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DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

ASSESSMENT OF THE PREVALENCE OF FAECAL COLIFORMS AND *ESCHERICHIA COLI* O157:H7 IN THE FINAL EFFLUENTS OF TWO WASTEWATER TREATMENT PLANTS IN AMAHLATHI LOCAL MUNICIPALITY OF EASTERN CAPE PROVINCE , SOUTH AFRICA

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CERTIFICATION

I hereby certify that this research work was carried out by **Mr Adefisoye Martins Ajibade** and supervised by me in the Department of Biochemistry and Microbiology, University of Fort Hare, Alice South Africa in accordance with the requirements for the award of Masters of Science degree in Microbiology and the work herein is original and has not been submitted at any other university for the award of any degree.

.....

PROF AI OKOH (Supervisor)

.....

Date

DEDICATION

"To God be the Glory, great thing He hath done!"

To my parents

Late Engr. (Overseer) MA & Deaconess AM Adefisoye,

and to all my siblings.

I love you all!

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LIST OF ABBREVATIONS

AEMREG	Applied and Environmental Microbiology Research Group
AMREF	African Medical and Research foundation
APHA	American Public Health Association
ANOVA	Analysis of Variance
BNR	Biological Nutrient Removal
BOD	Biological Oxygen Demand
CCME	Canadian Council of Ministers of the environment
CDC	Centers for Diseases control and Prevention
CFA	Colonisation Factor Antigen
CFU	Colony Forming Units
COD	Chemical Oxygen Demand
DAEC	Diffusely Adhering *E. coli*
DALY	Daily Adjusted Life Years
DO	Dissolved Oxygen
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
EAggEC	Enteroaggregative *E. coli*
EC	Electrical Conductivity

EHEC	Enterohemorrhagic *Escherichia coli* O157:H7
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
EU	European Union
FAO	Food and Agricultural Organisation of the United Nations
FC	Faecal Coliforms
FS	Faecal Streptococci
HUS	Haemolytic Ureamic Syndrome
HUSEC	Haemolytic Uremic Syndrome-associated Enterohemorrhagic *E. coli*
IMLR	Internal Mixed Liquor Recycle
INFOSAN	International Food Safety Authorities
JMP	Joint Monitoring Programme
KHD	Keiskammahoek Discharge Point
KHF	Keiskammahoek Final Effluent
KWWTW	Keiskammahoek Wastewater Treatment Works
LT	Heat Labile
MF	Membrane Filtration technique
NMEC	Neonatal Meningitis *E. coli*

NO_2	Nitrites
NO_3	Nitrates
NTU	Nephelometric Units
ONPG	*Ortho*-nitrophenyl- β-D-galactopyranoside
PAO	Phosphate Accumulating Organisms
PO_4	Phosphates
RBSs	Rotating Biological Contactors
SDWF	Safe Drinking Water Foundation
SPSS	Statistical Package for Social Science
ST	Heat Stable
STD	Stutterheim Discharge Point
SLTEC	Shiga-like Toxin Producing *E. coli*
STF	Stutterheim final Effluent
SWWTW	Stutterheim Wastewater Treatment Works
TC	Total Coliforms
TDS	Total Dissolved Solids
THM	Trihalomethanes
TSS	Total Suspended Solids
UNEP	United Nations Environmental Programme

UNICEF	United Nations Children's Fund
UNIDO	United Nations Industrial Development Organisation
UPEC	Uropathogenic *E. coli*
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
VTEC	Verocytotoxin-producing *E. coli*
WHO	World Health Organisation
WWTP	Wastewater Treatment Plant

ABSTRACT

The production of final effluents that meet discharged requirements and guidelines remain a major challenge particularly in the developing world with the resultant problem of surface water pollution. This study assessed the physicochemical and microbiological qualities of two wastewater treatment works in the Eastern Cape Province of South Africa in terms of the prevalence of faecal coliforms and *Escherichia coli* O157:H7 over a five month period. All physicochemical and microbiological analyses were carried out using standard methods. Data were collected in triplicates and analysed statistically using IBM SPSS version 20.0. The ranges of some of the physicochemical parameters that complied with set guidelines include pH (6.7 – 7.6), TDS (107 – 171 mg/L), EC (168 – 266 µS/cm), Temperature (15 – 24^{o}C), NO_3^- (0 – 8.2 mg/L), NO_2^- (0.14 – 0.71 mg/L) and PO_4 (1.05 – 4.50 mg/L). Others including Turbidity (2.64 – 58.00 NTU), Free Cl (0.13 – 0.65 mg/L), DO (2.20 – 8.48 mg/L), BOD (0.13 – 6.85 mg/L) and COD (40 – 482 mg/L) did not comply with set guidelines. The microbiological parameters ranged 0 – 2.7×10^4 CFU/100 ml for FC and 0 – 9.3×10^3 for EHEC CFU/100 ml, an indication of non-compliance with set guidelines. Preliminary identification of 40 randomly selected presumptive enterohemorrhagic *E. coli* isolates by Gram's staining and oxidase test shows 100% (all 40 selected isolates) to be Gram positive while 90% (36 randomly selected isolates) were oxidase negative. Statistical correlation between the physicochemical and the microbiological parameters were generally weak except in the case of free chlorine and DO where they showed inverse correlation with the microbiological parameters. The recovery of EHEC showed the inefficiency of the treatment processes to effectively inactivate the bacteria, and possibly other pathogenic bacteria that may be present in the treated wastewater. The assessment suggested the need for proper monitoring and a review of the treatment procedures used at these treatment works.

Keywords: Final effluent, guidelines, water pollution, faecal coliforms, enterohemorrhagic *Escherichia coli* O157:H7

CHAPTER ONE

1.1 Background study

Water is one of the basic needs of life and access to safe water is a fundamental human need and therefore, a basic right. However, water has also been recognised as a potential vehicle of disease since the beginning of recorded history (Prescott *et al*, 2008). Contaminated water jeopardises both the physical and social health of all people and it is an affront to human dignity (WHO, 2003). Several water-related outbreaks have been documented with reference to risk factors exposure through ingestion of contaminated drinking waters (WHO 2004), shellfish (Levine *et al.*, 1993; Bean *et al.*, 1996; Hlady and Klontz 1996; CDC 1998) and crops (Shuval *et al.*, 1986; WHO 1989; National Research Council, 1996). More than one billion people in the developing world have no safe drinking water, or water for domestic activities (AMREF, 2012). Also, about 2.4 billion people have no adequate sanitation thus leading to:

- water-borne diseases (e.g. cholera, typhoid);
- water-related diseases (e.g. malaria, yellow fever, river blindness, sleeping sickness);
- water-based diseases (e.g. guinea worm and bilharzia);
- water-washed diseases (e.g. trachoma and scabies) and;
- diarrhea; a leading killer of children in sub-Saharan Africa (AMREF, 2012).

In South Africa alone, more than 7 million people (approximately 17% of the population) do not have access to potable water supply and nearly 21 million (about 54% of the population) lack basic sanitation (DWAF, 1996; Zamxaka, 2004). South Africa water supply remains limited and unevenly distributed, and the country has been described as a

water-scarce country in terms of a commonly used definition, of the average "total actual renewable water resources" (TARWR) per person per year (Blignaut *et al.*, 2009; Muller *et al.*, 2009). Although, in a more recent statistics by the WHO/UNIFEC Joint Monitoring Programme (JMP) for Water Supply and sanitation (2010), there has been total improvement in both urban and rural drinking water accessibility in South Africa from 38,655,000 in the year 2000 to 45,792, 000 in the year 2010, however, much less improvement has been achieved on sanitation (WHO/UNICEF, 2010). While the South African government implemented many rural water supply schemes under the National Reconstruction and Development Programme, where rural water supply existed, drinking water mostly remain of poor quality and considered unsafe (Momba *et al.*, 2006). This highlights the potential of infection due to water-borne pathogens.

Poor water quality continues to pose a major threat to human health. Diarrheal disease alone amounts to an estimated 4.1 % of the total daily adjusted life years (DALY) global burden of disease and is responsible for the deaths of 1.8 million people every year (WHO, 2004). Although water-related diseases have largely been eliminated in wealthier nations, they remain a major concern in much of the developing world.

There are diverse effects of exposure to pathogenic bacteria in drinking water, and the most common symptom of waterborne illness remains gastrointestinal upset including nausea, vomiting and diarrhea. This often last only short period of time and is resolved. However, the effect of this may be more severe, chronic or even fatal in infant, immunocompromised individuals and elderly persons (Health Canada, 2006a). While data are incomplete, the World Health Organisation (WHO) estimated in its 2000 assessment that there are four billion cases of diarrhea each year in addition to millions of other cases of illness associated with the lack of access to clean water (WHO, 2000). Since many illnesses

are undiagnosed and unreported, the true extent of these diseases is unknown (Hlupheka and Hailemariam, 2001).

As the world population continue to grow rapidly, many developing countries face difficult choices between the growing demand for fresh water on one hand, and limited and increasingly polluted water supplies on the other. Improper waste disposal and poor water (wastewater) management are important factors responsible for the ever increasing pollution of freshwater bodies. The problems associated with sewage disposal have become a major problem for the developing world due to increase in human population and urbanisation. The commonality of sewage related problems throughout coastal areas of the world is significant since these areas are inhabited by over 60% of the human population (Owili, 2003).

Wastewater is any water that has been adversely affected in quality by several anthropogenic influences (Norzatulakma, 2010). It is made up of liquid waste discharges from domestic residences, commercial properties and industries, and can encompass a wide range of potential contaminants in different concentrations. Sewage, on the other hand, is a subset of wastewater contaminated mainly with biological wastes such as faeces, urine, food remnants and laundry waste. Sewage includes domestic, municipal or industrial liquid waste product disposed of, usually via a pipe or sewer or similar structure (Mittler, 2002; Darvishi and Farahani, 2011).

Wastewater (sewage) often contains a variety of organic and inorganic compounds of anthropogenic and natural origin. For instance, the composition of a typical municipal wastewater is roughly 99.93% water and 0.07% total (dissolved and suspended) solids. Furthermore, of the 0.07 % total solids (TS), only half are organic in nature, the other half are inert (Ellis, 2004). Municipal wastewater is a concern because of its composition and the total volumes discharged. Municipal wastewater effluent is comprised of grit, debris and

suspended solids; pathogens such as bacteria and viruses; decaying organic waste which reduces the amount of oxygen available in a water body; nutrients such as nitrogen and phosphorus and, household and industrial chemicals (CCME, 2006).

Although the collection of wastewater dates back to ancient times, its treatment is a relatively recent development dating from the late 1800s and early 1900s (Chow *et al*., 1972, Okoh *et al*., 2007). Wastewater treatment practices vary from country to country across the globe but its main objective remains the same; removing contaminants from wastewater. In line with the spirit and letter of the South African Constitution under the Bill of Rights which states that "*everyone has the rights to have access to sufficient food and water*" (Constitution of South Africa, 1996 section 27b) every South African deserves clean, safe and affordable water (Odjadjare, 2010). Since 1956, South Africa made it mandatory through the South African Water Act (Act 54 of 1956) that effluent be treated to acceptable standards and returned to the water course from where water was originally obtained (Morrison *et al*., 2001).

However, as the demand for water increased due to economic expansion and population growth, wastewater and sewage treatment plants increasingly operated under stress. This situation in turn exerted pressure on water and sanitation authorities to find ways to sustain the quality of water resources (Mema, 2009).

Several institutions and governmental agencies are often involved in the monitoring of water and wastewater final effluent qualities. Indicator microorganisms have traditionally been used to determine the possible presence of pathogens (Berg, 1978) for the purpose of these quality assessments. Members of bacteria groups used as indicators include the coliforms (which comprise of total coliforms and the thermotolerant or faecal coliform

groups) and the faecal streptococci or enterococci (Bitton, 2005; United States Environmental Protection Agency, 2012a).

1.2 Research problem

Presently, water contamination issues are of great concern worldwide. This environmental problem affects aquifers, water bodies and biodiversity; but most of all, public health (Rubio-Arias *et al*., 2010). There is a growing concern about South Africa's ability to develop and sustain water and sanitation infrastructures as a result of the ever increasing population, climatic change and global economic concerns. In addition to these are health, environmental and economic concerns from untreated or poorly treated wastewater effluent discharges to surface water bodies (Turton, 2008; Mema, 2009). Several incidents of water pollution due to sewage discharges from municipal wastewater and sewage treatment plants were reported in 2008 (Elie Fereche, 2010).

The Eastern Cape Province of South Africa is mostly rural and characterised by inadequate infrastructure. A significant proportion of the rural communities lack pipe-borne water and as such, depend on streams, rivers and groundwater bodies which are often impacted by poorly or inadequately treated effluents from municipal wastewater treatment plants for drinking and domestic purposes (Fatoki *et al*., 2003; Okoh *et al*., 2007). This increases the risks of the public being exposed to biological hazards from sub-quality wastewater effluents.

