*, 75(23), pp.7350–5.

Plessis, J. Du, 2008. *The assessment of the water quality of the Hex River Catchment-North West Province*. University of Johannesburg, pp.6–27.

- Pollice, A., Tandoi, V. and Lestingi, C., 2002. Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. *Water Research*, 36, pp.2541–2546.
- Prado, T., Fumian, T.M., Miagostovich, M.P. and Gaspar, A.M.C., 2012. Monitoring the hepatitis A virus in urban wastewater from Rio de Janeiro, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 106(2), pp.104–9.
- Prado, T., Gaspar, A.M.C. and Miagostovich, M.P., 2014. Detection of enteric viruses in activated sludge by feasible concentration methods. *Brazilian Journal of Microbiology*, 45(1), pp.343–349.
- Prasad, A. and Rao, K., 2011. Chemical analysis of textile effluent and decolorization of textile azo dye by *Bacillus endophyticus* strain. *IIOAB Journal*, 2(2), pp.55–62.
- Puig, M., Jofre, J., Lucena, F., Allard, a, Wadell, G. and Girones, R., 1994. Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. *Applied and environmental microbiology*, 60(8), pp.2963–70.
- Pusch, D., Oh, D.-Y., Wolf, S., Dumke, R., Schröter-Bobsin, U., Höhne, M., Röske, I. and Schreier, E., 2005. Detection of enteric viruses and bacterial indicators in German environmental waters. *Archives of virology*, 150(5), pp.929–47.
- Rached, E. and Brooks, D., 1996. *Water Management in Africa and the Middle East: Challenges and Opportunities*. IDRC, p.294.
- Raissy, M., Moumeni, M., Ansari, M. and Rahimi, E., 2012. Antibiotic resistance pattern of some *Vibrio* strains isolated from seafood. *Iranian Journal of Fisheries ...*
- Ramamurthy, T., Chowdhury, G., Pazhani, G.P. and Shinoda, S., 2014. *Vibrio fluvialis*: an emerging human pathogen. *Frontiers in microbiology*, 5, p.91.
- RAMP, 2014. *Water Quality Indicators: Temperature and Dissolved Oxygen - Regional Aquatics Monitoring Program (RAMP)*. [online] Available at: <<http://www.ramp-alberta.org/river/water+sediment+quality/chemical/temperature+and+dissolved+oxygen.aspx>> [Accessed 18 Jun. 2014].
- Ramteke, P.W., Awasthi, S., Srinath, T. and Joseph, B., 2010. Efficiency assessment of Common Effluent Treatment Plant (CETP) treating tannery effluents. *Environmental monitoring and assessment*, 169(1-4), pp.125–31.
- Ramudingana, P., 2010. *Detection of rotavirus and norovirus in children under 5 years of age in Pretoria, South Africa and molecular characterization of rotavirus strains*.