Reports of waterborne disease outbreaks in South Africa in general and the Eastern Cape Province in particular, have been linked to inadequately treated wastewater (Anderson and Strenstroma, 1987; Yao, 1989; Bosch *et al.,* 1991; Momba *et al.,* 2006). Whittington-

Jones (2005) reported that over 80% of treated effluents in the Eastern Cape Province do not meet the minimum legal discharge standards. This necessitates the need to evaluate the working efficiencies of the wastewater treatment plants prior to the discharge of their effluents into the receiving watersheds (Odjadjare, 2010). Although some studies have previously been done in this regard (Momba and Mfenyana, 2005; Momba *et al.,* 2006; Igbinosa, *et al.,* 2009), those studies have been restricted to just few wastewater treatment plants in Amathole District Municipality and the Eastern Cape Province as a whole. In addition to this, there is paucity of information on the overall efficiency of the selected wastewater treatment plants.

In view of this, the current study was therefore designed to assess the prevalence of faecal coliforms and enterohemorrhagic *Escherichia coli* O157:H7 in the final effluents of the two selected wastewater treatment plants in Amathole District Municipality in the Eastern Cape Province of South Africa.

1.3 Hypothesis

The working hypothesis set for this study was that the wastewater treatment plants under study produce final effluents of unacceptable physicochemical and microbiological qualities.

1.4 Research questions

In order to prove the set hypothesis, a number of research questions were formulated as follows:

1.4.1 To what extent do the wastewater treatment plants under study comply with set guidelines for final effluent qualities?

1.4.2 In what way(s) could the treated effluent discharge from the wastewater treatment plants affect the physicochemical qualities of the receiving watersheds?

1.4.3 What impact could the discharged effluents from the treatment plants have on the microbiological quality of the receiving watersheds in terms of the prevalence and distribution of faecal indicator bacteria (faecal coliforms) and pathogenic *E. coli* (*E. coli* O157:H7)?

1.4.4 What is the statistical correlation among the assessed characteristics of the final effluents produced by these wastewater treatment plants?

A set of aim and objectives were then formulated as shown below in order to answer these research questions.

1.5 Aim and objectives

The main aim of this study was to assess the working efficiency of the two selected wastewater treatment plants in Amathole District Municipality of the Eastern Cape Province of South Africa by assessing the physicochemical and microbiological (including faecal indicator bacteria and pathogenic *E. coli* O157:H7 strains) qualities of their final effluents. The **specific objectives** were set out as follows:

1. To assess the physicochemical qualities of the final effluents of the wastewater treatment plants.

2. To assess the occurrence of faecal coliforms and pathogenic *Escherichia coli* O157:H7 strains in the final effluents.

3. To determine the statistical correlation relationship among the assessed physicochemical and microbiological characteristics.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Clean, safe and readily available water is critical to the survival of human and other life forms. While safe water remains a critical resource world over, South Africa, being a country located in a semi-arid part of the world faces some challenges in preserving and conserving this scarce and limited resource. Compounding these challenges are the concerns over the environmental, health and economic implications of water pollution (Basson *et al*, 1997; Osode and Okoh, 2010). Water pollution arises from many sources, and occurs when pollutants or contaminants are discharged into water bodies without adequate treatment to remove harmful substances. Of major importance is the concern over pollution from untreated or inadequately treated municipal wastewater (sewage) effluents (Owili, 2003).

Although South Africa has a vibrant water industry with a track record of innovations. For instance, the WHO/UNICEF Joint Monitoring Programme (2008) reported that the country reached universal access to an improved source in urban areas, and in rural areas the share of those with access increased from 66% to 78% from 1990 to 2008. However, the concerns associated with discharged wastewater effluents qualities and its attendant problems remain a major challenge because much less progress has been achieved on sanitation. Contrary to the improvement in drinking water access; sanitation as access increased only from 55% to 59% during the same period (World Health Organisation/United Nations Children's Fund, 2008).

South Africa is one of the few countries in the world that enshrines the basic right to sufficient water in its Constitution, stating that "Everyone has the right to have access to

sufficient food and water" (Constitution of South Africa, 1996 Chapter 2, Section 27b). And, though the South Africa government made it mandatory through the South African Water Act (Act 54 of 1965) that effluent be treated to acceptable standards and returned to the water course where water was originally obtained, much remains to be done to fulfil that right (Morrison *et al*., 2001; Mema, 2009). Significant problems remain concerning the financial sustainability of service providers, leading to a lack of attention to maintenance. The uncertainty about the government's ability to sustain current funding levels in the sector is also a concern.

According to a WASH new Africa report in 2010, "*Many of South Africa's municipal wastewater treatment plants are not performing to acceptable water quality standards and there are several issues surrounding the performance of these plants. Contributing to the challenges experienced by municipalities is a lack of skills for the operation of facilities and a lack of infrastructures investment over the past decades. A lack of good-quality drinking water leads to health problems, which is serious, given the fact that many poor citizens source water directly from the rivers, where not only municipalities, but also industrial water users, discharge polluted water. Since South Africa does not have large rivers, the discharged effluents concentrate into small watercourses*" (WASH news Africa, 16 July, 2010).

2.2 Wastewater

In South Africa and other developing countries, many communities still depend on untreated surface water and ground-water sources for their daily water needs. Water from these sources is often contaminated by faecal pollution from wastewater effluents (Toze, 2004). Wastewater is a matrix consisting of raw sewage and primary, secondary and tertiary

treatment effluents. It primarily comprises of water in which solids exist as settleable particles, dispersed as colloids, which are materials that do not settle readily, or solids in a dissolved state (Mara and Horan, 2003; Omar and Barnard, 2010). Although wastewater and sewage are often used interchangeably; wastewater is any water that has been affected in quality by several anthropogenic influences, and includes domestic, industrial, agricultural and municipal liquid effluents. Sewage on the other hand is a subset of wastewater contaminated mainly with biological waste such as faeces, urine, food remnants and laundry waste (Norzatulakma, 2010; Darvishi and Farahani, 2011).

Municipal wastewater is mainly comprised of water together with relatively small concentrations of suspended and dissolved organic and inorganic solids (FAO, 1992), pathogens, nutrients, minerals and sediments which are all oxygen demanding (Sonune and Ghate, 2004). Among the organic substances present in sewage are carbohydrates, lignin, fats, soaps, synthetic detergents, proteins and their decomposition products, as well as various natural and synthetic organic chemicals from the process industries (Awad, 2011).

Wastewater quality is often described in terms of its physicochemical and/or microbiological characteristics. The qualitative characteristics of the wastewater generated by non-residential settlements can vary significantly between different types of settlements due to the extreme variation which can exist in the waste generating processes. Some of the physicochemical characteristics of wastewater often used in describing wastewater quality include temperature, colour, odour, suspended solids, turbidity, total dissolved solids, pH, dissolved oxygen (DO), biological oxygen demand (BOD), nutrients and toxic substances like metals and chlorides (Drinan and Whiting, 2001). Industries remain the major sources of pollution in all environments. Based on the type of industry, various levels of pollutants can be discharged into the environment directly or indirectly through public sewer lines (Kanu and Achi, 2011).

High levels of pollutants in river water systems cause an increase in biological oxygen demand BOD, COD, total dissolved solids (TDS), total suspended solids (TSS), toxic metals such as Cd, Cr, Ni and Pb and faecal coliforms. Such water thus becomes unsuitable for drinking, irrigation and aquatic life. Industrial wastewaters are characterised by high BOD from biodegradable wastes such as those from human sewage, pulp and paper industries, slaughter houses, tanneries and chemical industries. Others include those from plating shops and textiles, which may be toxic and require on-site physiochemical pre-treatment before discharge into municipal sewage system (Emongor *et al.*, 2005; Phiri *et al.*, 2005; Otokunefor and ObiUkwu, 2005; Kanu *et al.*, 2006).

2.2.1 History of collection and treatment of wastewater

Although the collection of wastewater dates back to ancient times, its treatment is a relatively modern practice dating from the late 1800s and early 1900s (Chow *et al*., 1972, Okoh *et al*., 2007). In the earlier human settlements, used waters (wastewater) were discharged directly into the environment, particularly, bodies of water. It was not until the late 19th century that large cities began to realise that they had to reduce the amount of polluting contaminants they were releasing into the environment. Populations had become so concentrated by 1850 that outbreaks of life-threatening diseases were traced to bacteria in the polluted water (Swedlund and Donta, 2003). Since that time, the practice of wastewater collection and treatment has been developed and perfected, using some of the most technically sound biological, physical, chemical, and mechanical techniques available (Denton Public Works, 2012).

Currently, there is a growing awareness of the impact of wastewater contamination on rivers, lakes and other water bodies. Wastewater treatment is now receiving greater attention

from the several international organisations and governmental regulatory agencies around the world. Resultantly, public health and water quality are better protected today than ever before (Volkman, 2003; Denton Public Works, 2012).

Wastewater treatment practices vary from country to country across the globe, but its main objective remains the same; removing contaminants from wastewater before discharging the water back into the environment.

2.2.2 Some important physicochemical parameters of wastewater effluents quality and their discharge limits

Discharged effluent quality guidelines are important because they help to protect human health and enhance the quality of the surface waters. Effluent standards guidelines help to identify water quality problems caused by, for example, improperly treated wastewater discharges, runoff or discharges from active or abandoned mining sites among others. Standard guidelines also support efforts to achieve and maintain protective water quality conditions (US EPA, 2012b). Effluent disposal standards have played a key role in controlling pollution discharges since the early 1970s in developed countries. Strict regulations of wastewater disposal have led some countries to a stage with only limited conventional water pollution problems. Developing countries, however, are under a constant and significant pressure to follow the international trends of frequently lowering pollutant concentration limits, while being unable to reverse the continuous trend of environmental degradation (Jining and Junying, 2011).

Several international, regional and governmental regulatory agencies are often involved in setting effluent standard guidelines or acceptable limits of contaminants permissible in discharged final effluents. Such regulatory agencies may include the WHO,

Food and Agricultural Organisation of the United Nations (FAO), the European Union (EU), the United State Environmental Protection Agency (US EPA) among others. In South Africa, the Department of Water and Forestry Affair (DWAF) is vested with both the responsibilities of setting and ensuring the standard guidelines for discharged effluents of municipal wastewater treatment plants in line with the National Water Act, 1998 (Act 36 of 1998) (DWAF, 2002; DWAF, 2003). The following sections give a brief description of some of the important physicochemical characteristics of wastewater discharged effluents and their recommended discharged limits. Table 2.1 below shows the different categories of wastewater constituents.

Table 2.1: Categories (strong, medium and weak) of domestic wastewater

Constituent	**Concentration (mg/L)**		
	Strong	**Medium**	**Weak**
Total solids	1200	700	350
Dissolved solids (TDS)	850	500	250
Suspended solids	350	200	100
Nitrogen (as N)	85	40	20
Phosphorus (as P)	20	10	6
Chloride	100	50	30
Alkalinity (as $CaCO_3$)	200	100	50
Grease	150	100	50
BOD_5	300	200	100

Source: UN Department of Technical Cooperation for Development (1985).

2.2.2.1 Turbidity

Turbidity is a principal physical characteristic of water and wastewater; and is an expression of the optical property that causes light to be scattered and absorbed by particles and molecules rather than transmitted in straight lines through a water or wastewater sample. It is caused by suspended matter or impurities that interfere with the clarity of the water. These impurities may include clay, silt, finely divided inorganic and organic matter, soluble coloured organic compounds, and plankton and other microscopic organisms (US EPA, 1999a; DWAF, 2002). A major implication of excessive turbidity in water is reduction in light penetration which may result in the decline of the rate of photosynthesis by the aquatic plants, and this may lead to less food being available for the aquatic animals (Palmer *et al.*, 2004). Also, high turbidity increases water temperatures because suspended particles absorb more heat. This, in turn, reduces the concentration of dissolved oxygen (DO) because warm water holds less DO than cold (US EPA, 2012c).

Turbidity is an important operational parameter in process control and can indicate problems with treatment processes, particularly coagulation or sedimentation and filtration (WHO, 2004). It is generally measured in nephelometric turbidity units (NTU) using turbidity meter (US EPA, 2012c). Turbidity in water directly correlates with the microbial load within water resources. Excessive turbidity can affect the effectiveness of chlorination during disinfection in wastewater treatment, resulting in the failure of the removal of microorganisms (Fatoki *et al.*, 2001; Obi *et al.*, 2007). The ineffectiveness of chlorination may also increase the chances of trihalomethanes (THM) precursor forming in the wastewater effluent (Fatoki *et al.*, 2001). THM is a carcinogenic compound that is formed as a by-product from chlorine and organic matter reaction, which may result in serious health implications for the aquatic life and humans exposed to it (Odjadjare and Okoh, 2009).

Although the Department of Water and Forestry (DWAF) has no specific limit for turbidity in the South African Guideline for General and Special Standards Requirements for the Purification of Wastewater or Effluent (Gazette No. 9225, Regulation No 991, 18 May 1984; Osode, 2010), the South African Target Water Quality Range for turbidity in water for domestic water supply is 0 to 1 NTU (DWAF, 1996) while the WHO standard is 5 NTU (WHO, 2004).

2.2.2.2 Nutrient load

One important characteristic of wastewater treatment plant effluents that often impacts receiving waters is its nutrient content. Excessive nutrient loading (considering nitrogen and phosphorus) is a major on-going threat to freshwater quality worldwide and particularly in South Africa that has been described as a water-scarce country (Carey and Migliaccio, 2009; Muller *et al.,* 2009). Many aquatic systems have very low ambient concentration and small shifts in the nutrient load can result in dramatic changes in the aquatic community structure (Miltner and Rankin 1998; Dodds and Welch, 2000; Rabalais, 2002). Many researchers investigating nutrient pollution from nonpoint sources have severally discovered that nutrient loads were often more strongly influenced by discharged effluent from wastewater treatment plants than the nonpoint source (Ahearn *et al.,* 2005; Popova *et al.,* 2006; Migliaccio *et al.,* 2007).

One major implication of excessive release of nutrient into freshwater bodies is eutrophication which was defined by the South African Department of Water Affairs and Forestry as the process of excessive nutrient enrichment of water which typically results in undesirable changes such as the stimulation of macrophyte (plants), algal or cyanobacterial growth, and other problems associated with the deterioration of water quality, thereby

interfering with water uses (DWAF, 2009). Uncontrolled inputs of phosphorus and/or nitrogen to aquatic environments leads to increased rates of eutrophication with the attendant problems.

Nitrogen in wastewater derives from breakdown products of proteins found in urine and faeces. These products are very soluble and often pass through sewage treatment process, and are subsequently discharged into rivers as a component of sewage treatment effluent. Nitrogen may exist in form of nitrate, nitrite, ammonia or ammonium salts in wastewater. All these forms of nitrogen can be used by macrophytes and algae thereby leading to increased rates of eutrophication. The differing forms of nitrogen are relatively stable in most river systems with nitrite slowly transforming into nitrate in well oxygenated rivers and ammonia transforming into nitrite and/or nitrate (Florescu, *et al.,* 2011). Studies have suggested that nitrate and nitrite ions act on many systems and could serve as direct precursors for the production of nitric oxide, a potent physiological regulator in vertebrates (Avery, 1999).

Nitrate and nitrite have been reported to be toxic in humans and animals for decades (Avery, 1999; Guillette and Edwards, 2005). As early as 1945, methemoglobinemia (Blue Baby syndrome) was associated with drinking nitrate-contaminated well water on farms from the Midwest USA. Methemoglobinemia is formed during nitrate-induced oxidation of haemoglobin. This prevents normal oxygen binding and leads to hypoxia. Methemoglobinemia, as well as additional concerns, continue today with increasing nitrate contamination of water bodies, ammonium ions also have been reported to have toxic effects on fish (Avery, 1999; Porter *et al*., 1999; Guillette and Edwards, 2005).

Uncontrolled discharge of phosphorus can also encourage excessive growths of aquatic plants and algae and thus contribute to eutrophication. Orthophosphates are the sole form of soluble inorganic phosphorus which can be directly utilised by the aquatic organisms,

mainly algae and other plants. Together with nitrates and nitrites, orthophosphates are found in high levels in discharged effluents of wastewater treatment plants and are important growth limiting factors in eutrophication, which result in undesirable ecological effects within the receiving water resource (DWAF, 1996a; Morrison *et al*., 2001).

The Department of Water Affairs and Forestry set the general limit for nitrate and nitrite (as nitrogen) in discharged wastewater effluent resource at 15 mg/L while the value was set at 1.5 mg/L was set as a special limit (DWAF, 2004). However, according to the USEPA's Ambient Water Quality Criteria for the Protection of Human Health, the maximum concentration of nitrate in discharged effluent is set at 10mg/L for non-cancer effects (USEPA, 2012e). DWAF set the general standard limits for Ortho-phosphate as phosphorus at 10 mg/L and a special limit of 1 mg/L (for median) and 2.5 mg/L (for maximum)(DWAF, 2004).

2.2.2.3 Dissolved oxygen, biochemical oxygen demand and chemical oxygen demand

Dissolved oxygen (DO) is very critical for the survival and reproduction of fish and other aerobic aquatic organism that cannot obtain oxygen directly from the atmosphere. DO in water ultimately comes from the atmosphere and photosynthesis by aquatic plants, and it is typically measured in and reported as milligram per litre (mg/L) or percent saturation (that is, the amount of oxygen the water holds compared to what it could absorb at that temperature) . DO is an important parameter used in water quality control. The amount of oxygen that can dissolve in water depends on several factors which include salinity, and atmospheric pressure among others. The effect of waste discharge on surface water sources is another factor that largely affects oxygen balance in water systems (DWAF, 1996; Wilson, 2010; United States Geological Survey, 2012a).

An indication of the organic oxygen demand content of wastewater can be obtained by measuring the amount of oxygen required for its stabilisation either as BOD or COD. Whilst BOD measurements determine the amount of oxygen required by microorganisms in breaking down organic matter, COD determination measures the amount of oxygen required for chemical decomposition of organic and inorganic contaminants, dissolved or suspended in water (Salem *et al.,* 2011). A general yardstick of evaluating the performance of sewage treatment plants is the degree of reduction of BOD (often determined as 5-day, 20^{o}C BOD) and the performance efficiency of treatment plant depends not only on proper design and construction but also on good operation and maintenance (Sundara Kumar *et al*., 2010).

The determination of BOD and COD is useful in evaluating the compliance of effluents with water quality requirements standards and also in the estimation of the potential of organic waste present in such effluent to deplete oxygen (DWAF, 1996a; Mazibuko, 2012). Since BOD measures the amount of oxygen required by bacteria to break down the organic matter present in the water, the greater the biodegradable organic matter present in water (effluent), the greater the BOD values (Akinlua and Asubiojo, 2006; Akan *et al*., 2008). Continuous discharge of effluent with high BOD into freshwater systems can have negative consequences on such water systems and may cause harm to the aquatic life of such water systems. (Morrison *et al*., 2001; Akinlua and Asubiojo, 2006).

High levels of COD in a water resource on the other hand may lead to drastic oxygen depletion, which may also greatly affect the aquatic biota (Fatoki *et al*., 2003; Mazibuko, 2012). COD measurements do not differentiate between biologically available and inert organic matter, and always have values greater than BOD values, though COD determination can be done in a few hours while BOD determination takes five days (Sundara Kumar *et al*., 2010).

Although the South African Government Gazette No. 20526 of 8 October 1999 did not specify a limit for the amount of DO in effluent, a previous Government Gazette of 18 May 1984 No. 9225 recommended a 75% saturation level of DO in discharged effluents. (Government Gazette, 1984; DWAF, 2004). According to WHO (2006), the minimum allowed limit of DO for the sustenance of aquatic life is 5 mg/L while the DO in water for drinking purposes is 6 mg/L (Rao, 2005; WHO, 2006; Igbinosa and Okoh, 2009). There is also no South African guideline for BOD in discharged effluent. However, for the protection of fisheries and the aquatic life, the EU guidelines stipulate the BOD target limits of 3.0 to 6.0 mg/L (Chapman, 1996; Momba *et al.*, 2006). In the United States regulations, secondary sewage treatment is generally expected to remove 85% of the BOD measured in sewage producing effluent BOD concentrations with a 30-day average of less than 30 mg/L and a 7-day average of less than 45 mg/L (US EPA, 2007).

The South African Department of Water and Forestry recommends a general limit of COD not exceeding 75 mg/L after algal removal in discharged effluent and a special COD limit not exceeding 30 mg/L (DWAF, 2004) while the World Health Organisation recommends a COD level not exceeding 1000 mg/L in discharged effluent (Akan *et al.*, 2008).

2.2.2.4 Temperature, pH and electrical conductivity

Temperature is an important physicochemical parameter of water bodies which not only affects the abundance, availability and distribution of aquatic organism but also influences their activities such as breathing, growth and reproduction. Studies have shown that temperature in water systems is inversely related to DO; which decreases with higher temperatures and vice versa. It has also been established that at 0^{o}C, DO is 14.62 mg/L; 11.33

mg/L at 10^{o}C; 9.17mg/L at 20^{o}C and 7.3 mg/L at 30^{o}C (Onuoha and Nwadukwe, 1989; Francis *et al.*, 2007).

Temperature is also important because it generally affects the rates of chemical reactions in water bodies. Water with higher temperature can dissolve more minerals from the rocks and will therefore have higher electrical conductivity. Some compounds are also more toxic to aquatic life at higher temperature (USGS, 2011).

A general standard maximum limit of 35^{o}C and special standard maximum limit of 25^{o}C discharged wastewater effluent were recommended by DWAF (Government Gazette, 1984). pH is a measurement of the relative amount of free hydrogen and hydroxyl ions in water or wastewater. Normally, pH is a routine measurement in water quality assessment and since it can be affected by chemicals in water, it is an important indicator of water that is changing chemically (FAO, 1992; USGS, 2012). Low pH values in rivers adversely affect aquatic life and impair recreational use of water while high pH could also alter toxicity of water pollutants in water body. Low pH can increases the solubility of many other elements such as Al, B, Cu, Cd, Hg, Mn and Fe (DWAF, 1996a; Akinlua and Asubiojo, 2006).

According to Chapman (1996), the European Union sets the pH protection limit for fisheries and other aquatic lives at 6.0 to 9.0 while the WHO has pH standard limits of 7.0 to 8.5 and 6.5 to 8.5 for drinking water and water meant for full contact recreational purposes respectively (WHO, 1984; WHO, 1989; Chapman, 1996; DWAF, 1996b; Igbinosa and Okoh, 2009). DWAF's general limit for pH in discharged effluent is between 5.5- 95 while the special limit is set at 5.5 – 7.5 (DWAF, 2004). While pH is an indicator of the acidity or basicity of water/ wastewater but seldom a problem itself; electrical conductivity on the other hand is a measure of the ability of water to conduct an electric current (FAO, 1992; US EPA, 2012b).

Electrical conductivity of water is a useful and easy indicator of its salinity. Wastewater effluents often contain high amounts of dissolved salts from domestic sewage. High salt concentrations in waste effluents increase salinity of the receiving water, resulting in adverse ecological effects on aquatic biota (Fried, 1991). Also, a very high salt concentration (> 1 000 mg/L) imparts a brackish, salty taste to water and is discouraged because of the potential health hazard (WHO, 1979). For this reason electrical conductivity can serve as a useful salinity indicator when considered with other factors (Morrison *et al.*, 2001).

Conductivity is also affected by temperature. Warmer water tends to have higher conductivity than cold water. For this reason, conductivity is reported as conductivity at 25 degrees Celsius (25 C). Conductivity is measured in microohms per centimetre (µmhos/cm) or microsiemens per centimetre (µs/cm) and can be measured in the field or the lab (US EPA, 2012b). DWAF recommended a general limit of 70 mS/m above intake to a maximum of 150 mS/m and special limit of 50 mS/m above background receiving water, to a maximum of 100 mS/m for electrical conductivity in effluent (DWAF, 2004).

2.2.3 Microbiological indicators of wastewater quality

Microbial indicators are often used as basic tools in water and wastewater quality assessment to detect and estimate faecal contamination levels in water sources. Although these bacterial indicators are not disease-causing themselves, their presence in water is often correlated to the possible presence of enteric viral and bacterial pathogens, and is used in the estimation of probable human health risk upon exposure to such contaminated water (Myers and Sylvester, 1997).

Microbial indicators are used in water quality assessment because it is usually an awfully hectic task to attempt enumerating/identify all pathogen that may be present in water.

To save time and resources, indicators are used based on their perceived correlation to the presence of pathogens. The bacteria species chosen as indicators are indigenous to the intestines of warm-blooded animals and indicate the potential presence of dangerous pathogens that can cause human illnesses (US EPA, 2012d).

According to the United States Environmental Protection Agency (2012d) and Prescott *et al.* (2008); the under listed criteria are necessary for an organism to be a suitable indicator of faecal contamination:

i. The organism should be easily detected using simple laboratory tests.

ii. It should be suitable for the analysis of all types of water and generally not present in unpolluted water.

iii. The organism should have a longer survival time than the hardiest enteric pathogen of concern.

iv. The organism should be found wherever enteric pathogens are found (e.g., in the gut of warm-blooded animals).

v. It should not reproduce in the contaminated water and produce an inflated value.

vi. The detection level of the indicator organism in contaminated water should have some direct correlation to the degree of faecal contamination.

Indicator bacteria including total coliforms (TC), faecal coliform (FC), enterococci, faecal streptococci (FS) and *E. coli* have traditionally been used for the purpose of microbial water quality monitoring (Myers and Sylvester, 1997; Ashbolt *et al*, 2001). However, the term ‘microbial indicator’ has been described as ambiguous by some authors. For instance, Grabow (1996) stated that there are myriads of possible reasons why indicators might be

present in a water sample while pathogens may be absent and vice versa. It has also been observed that there is no direct correlation between the number of any indicator and enteric pathogens (Grabow, 1996; Ashbolt *et al*, 2001).

In order to eliminate the ambiguity mentioned above, Ashbolt *et al*, (2001) classifies microbial indicator into three groups as follows:

a. General (process) microbial indicators,
b. Faecal indicators (such as *E. coli*)
c. Index organisms and model organisms.

Table 2.2: A description of microbial indicator classes.

Group	**Description**
Process indicator	A group of microorganisms that shows the efficiency of a process, such as total heterotrophic bacteria or total coliforms for chlorine disinfection.
Faecal indicator	A group of bacteria that indicates the presence of faecal contamination, such as the bacterial groups' thermotolerant coliforms or *E. coli*. Their presence may only infer the possible presence of pathogens.
Index and model organisms	**A group or species indicative of pathogen presence and behavior respectively, such as *E. coli* as an index for *Salmonella* and F-RNA coliphages as model of human enteric viruses.**

Source: Ashbolt *et al*, (2001).