- Rasheed, M.U., Thajuddin, N., Ahamed, P., Teklemariam, Z. and Jamil, K., 2014. Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. *Revista do Instituto de Medicina Tropical de São Paulo*, 56(4), pp.341–6.
- Rastogi, S., Kumar, A., Mehra, N.K., Makhijani, S.D., Manoharan, A., Gangal, V. and Kumar, R., 2003a. Development and characterization of a novel immobilized microbial membrane for rapid determination of biochemical oxygen demand load in industrial waste-waters. *Biosensors and Bioelectronics*, 18, pp.23–29.
- Rastogi, S., Rathee, P., Saxena, T.K., Mehra, N.K. and Kumar, R., 2003b. BOD analysis of industrial effluents: 5 days to 5 min. *Current Applied Physics*, 3(2-3), pp.191–194.
- Rech, Ž., 2008. *Biological Wastewater Treatment - Activated Sludge*. [online] Available at: <<http://www.riparia.org.rs/aqua/activated-sludge.htm>> [Accessed 16 Jun. 2014].
- Redda, M., 2008. *Studies of the performance, stability and reliability of various configurations of the activated sludge process at full-scale municipal wastewater treatment plants*. University of Texas at Arlington.
- Reis, R.S. Dos and Horn, F., 2010. Enteropathogenic *Escherichia coli*, *Salmonella*, *Shigella* and *Yersinia*: cellular aspects of host-bacteria interactions in enteric diseases. *Gut pathogens*, 2(1), p.8.
- Rich, L.G., 2003. *Nitrites and their impact on effluent chlorination*. [online] Available at: <<http://www.lagoonsonline.com/technote4.htm>> [Accessed 18 Jun. 2014].
- Richard, M. and Bowman, R., 1998. *Troubleshooting and Optimizing Wastewater Treatment in Small Communities: Lagoon Processes*.
- Rigotto, C., Victoria, M., Moresco, V., Kolesnikovas, C.K., Corrêa, A.A., Souza, D.S.M., Miagostovich, M.P., Simões, C.M.O. and Barardi, C.R.M., 2010. Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianopolis, South Brazil. *Journal of applied microbiology*, 109(6), pp.1979–87.
- Rodríguez, R.A., 2007. *Occurrence of enteric viruses on combined sewer overflows*. University of Arizona.
- Rogers, S. and Haines, J., 2005. Detecting and mitigating the environmental impact of fecal pathogens originating from confined animal feeding operations: Review.
- Rojas, Y.A. and Hazen, T.C., 1989. Survival of *Vibrio cholerae* in treated and untreated rum distillery effluents. *Water Research*, 23(1), pp.103–113.

La Rosa, G., Fratini, M., della Libera, S., Iaconelli, M. and Muscillo, M., 2012. Emerging and potentially emerging viruses in water environments. *Annali dell'Istituto Superiore di Sanità*, 48(4), pp.397–406.

Rosenblum, J., Ge, C., Bohrerova, Z., Yousef, A. and Lee, J., 2012. Ozonation as a clean technology for fresh produce industry and environment: sanitizer efficiency and wastewater quality. *Journal of applied microbiology*, 113(4), pp.837–45.

Safe Drinking Water Foundation, 2009. *TDS AND pH*.

Samie, A., Obi, C. and Dillingham, R., 2007. Enteroaggregative *Escherichia coli* in Venda, South Africa: Distribution of Virulence-Related Genes by Multiplex Polymerase Chain Reaction in Stool Samples of. *The American journal of tropical medicine and hygiene*, 77(1), pp.142–150.

Samie, A., Obi, C., Igumbor, J. and Momba, M., 2009. Focus on 14 sewage treatment plants in the Mpumalanga Province, South Africa in order to gauge the efficiency of wastewater treatment. *African Journal of Biotechnology*, 8(14), pp.3276–3285.

SANS, S.A.N.S., 2011. *Drinking water - Part 1: Microbiological, physical, chemical, aesthetic and chemical determinands*. 1st ed. Pretoria: SABS Standards Division.

Savichtcheva, O. and Okabe, S., 2006. Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Research*, 40(13), pp.2463–76.

Scott, R., 2014. *Lesson 21: Nitrates and Nitrites*. [online] Understanding the Basic Principles of Nitrogen. Available at: <http://water.me.vccs.edu/courses/env211/lesson21_print.htm> [Accessed 14 Jun. 2014].

Seidel, G., 2003. *Detection of non-CPE producing enteric viruses via ICC-PCR at wastewater land application sites in Arizona and California; endocrine disruption activity after wetland, pond, and soil aquifer treatment of wastewater*. University of Arizona.

Sengupta, S. and Pandit, A., 2011. Selective removal of phosphorus from wastewater combined with its recovery as a solid-phase fertilizer. *Water Research*, 45, pp.3318–3330.

Shabalala, A.N., Combrinck, L. and McCrindle, R., 2013. Effect of farming activities on seasonal variation of water quality of Bonsma Dam, KwaZulu-Natal. *South African Journal of Science*, 109(7/8), pp.1–7.

Shang, C., Qi, Y. and Lo, I.M.C., 2005. Factors Affecting Inactivation Behavior in the Monochloramination Range. *Journal of Environmental Engineering*, 131(1), pp.119–129.