2.2.3.1 The coliform group

Coliform bacteria otherwise described as total coliforms belong to the family *Enterobacteriaceae.* They are often found in the gut of humans and other warm-blooded animal, and have been widely used as indicator organisms. Coliforms are Gram-negative, facultative anaerobic, non-sporing bacilli that ferment lactose at 35-37^{o}C within 48 hours

with the production of acid and gas (APHA, 1995; Stevens *et al.,* 2003; Prescott, 2008).Other facultative anaerobic bacteria that form red colonies with metallic sheen within 24 hours of incubation at 35^{o}C on Endo-type lactose containing medium, as well as all bacteria that are able to cleave chromogenic containing medium with the aid of β-galactosidase enzyme (e.g., *ortho*-nitrophenyl-β-D-galactopyranoside or ONPG), are also included as coliforms (Leclerc *et al.*,2001; Health Canada, 2006a; Health Canada, 2011).

Total coliforms were originally described to consist of four genera including *Escherichia, Klebsiella, Enterobacter and Citrobacter;* all capable of lactose fermentation. Other genera included in the coliform group, but less common than these four genera are: *Budvicia, Erwinia, Leclercia* and *Serratia*. *Escherichia coli* is the most prominent coliform found in the gut of human (Health Canada, 2006a; Health Canada, 2011).

Unfortunately, TC includes a wide range of bacteria that may not necessarily originate from the intestinal tract of warm-blooded animals. These bacteria can be considered normal inhabitants of many natural environments including soil and aquatic environments which have not been impacted by faecal pollution (Leclerc *et al.,* 2001). And, although the presence of *E. coli* is considered an appropriate and specific indicator of faecal contamination, questions have often been raised in some quarters about the use of TC as health risk indicators in water and wastewater quality management (Health Canada, 2006a; Prescott, 2008). To address some of these questions, specific tests have been developed that allow water to be tested directly for the presence of FC also known as thermo-tolerant coliforms. These are coliforms specifically originating from the intestine of warm blooded animals, and grow at the more restrictive temperature of 44.5^{o}C (Prescott, 2008). Table 2.5 below give a list of some *Enterobacteriaceae* family and their origins.

Table 2.3: Some common coliforms in the *Enterobacteriaceae* family

Genera	Species	ONPG	Faecal origin	Non-faecal origin
Escherichia	*E. coli*	+	+	-
Klebsiella	*K. pneumonia*	+	+	+
Enterobacter	*E. amnigenus*	+	+	+
Citrobacter	*C. freundii*	+	+	+
Budvicia	*B. aquatic*	+	-	+
Erwinia	*E. amylovora*	+	-	+
Leclercia	*L. adecarboxylata*	+	-	+
Serratia	*S. liquefaciens*	+	-	+

Sources: (Leclerc *et al.*, 2001; Health Canada, 2006a; Health Canada, 2011)

2.2.3.2 *Escherichia coli* pathotypes

Escherichia coli (*E. coli*) is a Gram–negative, facultative anaerobic, rod (bacillus) bacterium that has long been known for its close association with the human digestive tract. *E. coli* was first discovered by Theodor Escherich (a German paediatrician) who called it *Bacterium coli commune* due to the fact that is was found in the colon and faeces of human. It was subsequently renamed *Escherichia coli* after the original discoverer (Castellani and Chalmers, 1919). Most strains of *E. coli* are non-pathogenic and are often considered part of normal flora of the human digestive tract where they are beneficial to the host by preventing the colonisation of the colon by other pathogenic bacteria and also producing some useful vitamin (e.g., vitamin K) which is useful to the host (Vogt and Dippold, 2005; CDC, 2012).

E. coli is the only member of the *Enterobacteriaceae* family that does not occur in the natural environment such as in water, soil or on vegetation. It is exclusively found in the gut of human and other warm-blooded animals; hence its presence in water samples is usually taken as an indication of faecal contamination of such water. *E. coli* has been estimated to be

present at a density of about 10^9 per gram of human and animal faeces; and comprises approximately 1% total biomass of human large intestine (Edberg *et al.,* 2000; Leclerc *et al.,* 2001; Health Canada, 2006b)

Although most *E. coli* strains are non-pathogenic members of the normal intestinal flora (commensal bacteria), some highly adapted strains of the bacteria cause diarrheal disease and other gastrointestinal illness along with other, more serious health problems (Vidal *et al.,*2005; Health Canada, 2006b; Prescott *et al.,* 2008). The pathogenic strains of *E. coli* that are capable of causing enteric infections are designated diarrheagenic *E. coli,* a group that includes emerging pathogen of public health importance worldwide (Nataro and Kaper, 1998; Vidal *et al.,* 2005). Some of the well characterised virulence factors that help the organism in evading the host defence mechanisms include: P-fimbriae, type 1 fimbriae, haemolysin, aerobactin, and serum resistance. The O and K antigens also shield *E. coli* from antimicrobial effects of complement and help it to escape phagocytosis in the absence of specific antibodies (Weinstein *et al.,* 1988; Baldwin *et al.,* 1992; Blanco *et al.,* 1992).

Six categories of the diarrheagenic *E. coli* that possess different virulence factors such as exotoxins have been described. The specific virulence factors produced by these strains, together with the type of diseases they cause have been used to separate them into different pathotypes (Vidal *et al.,* 2005; Prescott *et al.,* 2008; CFSPH, 2009). These include enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAggEC) and diffusely adhering *E. coli* (DAEC) (Vidal *et al.,* 2005; Prescott *et al.,* 2008). Uropathogenic *E. coli* (UPEC) and Neonatal Meningitis *E. coli* (NMEC) are two other extraintestinal *E. coli* strains that have been characterised (Wiles *et al.,* 2008; Dubois *et al.,* 2009). These are able to cause infections in the urinary tract, bloodstream (bacteremia) and in the central nervous system (sepsis and meningitis) (Nataro and Kaper, 1998).

Pathogenic *E. coli* strains have often been classified into different pathotypes based on their virulence factors and the specific gene patterns they possess (Palaniappan *et al,* 2006). Enteroaggregative *E. coli* (EAEC or EAggEC) and diffusely adherent *E .coli* (DAEC) are two classes that have been identified among the entero-adherent strains of *E. coli.* A stacked-bricklike attachment of bacterial cells to the Hep-2 cells is a characteristic of such aggregative adherence formations (Nataro *et al,* 1994; Nataro and Kaper, 1998; Rajendran *et al.,* 2010). The 60 MDa plasmid often harbours the genetic characteristics responsible for the virulence factor in EAEC (Baudry *et al*., 1990). Some of the virulence factors responsible for the pathogenicity of these strains of *E. coli* include the aggregative adherence factor II (*aafII*), a heat stable toxin (*ast*), the traditional activator (*aggR*) and an antiaggregation protein (dispersin) encoded by the *app* gene (Czeczulin *et al*., 1999; Vidal *et al,* 2005; Muller *et al.*, 2007; Nataro *et al.*, 1994; Sheikh *et al.*, 2002; Rajendran *et al.*, 2010). EAEC predominantly colonises the intestinal mucosa of the colon, followed by secretion of enterotoxins and cytotoxins. EAEC is also known to induce shortening of the villi, haemorrhagic necrosis of the villous tips and a mild inflammatory response with oedema and mononuclear infiltration of the submucosa (Weintraub, 2007).

Enterotoxigenic *E. coli* (ETEC) strains have long been associated with traveller's diarrhea and porcine and bovine diarrhea worldwide (Bekal *et al.,* 2003). It causes watery diarrhoea which often lasts up to a week, but can be protracted. The presence of one or more heat stable (STI and STII) and labile (LTI and LTII) toxin gene characterises ETEC strains (Rajendran *et al.*, 2010). At the onset of infection, it establishes itself by adhering to the epithelium of the small intestine with the aid of one or more colonisation factor antigens (CFA), followed by the expression of one or more of the heat stable (ST) or heat labile (LT) enterotoxins (O'Sullivan *et al.,* 2006; CDC, 2012).

ETEC was first recognised as a cause of human diarrheal illness in the 1960s but have since emerged as a major bacterial cause of diarrhea among travellers and children in the developing world (CDC, 2012a). Differences among the toxins i.e. heat stable, STa (STI) and STb (STII) – encoded for on plasmid – and for the heat labile, LTI and LTII – encoded for on the chromosome produced by these strains have been used to classify them into distinct groups (O'Sullivan *et al.,* 2006).

DAEC is characterised by the presence of *daaE* gene, which is essential for the expression of F1845 fimbriae, a putative virulence factor that mediates adherence of this pathotype (Bilge et al., 1989, Vidal, *et al*, 2005).

The invasiveness property of EIEC is mediated by the presence of a 140-MDa virulence plasmid (pINv), which encodes a number of genes for invasion including *virF* , *ipaH, ipaL* genes, etc. (Vidal *et al*, 2005; Johnson and Nolan, 2009). The initial steps in EIEC infection comprise of colonisation of epithelial cells, bacterial multiplication, and spread to adjacent cell (Sansonetti *et al.*, 1991; Colonna, *et al.*, 1995). EPEC was the first group of *E. coli* strains recognised as pathogens and continue to be a leading cause of diarrhea among infants from developing countries worldwide (Nataro and Kaper, 1998). A plasmid-encoded, type IV bundle-forming pilus (BFP) and the presence of intimin (*eae*) genes are critical in its virulence. The major structural subunit of BFP is bundlin, a highly polymorphic protein encoded by *bfpA* of the EAF plasmid-borne *bfp* operon (Donnenberg and Whittam, 2001; Vidal *et al*, 2005; Rajendran *et al*, 2010). EPEC is also known to produce verotoxin by means of the VT1 and VT2 verotoxic genes (Kong *et al.*, 1999). Typical EPEC is characterised by the presence of both eae and *bfp* genes, while atypical EPEC possess the eae gene only (Rajendran *et al*, 2010).

Uropathogenic *E. coli* (UPEC) is one of the extraintestinal strains of pathogenic *E. coli,* and it is known to be a cause of urinary tract infections including both cystitis and pyelonephritis in humans, dogs, and cats (Wiles *et al.,* 2008; Mora *et al.,* 2011). This strain of pathogenic *E. coli* has evolved to produce several virulence factors which facilitate the survival of the bacteria within the harsh condition that exist in the host urinary tract. It elaborates adhesion organelle e.g. type 1 and P pili which afford the bacteria to bind and colonize the host cells and tissues within the urinary tract, while also expressing an iron-chelating factor such as siderophones, enabling it to deplete host iron stores (Wiles *et al.,* 2008).

Another factor thought to be involved in the pathogenicity of the UPEC is its ability to resist the complement-dependent bactericidal effect of serum. The presence of K antigens in this organism is associated with upper urinary tract infections, and antibody to the K antigen has been shown to afford some degree of protection in experimental infections by forming biofilms (Todar, 2007). Biofilm-producing bacteria are known to be recalcitrant to immune factors and antibiotic therapy, and are often responsible for chronic urinary tract infections (Ehrlich *et al.,* 2005).

Neonatal meningitis *E. coli* (NMEC) is an *E. coli* pathotype which causes extraintestinal infection (meningitis) in neonates (Mora *et al.,* 2011; Todar, 2007). Neonatal meningitis is estimated to affect one in every 2000 to 4000 infants. Many (about 80%) of these strains are known to synthesize K-1 capsular antigens. NMEC strains invade the blood stream of infants from through the nasopharynx or gastrointestinal tract (GI) and are carried to the meninges. The K-1 antigen which is a homopolymer of sialic acid is considered the major determinant of virulence among NMEC. It prevents phagocytosis, complement, and responses from the host's immunological mechanisms. K-1 may not be the only determinant

of virulence in these strains however. Siderophore production and endotoxin are also thought to be involved in its virulence (Todar, 2007).

2.2.4 Enterohemorrhagic *Escherichia coli*

Enterohemorrhagic *E. coli* (EHEC) strains are characterised by verotoxin production which have been linked to life-threatening diseases such as severe haemolytic-uremic syndrome (HUS) complication; an important cause of acute renal failure in children and morbidity and mortality in adult humans and thrombo-cytopenic purpura (Karmali, 1989). In the elderly, the case fatality rate for HUS can be as high as 50% (CFSPH, 2009). Verotoxin-producing *Escherichia coli* have been described by a number of names, including enterohemorrhagic *E. coli* (EHEC), shiga-like toxin-producing *E. coli* (STEC or SLTEC), hemolytic uremic syndrome–associated enterohemorrhagic *E. coli* (HUSEC) and verocytotoxin- or verotoxin-producing *E. coli* (VTEC) (Karch, *et al.*, 2005; CFSPH, 2009).