Shaw, K.S., Rosenberg Goldstein, R.E., He, X., Jacobs, J.M., Crump, B.C. and Sapkota, A.R., 2014. Antimicrobial susceptibility of *Vibrio vulnificus* and *Vibrio parahaemolyticus* recovered from recreational and commercial areas of Chesapeake Bay and Maryland Coastal Bays. *PloS one*, 9(2), p.e89616.

Shih, 施孟欣 T A - Meng-Hsin, 2013. 台灣地區水體環境中腺病毒之親源分析 TT - *Phylogenetic Analyses of Human Adenoviruses (HAdVs) In Sewages of Taiwan Area*. 環境科學與工程學系, 東海大學, p.94 NI – 臺灣博碩士論文知識加值系統 LG – 英文.

Shon, H.K., Vigneswaran, S. and Snyder, S.A., 2006. *Effluent Organic Matter (EfOM) in Wastewater: Constituents, Effects, and Treatment*. *Critical Reviews in Environmental Science and Technology*, .

Sibanda, T., Chigor, V.N. and Okoh, A.I., 2013. Seasonal and spatio-temporal distribution of faecal-indicator bacteria in Tyume River in the Eastern Cape Province, South Africa. *Environmental monitoring and assessment*, 185(8), pp.6579–90.

Sibanda, T. and Okoh, A.I., 2012. Assessment of the incidence of enteric adenovirus species and serotypes in surface waters in the eastern cape province of South Africa: Tyume River as a case study. *TheScientificWorldJournal*, 2012, p.949216.

Sibanda, T. and Okoh, A.I., 2013. Real-time PCR quantitative assessment of hepatitis A virus, rotaviruses and enteroviruses in the Tyume River located in the Eastern Cape Province, South Africa. *Water SA*, 39(2), pp.295–304.

Simmons, F.J., 2010. *Fate of enteric viruses in advanced and conventional wastewater treatment*. *Masters Abstracts International*. Michigan State University.

Simmons, F.J., Kuo, D.H.-W. and Xagorarakis, I., 2011. Removal of human enteric viruses by a full-scale membrane bioreactor during municipal wastewater processing. *Water research*, 45(9), pp.2739–50.

Simmons, F.J. and Xagorarakis, I., 2011. Release of infectious human enteric viruses by full-scale wastewater utilities. *Water research*, 45(12), pp.3590–8.

Singleton, F.L., Attwell, R.W., Jangi, M.S. and Colwell, R.R., 1982. Influence of salinity and organic nutrient concentration on survival and growth of *Vibrio cholerae* in aquatic microcosms. *Applied and environmental microbiology*, 43, pp.1080–1085.

Slabbert, N., 2007. *The Potential Impact Of An Inter-Basin Water Transfer On The Modder And Caledon River Systems*. University of the Free State.

Sousa, O.V., Vieira, R.H.S.F., Patel, T.R., Hofer, E. and Mesquita, V.P., 2001. Effects of chlorine on cells of *Vibrio cholerae*. *Food Microbiology*, 18(3), pp.355–359.

Sowers, A., 2009. *The effects of wastewater effluent on a fish and amphibian species*. Clemson University.

Staley, J.T., Crosa, J., DeWalle, F. and Carlson, D., 1987. *Effect Of Wastewater Disinfectants On Survival Of R-factor Coliform Bacteria*. p.8.

Stedtfeld, R.D., Turlousse, D.M., Seyrig, G.G., Alm, E.W., Hashsham, S.A., Ahmad, F. and Duran, M., 2007. Detection and Occurrence of Indicator Organisms and Pathogens. *Water Environment Research*, 79(10), pp.898–928.

Steele, D., Reynecke, E., De Beer, M., Bos, P. and Smuts, I., 2002. Characterization of rotavirus infection in a hospital neonatal unit in Pretoria, South Africa. *Journal of Tropical Pediatrics*, 48, pp.167–171.

Stevik, T.K., Aa, K., Ausland, G. and Hanssen, J.F., 2004. *Retention and removal of pathogenic bacteria in wastewater percolating through porous media: A review*. *Water Research*, .

Stone, S., 2014. *Bloemhof E. coli outbreak under control, says MEC | Health | BDlive*. [online] Available at: <<http://www.bdlive.co.za/national/health/2014/06/02/bloemhof-e.-coli-outbreak-under-control-says-mec>> [Accessed 3 Jun. 2014].

Straton, A., 2013. *Committed to cleaning up the Zwartkops River*. [online] Available at: <<http://mype.co.za/new/2013/03/committed-to-cleaning-up-the-zwartkops-river/>> [Accessed 3 Jun. 2014].

Stringer, R., 1997. *The environment, economics and water policies*.

Sun William, L.L.W.J.J.D., 1973. Wastewater Disinfection of Trickle Filter Effluent with Bromine and Chlorine. *Am Chem Soc Div Environ Chem Prepr*, 13, pp.156–159.

Symonds, E.M., Griffin, D.W. and Breitbart, M., 2009. Eukaryotic viruses in wastewater samples from the United States. *Applied and environmental microbiology*, 75, pp.1402–1409.

Szymanski, N. and Patterson, R., 2003. Effective microorganisms (EM) and wastewater systems. In: *uture Directions for On-site Systems: Best Management Practice Proceedings of On-site '03 Conference*. pp.347–354.

- Tarr, C.L., Patel, J.S., Puhr, N.D., Sowers, E.G., Bopp, C.A. and Strockbine, N.A., 2007. Identification of *Vibrio* isolates by a multiplex PCR assay and *rpoB* sequence determination. *Journal of clinical microbiology*, 45(1), pp.134–40.
- Taylor, M.B., Marx, F.E. and Grabow, W.O., 1997. Rotavirus, astrovirus and adenovirus associated with an outbreak of gastroenteritis in a South African child care centre. *Epidemiology and infection*, 119(2), pp.227–30.
- Terry, P.A., 2010. Application of Ozone and Oxygen to Reduce Chemical Oxygen Demand and Hydrogen Sulfide from a Recovered Paper Processing Plant. *International Journal of Chemical Engineering*, 2010, pp.1–6.
- Thomas, K.U., Joseph, N., Raveendran, O. and Nair, S., 2006. Salinity-induced survival strategy of *Vibrio cholerae* associated with copepods in Cochin backwaters. *Marine Pollution Bulletin*, 52, pp.1425–1430.
- Thurston-Enriquez, J.A., Haas, C.N., Jacangelo, J. and Gerba, C.P., 2003. Chlorine Inactivation of Adenovirus Type 40 and Feline Calicivirus. *Applied and Environmental Microbiology*, 69(7), pp.3979–3985.
- Thurston-Enriquez, J.A., Haas, C.N., Jacangelo, J. and Gerba, C.P., 2005. Inactivation of enteric adenovirus and feline calicivirus by chlorine dioxide. *Applied and environmental microbiology*, 71(6), pp.3100–5.
- Tiemessen, C. and Nel, M., 1996. Detection and typing of subgroup F adenoviruses using the polymerase chain reaction. *Journal of virological methods*.
- TLC, 2007. *Water & Wastewater Sampling Course*. Technical Learning College.
- Trang, D.T., Hien, B.T.T., Mølbak, K., Cam, P.D. and Dalsgaard, A., 2007. Epidemiology and aetiology of diarrhoeal diseases in adults engaged in wastewater-fed agriculture and aquaculture in Hanoi, Vietnam. *Tropical Medicine and International Health*, 12, pp.23–33.
- Tree, J.A., Adams, M.R. and Lees, D.N., 2003. Chlorination of indicator bacteria and viruses in primary sewage effluent. *Applied and environmental microbiology*, 69, pp.2038–2043.
- Tyagi, V.K., Chopra, A.K., Kazmi, A.A. and Kumar, A., 2006. Alternative microbial indicators of faecal pollution: current perspective. *Iranian Journal of Environmental Health, Science and Engineering*, 3(3), pp.205–216.
- Tyrrell, S.A., Rippey, S.R. and Watkins, W.D., 1995. Inactivation of bacterial and viral indicators in secondary sewage effluents, using chlorine and ozone. *Water Research*, 29, pp.2483–2490.