Several serological groups (serotypes) have been identified within the EHEC pathotype. These include members of O26, O48, O91, O98, O103, O104, O111, O113, O117, O118, O121, O128, OX_3, O159 and O145, and the highly virulent serotype O157:H7 (Donnenberg and Whittam, 2001; Bugarel, *et al.*, 2010; Debroy, *et al.*, 2011; Chattaway, *et al.*, 2011). Enterohemorrhagic *E. coli* O157:H7 (designated by its somatic, O, and flagellar, H, antigens) is the most important EHEC serotype in relation to public health (IFT, 1997). *E. coli* O157:H7 was first discovered in 1982 as a highly virulent human pathogen following two outbreaks of hemorrhagic colitis (Riley *et al.*, 1983; IFT, 1997). Other members of some non-O157 serotypes have frequently been involved in sporadic cases and outbreaks, and are increasingly recognised as causes of hemorrhagic colitis and HUS (Woodward, *et al.*, 2002; Hussein, 2007). Some of these non O-157 serotypes may be as significant in human disease

as EHEC O157:H7. For instance, EHEC O153 has been linked to a disease that resembles HUS in rabbits (Paton and Paton, 2002; CFSPH, 2009; WHO, 2013a).

The pathogenesis in enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 infections has been characterised in terms of virulence factors encoded on multiple pathogenicity islands. Several studies (Ceponis *et al.,* 2003; Jandu, *et al.,* 2009; Gareau *et al.,* 2011) in the past have shown that EHEC O157:H7 controls host cell signal transduction cascades, independent of toxins and rearrangement of the cytoskeleton. However, the virulence factors and mechanisms responsible for EHEC-mediated subversion of signal transduction are still being studied (Jandu *et al.,* 2009). EHEC O157:H7 strains produce one or both of two very important types of Shiga toxin (because of its similarity to the types of toxin produced by *Shigella dysenteriae*), designated stx1 and stx2 (Feng *et al*, 2001; Abu-Ali *et al*, 2010; Pierard *et al*, 2012). The production of stx2 has been associated with the increased risk of developing HUS (Karpman, *et al*., 2001; Gamage *et al*., 2003). The presence of stx_1 *and* stx_2 genes which encode the shiga toxin allow its production. Other virulent genes harboured by this serotype include *eaeA* (encodes intimin, a putative accessory virulent factor), *hlyA* (a plasmid encode enterohemolysin), *rfb*; which encodes O-antigens peculiar to O111 and O157 enterohemorrhagic *E. coli* strains (Paton and Paton, 2002; Rajendran *et al.*, 2010).

Two other putative virulence factors that have been described for *E. coli* O157:H7 serotype include: a serine protease (*EspP*) which is capable of cleaving human coagulation factor V and a bifunctional catalase peroxidase *Kat (*Pradel *et al.,* 2000). It should be noted that the experimental proof of the role of these factors in *E. coli* O157:H7 virulence is yet to be established (Pradel *et al.,* 2000)

Some of the symptoms of infections caused by *E. coli* O157:H7 include severe or acute hemorrhagic diarrhea which may in some cases advance to bloody diarrhea

(haemorrhagic colitis), and abdominal cramp (Havelaar, *et al*., 2003). Fever and vomiting may also occur and, nonhemorrhagic diarrhea has been reported in some cases also. Although most infected people recover within ten days, in a small proportion of patients; young children and the elderly in particular, the infection may progress to a life-threatening disease, such as haemolytic uraemic syndrome (HUS) (Rahal, *et al.*, 2012). HUS is a disease characterised by acute renal failure, haemolytic anaemia and thrombocytopenia. It is estimated that up to 10% of infected people with EHEC infection may develop HUS, with a case-fatality rate ranging from 3 to 5% (WHO, 2013a). Overall, HUS is the most common cause of acute renal failure in young children. It can cause neurological complications (such as seizure, stroke and coma) in 25% of HUS patients and chronic renal sequelae, usually mild, in around 50% of survivors (IFT 1997; WHO, 2013a). Insulin-dependent diabetes may also persist in HUS and a few cases of HUS may be recurrent (Siegler *et al.*, 1993).

2.2.4.1 Some outbreaks of hemorrhagic disease linked to EHEC O157:H7

Increasing cases of hemorrhagic outbreaks caused by enterohemorrhagic *E. coli* O157:H7 are recorded worldwide almost every year (Hofinger *et al.*, 1998; Scott *et al.*, 2005; Okeke, 2009). Many of these outbreaks have often been linked to the consumption of fruits and vegetables, most especially sprouts, spinach, lettuce, coleslaw and salads; that have come in contact with sewage or faeces from domestic or wild animals at some stages during the cultivation or handling processes (Sy, 2004). Faecal contamination of water and other food items, as well as cross-contamination during food preparation are other risk factor that can also cause infection (WHO, 2013a). While some outbreaks resulting from recreational activities in faecal contaminated surface waters have also been reported (WHO, 2011; WHO, 2013a).

The first recognised outbreak caused by E. coli O157:H7 was recorded in 1982 in Oregon (United State), with 26 cases, out of which 19 persons were hospitalised (Riley *et al*., 1983). The second case of outbreak was recorded about three months later in Michigan (United States), involving 21 cases out of which 14 persons were hospitalised (Riley *et al*., 1983). Undercooked hamburger from a particular fast food restaurant chain was implicated as the vehicle for the outbreaks, and *E. coli* O157:H7 was isolated from infected patients and frozen ground beef patty on both occasions (IFT, 1997). A retrospective study by the Centers for Disease Control and Prevention (CDC) indicated evidence of a rare sporadic infection prior to 1982 (Centers for Epidemiology and Animal Health, 1997). In the study *E. coli* O157:H7 was detected only once (isolated from a 50 year old California woman in 1975), out of over 3000 *E. coli* serotypes identified from 1973-1983(Riley *et al.,* 1983; Centers for Epidemiology and Animal Health, 1997).

Another outbreak of EHEC O157:H7 was recorded on a sugar plantation in Swaziland 1992 (Okeke, 2009). A team of physicians working on about 200 patients infected with bloody diarrhea and severe abdominal pain had difficulty in linking the outbreak of the dysentric illness to a common parasite or bacterial pathogens including *Shigella* spp. With the etiologic agent still unidentified after the second week of the outbreak, stool samples from the infected individuals were forwarded to a reference laboratory in South Africa, where a surprising discovery was made: "*Escherichia coli* O157:H7 had emerged in Africa!"(Isaacson *et al.,* 1993; Effler *et al.,* 2001). Before this discovery, an outbreak of *E. coli* O157:H7 had never been recorded in Africa as a whole or any other country of the developing world. However, *E. coli* O157:H7 had only once been previously isolated in Africa, from an elderly man undergoing surgery for lower gastrointestinal bleeding in Johannesburg in 1990 (Browning *et al.,* 1990; Effler, *et al.,* 2001). Subsequent occurrences of large scale outbreak

and increasing widespread distribution of infection cases has led to the designation or enterohemorrhagic *E. coli* O157:H7 as a new emerging pathogen (Effler, *et al.*, 2001)

The United States experienced another outbreak of *E. coli* O157:H7 in 2006, involving bagged spinach with 205 cases of illness where 104 patients were hospitalised (INFOSAN Information Note No. 01/2007, 2007). Thirty one cases of kidney failure and 3 deaths were recorded (INFOSAN Information Note No. 01/2007, 2007). In 1996, Sakai city (Japan) recorded a large outbreak of *E. coli* O157:H7 involving a total number of 6309 school children and 92 school staff members from about 62 municipality elementary schools. By the month of July in that year, 101 cases of haemolytic uremic syndrome were recorded and the number of hospitalised persons increased to 534 with 31 deaths cases (WHO, 2013b).

2.3 Wastewater treatment

Wastewater collected from municipalites and communities must ultimately be returned to receiving water or to the land or reuse. A very important question from the point of view of a public health official and a design engineer is "*What level of treatment must be acheived in a given application-beyond those prescibed by discharge permits-to ensure protection of the public health and the environment?*" The answer to this question requires detailed analysis of local conditions and needs, application of scientific knowledge and engineering judgemnet based on past experience and consideration of national, provincial and local regulations (Tchobanoglous *et al*, 2002).

The principal objective of wastewater treatment is generally to allow human and industrial effluents to be disposed of without danger to human health or unacceptable damage to the natural environment (FAO, 1992). Wastewater treatment often involves the collection

of large volumes of wastewater in a treatment plant (often called wastewater treatment plant; WWTP) and subjecting the collected wastewater to a series of physical, chemical and biological processes to remove as much as possible the various organic and inorganic contaminants contained in the water. A wastewater treatment plant (WWTP) can be described as an installation of several mechanical and electrical devices that use a combination of physical/mechanical, chemical and biological processes in treating sewage and other forms of wastewater, in order to remove or reduce physical, chemical and biological contaminants from wastewater (FAO, 1992, USEPA, 2004).

Wastewater treatment often produces a safe waste stream (treated effluent) which is often discharged back into receiving bodies of water and a semi-solid waste (treated sludge) for disposal or reuse (usually as farm fertilizer or in land-fills) (PUB, 2011). More recently, using advanced technology, it is now possible to reuse wastewater or sewage effluent for drinking water, although Singapore is the only country to implement such technology on a production scale in its production of NEWater (PUB, 2011), several other countries often use treated effluent for aquaculture and irrigation purposes.

2.3.1 Stages in wastewater treatment

The Conventional wastewater treatment consists of a combination of physical, chemical, and biological processes and operations to remove solids, organic matter and, sometimes, nutrients from wastewater. Generally, the terms used to describe different degrees of treatment, in order of increasing treatment level are: preliminary, primary, secondary, and tertiary and/or advanced wastewater treatment. Most municipal wastewater treatment facilities use primary and secondary levels of treatment, and some also use tertiary treatments, and the type and order of treatment may vary from one treatment plant to another.

In some countries, disinfection to remove pathogens sometimes follows the last treatment step (FAO, 1992; SDWF, 2012). A brief description of some of the stages involved in wastewater treatment processes are given below.

2.3.1.1 Preliminary treatment

The aim of preliminary treatment is to remove coarse and other large materials from raw wastewater in order to enhance the operation and maintenance of subsequent treatment units. Preliminary treatment operations typically include coarse screening, grit removal and, in some cases, comminution of large objects. The velocity of the water is maintained sufficiently high through the grit chambers so as to prevent the settling of most organic solids. Grit removal is not often included as a preliminary treatment step in most small wastewater treatment plants (FAO, 1992). Comminutors or barminutors are also sometimes adopted to supplement coarse screening and serve to reduce the size of large particles so that they will be removed in the form of sludge in subsequent treatment processes (FAO, 1992; US EPA, 2004).

Some treatment plants incorporate fat and grease removal by allowing the sewage to pass through a small tank where skimmers collect the fat floating on the surface. Air blowers in the base of the tank may also be used to help recover the fat as froth. Many plants, however, use primary clarifiers with mechanical surface skimmers for fat and grease removal (Lead *et al*, 2005). Flow measurement devices, often standing-wave flumes, are always included at the preliminary treatment stage (FAO, 1992).

2.3.1.2 Primary treatment (settlement)

After preliminary treatment the sewage flows into large round or rectangular tanks, commonly called pre-settling basins, primary sedimentation tanks or primary clarifiers (Water UK, 2006). At the primary treatment level, about 20 to 30% of the BOD that is present in the sewage is physically removed in particulate form (Prescott *et al*, 2008). In this treatment, the particulate materials are removed by screening, precipitation of small particulates or suspended materials, and settling in the basin or tanks. The resulting solid or semi-solid material is called sludge. The tanks are used to settle sludge while grease and oils rise to the surface and are skimmed off (often recovered for use in saponification) (Lead *et al*, 2005). Primary settling tanks are usually equipped with mechanically driven scrapers that continually drive the collected sludge towards a hopper in the base of the tank where it is pumped to sludge treatment facilities (USEPA, 2004). The liquid element (settled sewage) flows over a weir to the next stage of treatment (Tchobanoglous, 2003).

2.3.1.3 Secondary treatment

Naturally occurring bacteria in the receiving watercourse use organic material as a food source and need oxygen dissolved in the water to do this. Discharges of large quantities of organic matter (often present in primary effluents) will therefore result in oxygen in the water being rapidly used up with consequent harm to fish and other aquatic organisms (Water UK, 2006). Primary effluents are often subjected to secondary treatments to promote the biological transformation of dissolved organic matter to microbial biomass and carbon dioxide (Prescott, 2008).

Secondary (biological) treatment of wastewater takes place in fixed or suspended growth reactors using any or variants of activated sludge, biofiltration, rotating biological contactors, and constructed wetlands processes (USEPA, 1997). Under ideal conditions the microorganisms produced by this process will aggregate to form a settleable stable floc structure. About 90 to 95% of the BOD and many bacterial pathogens are removed by this process (Prescott, 2008). The various techniques used in the secondary treatment to biologically removed dissolved organic matter incorporate similar microbial activities (FAO, 1992; Prescott, 2008).

2.3.1.4 Tertiary treatment

In regions where the final effluents from the wastewater treatment plants are expected to be of the highest quality, the treated effluent from the secondary treatment stages are often subjected further to advanced or tertiary treatments sometimes referred to as 'polishing' (Water UK, 2006). These treatments usually involve improved removal of dissolved and suspended solids as well as the removal of nutrients such as nitrogen, phosphorus, heavy metals, etc. (FAO, 1992). More than 99% of all forms of contaminants can be removed through tertiary treatment processes; however, the related technologies are very expensive and require high level of technical know-how, steady energy supply, and chemical and specific equipment which may not be readily available (The World Bank Group, 2012). Tertiary treatment may include processes such as filtration, coagulation and flocculation (EPA Victoria, 2002).