U S Environmental Protection Agency, 2002. *Onsite Wastewater Treatment Systems Manual*.

U.S. Environmental Protection Agency, 1985. *Perspectives on nonpoint source pollution: proceedings of a national conference, Kansas City, Missouri, May 19-22, 1985*. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, p.514.

U.S. Environmental Protection Agency, 2004. *NPDES compliance inspection manual*.

Umbreit, W.W., 1966. *Advances in applied microbiology, Volume 8 (Google eBook)*. Academic Press, p.377.

UNEP/WHO, 1996. *Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. Military Operations Research*, .

US EPA, O., 2012a. *5.11 Fecal Bacteria*. [online] Available at: <<http://water.epa.gov/type/rsl/monitoring/vms511.cfm>> [Accessed 3 Jun. 2014].

US EPA, O., 2012b. *5.9 Conductivity*. [online] Available at: <<http://water.epa.gov/type/rsl/monitoring/vms59.cfm>> [Accessed 13 Aug. 2014].

US EPA, OW, 2012. *5.2 Dissolved Oxygen and Biochemical Oxygen Demand*.

Valiela, I., Alber, M. and LaMontagne, M., 1991. Fecal coliform loadings and stocks in Buttermilk Bay, Massachusetts, USA, and management implications. *Environmental Management*, 15(5), pp.659–674.

Vaseeharan, B., Ramasamy, P., Murugan, T. and Chen, J.C., 2005. In vitro susceptibility of antibiotics against *Vibrio* spp. and *Aeromonas* spp. isolated from *Penaeus monodon* hatcheries and ponds. *International journal of antimicrobial agents*, 26(4), pp.285–91.

Velasco, H.G., 2001. Water rights administration. Experience, issues and guidelines. *FAO Legislative Study (FAO)*.

Venter, J.M.E., van Heerden, J., Vivier, J.C., Grabow, W.O.K. and Taylor, M.B., 2007. Hepatitis A virus in surface water in South Africa: what are the risks? *Journal of water and health*, 5, pp.229–240.

Verma, T., Ramteke, P.W. and Garg, S.K., 2008. Quality assessment of treated tannery wastewater with special emphasis on pathogenic *E. coli* detection through serotyping. *Environmental Monitoring and Assessment*, 145, pp.243–249.

Vidal, M., Kruger, E., Durán, C., Lagos, R., Levine, M., Prado, V., Toro, C. and Vidal, R., 2005. Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic *Escherichia coli* associated with enteric infections. *Journal of clinical microbiology*, 43(10).

Virto, R., Mañas, P., Alvarez, I., Condon, S. and Raso, J., 2005. Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. *Applied and environmental microbiology*, 71, pp.5022–5028.

WAMTechnology, n.d. *Wastewater Treatment Process*.

Wang, J., 2005. *Microscopic Study of Phosphorous Removal Process in an Activated Sludge System*. University of Cincinnati.

Watt, S. and Lanotte, P., 2003. *Escherichia coli* strains from pregnant women and neonates: intraspecies genetic distribution and prevalence of virulence factors. *Journal of clinical microbiology*, 41(5).

WDNR, W.D. of N.R., 2010. *Advanced On-site Laboratory Testing Study Guide*. Madison,.

Wennberg, A.C., Tryland, I., Ostensvik, O., Secic, I., Monshaugen, M. and Liltved, H., 2013. Effect of water treatment on the growth potential of *Vibrio cholerae* and *Vibrio parahaemolyticus* in seawater. *Marine environmental research*, 83, pp.10–5.

Wéry, N., Lhoutellier, C., Ducray, F., Delgenés, J.P. and Godon, J.J., 2008. Behaviour of pathogenic and indicator bacteria during urban wastewater treatment and sludge composting, as revealed by quantitative PCR. *Water Research*, 42, pp.53–62.