2.3.1.5 Disinfection

The disinfection stage is the ultimate mechanism for the inactivation of pathogenic microorganisms that may persist in the treated effluent in order to prevent the spread of waterborne disease to downstream user of receiving water step in the treatment of wastewater before the final effluent is released back into the environment via streams, rivers and other water bodies or reused for agricultural purposes such as irrigation or aquacultures (USEPA, 1999b; EPA Victoria, 2002). Disinfection is an important bodies as well as protect the environment (US EPA, 1999b). Table 2.4 below shows stages of wastewater treatment.

Table 2.4: Summary of levels (stages) of wastewater treatment

Treatment level	Description
Preliminary	Involves removal of wastewater debris such as rags, sticks, floatables, grit, and grease that may cause maintenance or operational problems with the treatment operations, process, and ancillary systems
Primary	Separation of a portion of the suspended solids and organic matter from the wastewater
Advanced primary	Enhanced removal of suspended solids and organic matter from the wastewater. Typically accomplished by chemical addition or filtration
Secondary	Removal of biodegradable organic matter and suspended solids. Disinfection is also typically included in the definition of conventional secondary treatment
Secondary with nutrient removal	Removal of biodegradable organics, suspended solids, and nutrients (nitrogen, phosphorus, or both nitrogen and phosphorus)
Tertiary	Removal of residual suspended solids (after secondary treatment), usually by granular medium filtration or micro-screens. Disinfection is also typically a part of tertiary treatment. Nutrient removal is often included in this disinfection
Advanced	Removal of dissolved and suspended materials remaining after normal biological treatment when required for various water reuse applications

Source: Tchobanoglous *et al.*, 2002.

2.3.2 Types of wastewater treatment technologies

Descriptions of various technological options used in the treatment of wastewater are given below.

2.3.2.1 Activated sludge

In general, activated sludge systems encompass a combination mechanisms and processes that utilise dissolved oxygen and suspended growth of microorganisms to promote the development of biological flocs which substantially removes dissolved organic matter from sewage (Gibb *et al.*, 1993; USEPA, 2004). In this system, the settled wastewater is mixed with a blend of bacteria and other microorganisms known as activated sludge (the active biomass that is formed when organic matter is oxidised and degraded by microorganisms) and aerated by agitators or air blowers in large tanks (Water UK, 2006; Prescott, 2008). Aeration devices commonly used include submerged diffuser that release air and mechanical surface aerators that introduce air by agitating the mixed liquor surface (FAO, 1992).

Anaerobic activated sludge systems involve a horizontal flow of materials with recycling of sludge. The system can be designed with variation in mixing and the ratio of organic matter added to the active biomass to form a low-rate system (with lower nutrient input per unit of microbial biomass) or a high-rate system (with high nutrient per unit of microbial biomass) (Prescott, 2008). Several variations of the basic activated sludge process, such as conventional aeration, extended aeration and oxidation ditches, are in common use, but the principles are similar (FAO, 1992; USEPA, 1997).

2.3.2.2 Trickling filters

Trickling filters are beds of packed or fixed media such as stones, gravels, ceramic, plastic shapes, or wooden slats (Smith, 2003). The packed medium provides support for the growth of microorganisms to form microbial biofilms which degrade dissolved and suspended organic matter in wastewater (FAO, 1992; USEPA, 1997; Prescott, 2008). Pre-treated wastewater is sprayed intermittently, or sometimes continuously, over the top of media and trickles down to the bottom, where it is collected for further treatment. As the wastewater trickles down, microorganisms become attached to the media and multiply. Organic matter in the wastewater diffuses into the film, where it is metabolised, thus lowering the BOD of the sewage (FAO, 1992; Encyclopaedia Britannica, 2012).

Oxygen is normally supplied through the films by the natural flow of air either up or down through the spaces among the fixed media, depending on the relative temperatures of the wastewater and ambient air. Periodically, portions of the film 'slough off the media. The sloughed material is separated from the liquid in a secondary clarifier and discharged to sludge processing (FAO, 1992; Encyclopaedia Britannica, 2012). Many variations on the arrangement of structure, media and distribution systems are available (USEPA, 1997).

2.3.2.3 Oxidation pond (lagoons)

Oxidation ponds, also called lagoons or stabilisation ponds are large, shallow ponds designed to treat wastewater through the interaction of sunlight energy and microorganisms (such as bacteria and algae). They are a simple secondary treatment of sewage that provides settlement and further biological improvement of treated wastewater through storage in large man-made ponds or lagoons (Kurian and McCarney, 2010; Encyclopeadia Britannica Online,

2013). These oxidation ponds are highly aerobic allowing the growth of heterotrophic bacteria and algae. The heterotrophic bacteria degrade organic matter in the wastewater resulting in the production of cellular material and minerals (Sharma, 1989).

The production of these supports the growth of algae in the oxidation pond. Growth of algal populations allows further decomposition of the organic matter by producing oxygen. The production of this oxygen replenishes the oxygen used by the heterotrophic bacteria. Typically oxidation ponds need to be less than 10 feet deep in order to support the algal growth (Atlas, 1995; Ghangrekar, 2007; Encyclopeadia Britannica Online, 2013).

In addition, the use of oxidation ponds is largely restricted to warmer climate regions because they are strongly influenced by seasonal temperature changes (Kurian and McCarney, 2010). Mechanical aerators are sometimes installed to supply yet more oxygen, thereby reducing the required size of the pond. Sludge deposits in the pond must eventually be removed by dredging. Algae remaining in the pond effluent can be removed by filtration or by a combination of chemical treatment and settling (Curley, 2011). The benefits of treating sewage by pond systems offer, through a simple and low-cost technology, social and commercial benefits, from the waste raw materials (Atlas, 1995; Ghangrekar, 2007).

2.3.2.4 Biological nutrient removal (BNR)

Biological nutrient removal in wastewater involves the steps or processes that remove plant nutrients including nitrogen compounds (nitrate and nitrite) and phosphorus from the treated wastewater (Stevens, 2006). Nitrogen is often present in sewage as ammonia and nitrates. Ammonia is toxic to fish and other aquatic animals as it exerts high oxygen demand in receiving water in its conversion to nitrate. A two-step biological process known as

nitrification-denitrification can be used to remove nitrate from sewage (Philips *et al*, 2002). In this process, ammonia is first converted to nitrates by microorganisms; this is followed by the metabolisation of the nitrate by another species in microorganism forming nitrogen gas that escapes into the atmosphere. This process usually requires the construction of more aeration and settling tanks and significantly increases the cost of treatment (Washington State Department of Health, 2005).

Phosphorus, another important nutrient causing eutrophication can be removed biologically from sewage through a process called enhanced biological phosphorus removal (USEPA, 2004). In this process, specific bacteria, called polyphosphate accumulating organisms (PAOs), are selectively enriched and accumulate large quantities of phosphorus within their cells (up to 20% of their mass). When the biomass enriched in these bacteria is separated from the treated water, these biosolids have a high fertilizer value (Renneberg, 2008). Phosphorus removal can also be achieved by chemical precipitation, usually with salts of iron (e.g. ferric chloride), aluminium (e.g. alum), or lime. Phosphorus in wastewater is usually present in the form of organic compounds and phosphate that can easily be removed by chemical precipitation (Encyclopeadia Britannica Online, 2013).

Different treatment processes are required to remove nitrogen and phosphorus. Many sewage treatment plants use centrifugal pumps to transfer the nitrified mixed liquor from the aeration zone to the anoxic zone for denitrification. These pumps are often referred to as *Internal Mixed Liquor Recycle* (IMLR) pumps. Once removed, phosphorus, in the form of a phosphate-rich sludge, may be stored in a land fill or resold for use in fertilizer (Black and Veatch, 1971; USEPA, 2004). Other different technologies used in secondary wastewater treatment include rotating biological contactors (RBSs), Extended Aeration Systems, Anaerobic Digester among others. Table 2.5 below shows some advantages and disadvantages attached to the use of different wastewater treatment technologies.

Table 2.5: Some advantages and disadvantages of various sewage treatment systems

	Criteria	Package plant	Activated sludge plant	Extended aeration activated sludge	Biological filter	Oxidation ditch	Aerated lagoon	Waste stabilization pond system
Plant performance	BOD removal	F	F	F	F	G	G	G
	Faecal coliform removal	P	P	F	P	F	G	G
	Suspended solid removal	F	G	G	G	G	F	F
	Helminth removal	P	F	P	P	F	F	G
	Virus removal	P	F	P	P	F	G	G
Economic factors	Simple and cheap construction	P	P	P	P	F	F	G
	Simple operation	P	P	P	F	F	P	G
	Land requirement	G	G	G	G	G	F	P
	Maintenance cost	P	P	P	F	P	P	G
	Energy demand	P	P	P	F	P	P	G
	Sludge removal costs	P	F	F	F	P	F	G

Source: Arthur 1983. Key: G = Good; F = Fair; P = Poor

Although various technological options are available for wastewater treatment, the choice of a particular treatment technology depend on several factors such as the microbial pathogen present, their resistance to treatment, sensitivity of receiving waterbody/land, water use license requirements, funding to construct facility, running cost and projected population, reuse opportunity and the potential for contact with workers and the general public among others (Toze, 1997; Bhagwan, 2011).

CHAPTER THREE

METHODOLOGY

3.1 Study site and plant description

The two selected wastewater treatment plants are located in Amahlati Local Municipality of Amatole District Municipality in the Eastern Cape Province of South Africa.

Stutterheim wastewater treatment works (SWWTW) is located within the geographical coordinates of 32º34.285´S and 027º26.095´E. It uses activated sludge and drying beds technology to treat its sewage, and discharges its final effluent into Cumkala River (DWA, 2009; DWA, 2012). According to the Green Drop Report 2012, the plant has a designed capacity of 4.0 Mℓ/day and an operational capacity of 62.5%. The plant has poor effluent compliance with microbiological compliance of 5%, chemical compliance of 5% and physical compliance of 5%. According to the same report, the wastewater risk rating of this wastewater treatment plant is 76.5%, and has a value of 5% for its annual average effluent quality compliance. The Green Drop Progress Report (2012) places the plant on a high risk level (DWA, 2012).

Keiskammahoek wastewater treatment works (KWWTW) is located within the coordinates of longitude 32º41.519´S and 027º08.615´E. It has a design capacity of 0.67 Mℓ/day. It uses activated sludge and sludge lagoon technology and discharges its effluent into the Keiskamma River (DWA, 2009; DWA, 2012). This plant is in a high risk state according to the Green Drop Progress Report (2012) with microbiological, physical and chemical compliances of 70%, 56.7% and 70% respectively. The plant has a wastewater risk rating of 88.2% and an annual average effluent quality compliance of 65.6%. Problem areas include lack of influent monitoring and poor effluent compliance (DWA, 2012).

3.2 Sampling

Wastewater samples were collected once monthly for a period of five months (September 2012 to January 2013) from the final effluent tanks of the wastewater treatment plants and the discharge points to the receiving watersheds using grab method. Sterile plastic bottles of about 1.7 L capacity to which 1.7 ml of 0.1 M Sodium thiosulphate has been added were used for the collection of the samples. After collection, the sample bottles were covered tightly with screw caps and transported in cooler boxes containing ice to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory at the University of Fort Hare, Alice for analyses within 6 h of collection.

3.3 Physicochemical analysis

All field meters were calibrated according to the manufacturers' specifications. CRISON multi-parameter meter (MM40) was used to determine pH, electrical conductivity (EC), temperature, total dissolved solids (TDS) on site. Dissolved oxygen (DO), turbidity and free residual chlorine were also determined on site using HACH BOD multi parameter meter (HACH Company, model HQ 40d), microprocessor turbidimeter (HACH Company, model 2100P) and a free and total ion specific meter (Hanna-BDH laboratory supplies, HI 93711) respectively.

Nutrients concentrations (phosphate, nitrate and nitrite) and chemical oxygen demand (COD) were determined in the laboratory by standard photometric method using Spectroquant Pharo 100 (Merck Pty Ltd). The biochemical oxygen demand (BOD) was determined by measuring the amount of dissolved oxygen left in the collected samples after a 5 day incubation period and subtracting the remain amount of oxygen from the amount of oxygen present in the sample on the day 1 of sample collection, according to standard

methods (APHA/AWWA/WEA (1998); Standard Methods for the Examination of Water and Wastewater, (21st ed.)). The analysis of all parameter measured were done in triplicates and the values are reported as means of the triplicate values plus/minus the standard deviations.

3.3.1 Determination of phosphate (PO_4) concentration in the samples

Phosphate concentrations (a nutrient responsible for eutrophication) in the collected samples were determined as orthophosphate (PO_4). Method number 14848 with measuring range 0.05 to 5.00 mg/L was used to measure the concentration in all of the samples. To use this method, 5 ml of the sample was accurately pipetted into a clean test tube. This was followed by the addition of 5 drops of reagent PO_4^{-1} and mixing. One level spoonful (provided in the cap of the PO_4^{-2}) of reagent PO_4^{-2} was added followed by vigorous mixing using a vortex machine (Vortex Mixer Model VM- 1000, Digisystem Laboratory Instruments INC.) until the reagent was completely dissolved. The reaction mixture was allowed to stand on the bench (reaction time) for 5 min after which it was filled into a cuvette and the concentration of phosphate was determined using Spectroquant Pharo 100 (Merck Pty Ltd) in line with the manufacturer's instruction. The method outlined above is similar to EPA 365.2+3, US Standard Methods 4500-PE, ISO 6878/1, and EN 1189.

3.3.2 Determination of nitrates (NO_3) concentration in the samples

Determination of Nitrate (NO_3) was done using method number 14773 (NO_3^-) with measuring range from 0.5 to 20.00 NO_3-N mg/L of the Spectroquant Pharo 100 (Merck Pty Ltd) photometer. Briefly, one spoonful of reagent NO_3^{-1} was placed in a dry clean test tube followed by the addition of 5 ml of reagent NO_3^{-2} and shaken vigorously until reagent NO_3^{-1}

was completely dissolved. This was followed by the addition of 1.5 ml of the pre-treated sample and vigorous mixing using a vortex (Vortex Mixer Model VM- 1000, Digisystem Laboratory Instruments INC). The reaction mixture was then allowed to stand for 10 min (reaction time) at room temperature after which it was filled into a cuvette and the concentration was determined in the photometer.

3.3.3 Determination of nitrites (NO_2) concentration in the samples

Nitrite concentration in all the samples was determined using method number 14776 (NO_2^-), measuring range of 0.20 to 1.00 NO_2-N mg/L of the Spectroquant Pharo 100 (Merck Pty Ltd) photometer. This method is analogous to EPA 354.1, US Standard Methods 4500-NO_2-B, and EN 26 777. To carry out the assay, 5 ml of the sample was pipetted into test tube followed by the addition of 1 level microspoon (provided in the cap of the reagent bottle) of reagent NO_2^{-1} and mixed vigorously using vortex machine (Vortex Mixer Model VM- 1000, Digisystem Laboratory Instruments Inc.) until the reagent was completely dissolved. The sample was allowed reaction time of 10 min, after which it was filled into a cuvette and measured in the Spectroquant Pharo 100 (Merck Pty Ltd) photometer.

3.3.4 Determination of biological oxygen demand (BOD) of the samples

Biological oxygen demand (mg/L) was determined by filling about 300 ml of the samples into standard plastic BOD bottles and the amount of dissolved oxygen present the samples were measured using HACH DO/BOD meter (HACH Company, model 2943900) to give DO_1. The 300 ml samples were then incubated in dark cupboard (to prevent light and oxygen) for a period 5 day after which the amount of dissolved oxygen left was again

measured using the HACH BOD meter (HACH Company, model 2943900). The amount of BOD of the sample was then determined using the formula below according to standard method:

BOD mg/L = $DO_1 – DO_5$

Where: DO_1 = dissolved oxygen on day one of sample collection and;

DO_5 = dissolved oxygen after 5 day incubation period.

3.3.5 Determination of chemical oxygen demand (COD) of the samples

Chemical oxygen demand (COD) was determined using standard photometric method (DWAF, 1999). Method number 09773 of Spectroquant Pharo 100 (Merck Pty Ltd) photometer with concentration range of 100 to 1500 mg/L was used to determine the amount of COD after the samples were digested in a thermoreactor (Spectroquant TR 420) at 148^{o}Cfor 120 minutes. To carry out this procedure, 300 µl of solution A and 2300 µl of solution B were carefully pipetted into clean dry screw-capped COD reaction cells (Cat No. 114724) and swirl to allow proper mixing of these reagents. This was then followed by the addition of 3000 µl of the sample (Blanks cell prepared with distilled water were included in the preparations). The reaction cells were then mixed vigorously by means of vortex machine (Vortex Mixer Model VM- 1000, Digisystem Laboratory Instruments Inc.). The reaction cells were loaded into the pre- thermoreactor at for 148^{o}C and digested for 120 min. After the digestion period, the cells were removed from the thermoreactor and allowed to cool in a test tube rack for 10 min. After the 10 min cooling the cells where swirled and returned back into the test tube rack for complete cooling to room temperature, followed by measurement in the

photometer. The COD of the samples were determined by subtracting the absorbance values obtained for the blanks from the absorbance values obtained for the sample as shown below:

COD mg/L = Absorbance of sample – Absorbance of blank

3.4 Bacteriological analysis

The bacteriological analyses of the collected samples were done using internationally accepted standard methods as follows:

3.4.1 Detection and enumeration of faecal coliforms and enterohemorrhagic *Escherichia coli* (EHEC)

3.4.1.1 Membrane filtration (MF) method

Different dilutions of each of the collected samples were prepared at ten-fold (10^{-1}), hundred-fold (10^{-2}) and a thousand-fold (10^{-3}) and 100 ml from each of the dilutions were filtered through cellulose membrane filter (MF) of 0.45 µm pores, with the aid of vacuum pump. The membrane filters were then aseptically transferred onto already prepared petri-dishes containing m-FC agar (Biolab, Merck) (for faecal coliform) and *E. coli* O157:H7 chromogenic agar base (Conda; Cat. No. 1588.00) (for enterohemorrhagic *E. coli* O157:H7); to which Cefixime Tellurite supplements (Mast; Ref. SV48) had been added. The agar medium is a selective and differential medium for the detection of *E. coli* O157:H7. The petri dishes for *E. coli* O157:H7 were then incubated in inverted positions at 37^{o}C for 24 h while the m-FC plates (for faecal coliforms) were incubated at 44.5^{o}C for 24 h.

3.4.1.2 Identification and counting of colonies

After the recommended incubation period, the typical target bacteria (*E. coli* O157:H7) colonies formed on the plates were identified as pale pink (based on the manufacturers' instructions). These were counted and recorded as presumptive colony forming units per 100 ml (cfu/100 ml) of the effluent samples analysed. The putative *E. coli* isolates obtained from the chromogenic plates were subcultured and purified further on fresh chromogenic agar plates. Single colonies of the purified isolates obtained from the subsequent subculturing were aseptically inoculated into Luria Bertani (Merck) and persevered in 15% sterile glycerol at -80^{o}C for further analysis.

3.4.1.3 Presumptive identification of enterohemorrhagic *E. coli* isolate

The Gram's staining procedure and oxidase test were carried out in order to characterise the presumptive EHEC isolates purified from the *E. coli* O157:H7 chromogenic agar plates. All *E. coli* pathotypes including EHEC are Gram negative and oxidase negative.

3.5 Statistical analysis

All data obtained in this study were subjected to statistical analysis at 95% confidence limit using SPSS 20.0 version for windows program (SPSS, Inc.). The means and standard deviation were determined and One way analysis of variance (ANOVA) (SPSS 20.0 version for Windows Program) was performed to determine differences between different pairs of treatment. The statistical correlations between the measured physicochemical and bacteriological parameters were also determined.

CHAPTER FOUR

RESULTS

4.1 Physicochemical analysis

The results obtained for the physicochemical quality parameter analysis of samples collected from both the final effluent tanks and the discharge points of the two wastewater treatment plants (Stutterheim wastewater treatment work (SWWTW) and Keiskammahoek wastewater treatment works (KWWTW) assessed are presented in Tables 4.1 and 4.2 respectively. The pH of SWWTW ranged between 6.8 at the final effluent tank in September and 7.6 at the discharge point in October. These pH values fell within the guideline of 5.5 – 9.5 recommended by DWAF. The temperature at this treatment works ranged from a low of 15^{o}C in September to a high of 24^{o}C in October throughout the sampling months. This also fell within the specified maximum limit of 35^{o}C recommended by DWAF.

The ranges of other measured parameters that complied with the set guidelines are: total dissolved solids (107 – 154 mg/L); electrical conductivity (168 – 239 µS/cm); and dissolved oxygen (DO) which ranged between 4.88 mg/L at the final effluent tank in October and 8.48 mg/L at the discharge point in September. Generally, the DO measurements complied with the stipulated WHO standards throughout the sampling period.

Turbidity measurements for the SWWTW ranged from 2.64 NTU at the final effluent tank in September 2012 to 9.80 NTU also at the final effluent tank in November 2012. The upper limit for the turbidity was above the limit of 5 NTU stipulated by WHO (WHO, 2008). The biological oxygen demand (BOD) and chemical oxygen demand (COD) ranged between 0.13 and 6.85 mg/L and, 46 and 460 mg/L respectively. The BOD values complied with the

set limits by the European Union (EU) (Chapman , 1996); except at the discharge point in October where the value (6.85 mg/L) was slightly above the recommended limit.

Table 4.1: Physicochemical qualities of the final effluent (STF) and discharge point (STD) of Stutterheim wastewater treatment works (SWWTW)

Parameter	September		October		November		December		January		Range		Regulatory Guidelines (General limit)
	STF	STD	STF	STD	STF	STD	STF	STD	STF	STD	STF	STD	
pH	6.8 ± 0.1	7.1 ± 0.2	7.1 ± 0.0	7.6 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	6.9 ± 0.0	6.9 ± 0.0	7.2 ± 0.0	7.3 ± 0.0	6.8 – 7.2	6.9 – 7.6	5.5 – 9.5 (DWAF,2004)
TDS (mg/L)	112 ± 14	121 ± 1	143 ± 3	147 ± 1	154 ± 1	153 ± 0	129 ± 3	127 ± 0	109 ± 3	107 ± 0.0	109 - 154	107 – 153	450 mg/L (DWAF, 1996b)
EC (µS/cm)	174 ± 23	189 ± 1	224 ± 5	230 ± 2	241 ± 1	239 ± 0.6	201 ± 5	170 ± 3	170 ± 4	168 ± 1	170 – 241	168 – 239	7000 µS/cm above intake to a maximum of 15000 µS/cm (DWAF, 2004)
Temp (^oC)	17 ± 1	15 ± 1	21 ± 1	24 ± 1	22 ± 0	20 ± 0	23 ± 1	23 ± 1	22 ± 1	23 ± 1	17 – 23	15 – 24	Maximum of 35^oC (DWAF, 2004)
Turbidity (NTU)	2.64 ± 0.20	6.21 ± 1.43	3.97 ± 0.18	4.40 ± 0.27	9.80 ± 0.71	8.82 ±0.36	3.52 ± 0.29	3.34 ±0.16	3.49 ± 0.11	4.09 ± 0.27	2.64 – 9.80	3.34 – 8.82	<5 NTU (WHO, 2008)
Free Cl (mg/L)	0.23 ± 0.10	0.41 ± 0.71	0.13 ± 0.03	0.14 ± 0.01	0.32 ± 0.03	0.34 ± 0.01	0.27 ± 0.04	0.17 ± 0.03	0.19 ± 0.03	0.21 ± 0.03	0.13 – 0.32	0.14 – 0.41	0.25 mg/L (DWAF, 2004)
DO (mg/L)	5.78 ± 0.42	8.48 ± 0.07	4.88 ± 0.07	7.81 ± 0.22	5.24 ± 0.06	8.07 ± 0.20	5.58 ± 0.26	7.82 ± 0.11	5.43 ± 0.11	7.57 ± 0.08	4.88 – 5.78	7.57 – 8.48	≥5 mg/L (WHO, 2006)
BOD_5 (mg/L)	1.08 ± 0.77	0.13 ± 0.01	3.63 ± 0.23	6.85 ± 0.35	3.6 ± 0.41	1.7 ± 1.22	4.02 ± 0.10	4.26 ± 0.06	3.39 ± 0.09	3.77 ± 0.52	1.08 – 4.02	0.13 – 6.85	3-6 mg/L (EU standard) (Chapman , 1996)
NO_3 (mg/L)	0.00 ± 0.00	0.00 ± 0.00	3.10 ± 0.17	2.97 ± 0.21	4.3 ± 0.23	4.20± 0.32	5.03 ± 0.21	4.06 ± 0.38	4.17 ± 0.46	4.23 ± 0.32	0.0 – 5.03	0.0 – 4.23	15 mg/L (DWAF, 2004)
NO_2 (mg/L)	0.53 ± 0.09	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.19 ± 0.01	0.20 ± 0.02	0.20 ± 0.04	0.20 ± 0.03	0.19 ± 0.04	0.18 ± 0.03	0.14 – 0.53	0.14 – 0.20	15 mg/L (DWAF, 2004)
PO_4 (mg/L)	1.05 ± 0.03	1.31 ± 0.15	1.58 ± 0.02	1.59 ± 0.01	14.6 ± 0.25	15.7 ± 0.45	2.72 ± 0.06	2.52 ± 0.16	1.73 ± 0.15	1.85 ± 0.15	1.05 – 2.72	1.31 – 2.52	10 mg/L (DWAF, 2004)
COD (mg/L)	335 ± 41	460 ± 215	73 ± 15	49 ± 42	140 ± 29	131 ± 36	89 ± 13	62 ± 9	46 ± 12	96 ± 31	46 – 335	62 – 460	75 mg/L after removal of algae (DWAF, 2004)

*All values are means of triplicate determination ± standard deviation.

Key: STF- Stutterheim final effluent; STD- Stutterheim discharge point; TDS- Total dissolved solid; EC- Electrical conductivity; Temp- Temperature; Free Cl- Free chlorine; DO- Dissolved oxygen; BOD- Biological oxygen demand; NO_3- Nitrate; NO_2- Nitrite; PO_4- Phosphate; COD- Chemical oxygen demand.