Westminster College, 1993. Determining the Amount of Nitrate in Water. (3), pp.122–123.

WHO, 1997. *Guidelines for Drinking-Water Quality*. Second ed. World Health Organization, pp.51–72.

WHO, W.H.O., 2008. Guidelines for drinking-water quality: incorporating first and second addenda to third edition, Vol. 1, Recommendations.

WHO, W.H.O., 2011. *WHO | Guidelines for drinking-water quality, fourth edition*. World Health Organization.

Wilde, F.D. and Ed, 2008a. Field measurements: U.S. Geological Survey Techniques of Water-Resources Investigations. In: *National Field Manual for the Collection of Water-Quality Data*. pp.1–48.

- Wilde, F.D. and Ed, 2008b. pH Field measurements: U.S. Geological Survey Techniques of Water-Resources Investigations; In: *National Field Manual for the Collection of Water-Quality Data*. pp.1–30.
- Winward, G.P., Avery, L.M., Stephenson, T. and Jefferson, B., 2008. Chlorine disinfection of grey water for reuse: Effect of organics and particles. *Water Research*, 42, pp.483–491.
- Wochinger, A.V., 2012. *Human Enteric Viruses in Recreational Coastal Waters*. Stony Brook University.
- Wong, H., You, W. and Chen, S., 2012. Detection of Toxigenic *Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus* in Oyster by Multiplex-PCR with Internal Amplification Control. *Journal of Food & Drug Analysis*, 20(1), pp.48–58.
- Woods, J.S., 2010. *Determining the relationship of human enteric viruses in clinical, wastewater, and environmental samples utilizing molecular and cell culture techniques*. The University of Southern Mississippi.
- World Water Assessment Programme, 2009. *The United Nations World Water Development Report 3: Water in a Changing World*. Paris: UNESCO.
- WRF:Water Research Foundation, 2010. *Challenge Organisms for Inactivation of Viruses by Ultraviolet Treatment*.
- Wright-Walters, M. and Volz, C., 2009. Municipal wastewater concentrations of pharmaceutical and xeno-estrogens: wildlife and human health implications. *Proceedings of the 2007 National Conference on Environmental Science and Technology*, (1), pp.1–9.
- Xagorarakis, I., Harrington, G.W., Assavasilavasukul, P. and Standridge, J.H., 2004. Removal of emerging waterborne pathogens and pathogen indicators. *Journal - American Water Works Association*, 96, p.102.
- Y S I Environmental, 2006. Improving Microbial Efficiency at Wastewater Treatment Plants with ROX™ Optical Dissolved Oxygen Sensor.
- Yadav, R. and Dagar, N., 2012. Variability in Physico-Chemical Parameters of Ground Water of North-East Zone of the Bhiwadi Industrial Area (Alwar). *J. Curr. Chem. Pharm. ...*, 2(3), pp.198–208.
- Ye, L. and Zhang, T., 2013. Bacterial communities in different sections of a municipal wastewater treatment plant revealed by 16S rDNA 454 pyrosequencing. *Applied microbiology and biotechnology*, 97(6), pp.2681–90.

Yildiz, F.H. and Schoolnik, G.K., 1998. Role of rpoS in stress survival and virulence of *Vibrio cholerae*. *Journal of bacteriology*, 180, pp.773–784.

Zamxaka, M., Pironcheva, G. and Muyima, N., 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), pp.333–340.

Zanetti, S., Spanu, T., Deriu, A., Romano, L., Sechi, L.A. and Fadda, G., 2001. In vitro susceptibility of *Vibrio* spp. isolated from the environment. *International journal of antimicrobial agents*, 17(5), pp.407–9.

Zhao, D. and Sengupta, A.K., 1998. Ultimate removal of phosphate from wastewater using a new class of polymeric ion exchangers. *Water Research*, 32, pp.1613–1625.

Zuma, B., 2010. *Microbial ecology of the Buffalo River in response to water quality changes*. Rhodes University.