The COD values varied widely over the sampling period. The lowest value of 46 mg/L was observed in January 2013 at the final effluent tank while the highest value (460 mg/L) was observed in September 2012 at the discharge point. The free chlorine measurements within the sampling period varied from 0.13 mg/L to 0.41 mg/L. There were variations in the free chlorine concentration which in most cases do not conform DWAF set limit of 0.25 mg/L of free chlorine in discharged effluent of wastewater treatment plants. With regard to nutrient components, the measured values ranged between 0 - 5.03 mg/L, 0.14 - 0.53 mg/L and, 1.05 - 2.72 mg/L for nitrates, nitrites and phosphate respectively.

At KWWTW, the pH of the effluents ranged from 6.7 to 6.9 while temperature ranged from 15^{o}C to 23^{o}C. Total dissolved solids ranged between 129 mg/L and 171 mg/L. The values obtained for other physicochemical parameters measured are; EC (201 – 266 µS/cm), turbidity (13.74 – 58.00 NTU), DO (2.20 – 5.94 mg/L), BOD (1.04 – 4.76 mg/L) and COD (40 – 482 mg/L).

High levels of variation were observed in the free chlorine regime at this treatment plant over the entire sampling period. The least chlorine concentration (0.14 mg/L) was observed in the month of September 2012 and the highest chlorine concentration (0.65 mg/L), in January 2013. In terms of the nutrient load, nitrates concentration over the sampling period ranged from 4.63 mg/L to 8.20 mg/L while nitrites concentration ranged between 0.23 mg/L and 0.71 mg/L. The concentration of phosphates over the sampling period was between 2.27 mg/L and 4.50 mg/L.

Table 4.2: Physicochemical qualities of the final effluent (KHF) and discharge point (KHD) of Keiskammahoek wastewater treatment works (KWWTW)

Parameter	September		October		November		December		January		Range		Regulatory Guidelines (General limit)
	KHF	KHD	KHF	KHD	KHF	KHD	KHF	KHD	KHF	KHD	KHF	KHD	
pH	7.0 ± 0.1	7.1 ± 0.0	6.7 ± 0.2	6.9 ± 0.4	7.0 ± 0.0	6.9 ± 0.0	6.9 ± 0.0	6.9 ± 0.0	7.3 ± 0.0	7.3 ± 0.0	6.7 – 7.3	6.9 – 7.3	5.5 – 9.5 (DWAF,2004)
TDS (mg/L)	157 ± 1	155 ± 0	150 ± 1	148 ± 2	171 ± 0	170± 0	140 ± 3	143 ± 3	129 ± 2	129 ± 1	129 – 171	129 – 170	450 mg/L (DWAF, 1996b)
EC (µS/cm)	246 ± 3	242 ± 1	235 ± 1	232 ± 4	266 ± 0	266 ± 6	219 ± 4	223 ± 4	201 ± 3	200 ± 2	201 – 266	200 – 266	7000 **µ**S/cm above intake to maximum of 15000 **µ**S/cm (DWAF, 2004)
Temp (^oC)	16 ± 2	15 ± 0.1	18 ± 1	18 ± 0	23 ± 1	21 ± 2	22 ± 1	21 ± 1	22 ± 0	21 ± 0	16 – 23	15 – 21	Maximum of 35^{o}C (DWAF, 2004)
Turbidity (NTU)	23.47 ± 1.65	20.73 ± 0.15	51.27 ± 1.88	49.90 ± 3.46	57.47 ± 0.21	58.0 ± 0.7	20.43 ± 1.8	20.03 ± 0.7	13.74 ± 1.76	16.34 ± 0.98	13.74 – 57.47	16.34 – 58.00	<5 NTU (WHO, 2008)
Free Cl (mg/L)	0.15 ± 0.03	0.14 ± 0.02	0.49 ± 0.05	0.41 ± 0.04	0.21 ± 0.1	0.18 ± 0.0	0.37 ± 0.05	0.39 ± 0.01	0.61 ± 0.04	0.65 ± 0.10	0.15 – 0.61	0.14 – 0.65	0.25 mg/L (DWAF, 2004)
DO (mg/L)	2.41 ± 0.32	5.58 ± 0.10	2.60 ± 0.03	5.94 ± 0.07	2.2 ± 0.09	5.01 ± 0.04	2.57 ± 0.11	5.26 ± 0.04	2.64 ± 0.17	5.30 ± 0.27	2.20 – 2.64	5.01 – 5.94	≥5 mg/L (WHO, 2006)
BOD_5 (mg/L)	1.67 ± 0.25	4.76 ± 0.24	1.04 ± 0.16	4.62 ± 0.32	1.71 ± 0.15	4.39 ± 0.22	2.43 ± 0.14	3.86 ± 0.91	1.57 ± 0.07	2.52 ± 0.32	1.04 – 2.43	2.52 – 4.76	3-6 mg/L (EU standard) (Chapman , 1996)
NO_3 (mg/L)	6.57 ± 0.72	6.77 ± 0.61	5.33 ± 1.27	4.63 ± 0.21	4.67 ± 0.40	4.7 ± 0.15	5.60 ± 0.17	5.70 ± 0.26	6.70 ± 0.50	8.2 ± 1.2	4.67 – 6.70	4.63 – 8.20	15 mg/L (DWAF, 2004)
NO_2 (mg/L)	0.41 ± 0.03	0.43 ± 0.02	0.34 ± 0.01	0.35 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.04	0.70 ± 0.01	0.71 ± 0.05	0.23 – 0.70	0.23 – 0.71	15 mg/L (DWAF, 2004)
PO_4 (mg/L)	3.04 ± 0.13	2.75 ± 0.60	2.27 ± 0.02	2.26 ± 0.08	4.50 ± 0.02	4.44 ± 0.08	2.86 ± 0.01	2.74 ± 0.15	2.48 ± 0.14	2.46 ± 0.15	2.27 – 4.50	2.26 – 4.44	10 mg/L (DWAF, 2004)
COD (mg/L)	482 ± 175	219 ± 119	237 ± 12	301 ± 113	247 ± 32	261 ± 19	66 ± 12	40 ± 7	107 ± 40	86 ± 24	66 – 482	40 - 301	75 mg/L after removal of algae (DWAF, 2004**)**

*All values are means of triplicate determination ± standard deviation.

Key: KHF- Keiskammahoek final effluent; KHD- Keiskammahoek discharge point; TDS- Total dissolved solid; EC- Electrical conductivity; Temp- Temperature; Free Cl- Free chlorine; DO- Dissolved oxygen; BOD- Biological oxygen demand; NO_3- Nitrate; NO_2- Nitrite; PO_4- Phosphate; COD- Chemical oxygen demand.

4.2 Bacteriological analysis

4.2.1 Prevalence and distributions of faecal coliforms and enterohemorrhagic *E. coli*

Data for the prevalence and distribution of faecal coliforms and presumptive enterohemorrhagic *E. coli* over the sampling period for both the SWWTW and the KWWTW is shown in Tables 4.3 and 4.4 respectively. At the SWWTW final effluent tank, the presumptive faecal coliforms count varied between 2 CFU/100 ml in September 2012 and 2.7 × 10^4 CFU/100 ml in October 2012 while the faecal coliform counts at the discharge point ranged between 0 CFU/100 ml in January 2013 and 1.2 × 10^3 CFU/100 ml in October 2012. The counts for presumptive EHEC at the final effluent tank ranged between 0 CFU/100 ml in September 2012 and 4.7 × 10^4 CFU/100 ml in January 2013 while the presumptive EHEC counts at the discharge point ranged between 0 CFU/100 ml in September 2012 and 1.5 × 10^3 CFU/100 ml in November 2012.

Faecal coliform counts at the final effluent tank of KWWTW plant ranged between 55 CFU/100 ml in December 2012 and 8.5 × 10^3 CFU/100 ml in November 2012 while at the discharge point the counts ranged between 0 CFU/100 ml in January 2013 and 8.4 × 10^3 CFU/100 ml in November 2012. The presumptive EHEC counts varied from 7.0 × 10^3 CFU/100 ml in December 2012 to 4.6 × 10^3 CFU/100ml at the final effluent tank while counts at the discharge point varied from 0 CFU/100 ml in January 2013 to 9.3 × 10^3 CFU/100ml in October 2012.

Table 4.3: Presumptive faecal coliforms and enterohemorrhagic *E. coli* counts for the final effluent and discharge point of the SWWTW

Sampling Month	Microbial counts (CFU/100ml)			
	Faecal coliform		EHEC bacteria counts	
	STF	STD	STF	STD
September	2	1	0	0
October	2.7×10^4	1.2×10^3	6.9×10^2	3.9×10^2
November	1.9×10^1	2.0×10^1	1.7×10^3	1.5×10^3
December	3	2	1.6×10^2	1.6×10^2
January	1.0×10^3	0	4.7×10^3	2.3×10^2

STF- Stutterheim final effluent, STD- Stutterheim discharge point. *All values are means of triplicate determination.

Table 4.4: Presumptive faecal coliforms and enterohemorrhagic *E. coli* counts for the final effluent and discharge point of the KWWTW

Sampling Month	Microbial counts (CFU/100ml)			
	Faecal coliform		EHEC bacteria counts	
	KHF	KHD	KHF	KHD
September	9.1×10^2	8.8×10^2	7.3×10^2	4.5×10^2
October	1.5×10^3	1.7×10^3	4.6×10^3	9.3×10^3
November	8.5×10^3	8.4×10^3	7.2×10^3	7.9×10^3
December	5.5×10^1	1.8×10^1	7.0×10^2	3.8×10^2
January	3.0×10^2	0	1.5×10^3	0

KHF- Keiskammahoek final effluent, KHD- Keiskammahoek discharge point. *All values are means of triplicate determination.

4.2.2 Preliminary identification of presumptive EHEC isolates

All the forty randomly selected isolates (100%) subjected to Gram's staining procedure were Gram negative. Some cells appeared as short-rods when viewed under the 100× magnification of the light microscope. Ten percent of the isolates were oxidase positive while the remaining 90% were oxidase negative.

4.3 Correlation profiles among the physicochemical and microbiological parameters

The correlation coefficients among all tested parameters for the four sampling points are presented in Tables 4.5 to 4.8. Tables 4.5 and 4.6 show the correlation coefficients of the SWWTW final effluent tank and discharge point respectively. At the final effluent tank (STF), some of the physicochemical parameters exhibited strong significant correlation with each other. For example BOD_5 and temperature exhibit a very strong direct correlation with each other ($p<0.05$; $r^2=0.97$). This implies that as BOD increases, the temperature of the water also increases.

The statistical correlations between the physicochemical parameters and the microbiological parameters were generally weak except in the cases of free chlorine and DO where they showed inverse correlations with the microbiological parameters. While free chlorine showed a fairly strong inverse correlation with FC counts ($p<0.05$; $r^2=-0.751$), there was a weak inverse correlation between free chlorines and EHEC counts.

Table 4.5: The correlation half matrix between the measured physicochemical and microbiological qualities for the SWWTW final effluent tank (STF).

Parameters	pH	TDS (mg/L)	EC (mS/cm)	Temp (^oC)	Turbidity (NTU)	Free Cl (mg/L)	DO (mg/L)	BOD_5 (mg/L)	NO_3 (mg/L)	NO_2 (mg/L)	PO_4 (mg/L)	COD (mg/L)	FC (CFU/100ml)	EHEC (CFU/100ml)
pH	1													
TDS (mg/L)	.076	1												
EC (mS/cm)	.078	1.000**	1											
Temp (oC)	.547	.421	.421	1										
Turbidity (NTU)	.117	.781	.784	.378	1									
Free Cl (mg/L)	-.477	.282	.284	.191	.630	1								
DO (mg/L)	-.644	-.694	-.694	-.417	-.369	.433	1							
BOD_5 (mg/L)	.569	.534	.533	.973**	.359	.051	-.585	1						
NO_3 (mg/L)	.512	.398	.398	.997**	.390	.250	-.357	.956*	1					
NO_2 (mg/L)	-.739	-.544	-.544	-.909*	-.365	.130	.747	-.963**	-.878	1				
PO_4 (mg/L)	.006	.724	.726	.342	.989**	.736	-.230	.297	.363	-.273	1			
COD (mg/L)	-.808	-.269	-.269	-.898*	-.133	.247	.604	-.921*	-.871	.954*	-.048	1		
FC (CFU/100ml)	.354	.400	.398	.009	-.137	-.751	-.817	.231	-.062	-.389	-.266	-.305	1	
EHEC (CFU/100ml)	.812	-.291	-.287	.373	.107	-.147	-.124	.264	.377	-.382	.068	-.525	-.220	1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Key: STF- Stutterheim final effluent; TDS- Total dissolved solid; EC- Electrical conductivity; Temp- Temperature; Free Cl- Free chlorine; DO- Dissolved oxygen; BOD- Biological oxygen demand; NO_3- Nitrate; NO_2- Nitrite; PO_4- Phosphate; COD- Chemical oxygen demand; FC- Faecal coliforms; EHEC- Enterohemorrhagic *E. coli*.

Table 4.6: The correlation half matrix between the measured physicochemical and microbiological qualities for the SWWTW discharge point (STD).

Parameters	pH	TDS (mg/L)	EC (mS/cm)	Temp (^oC)	Turbidity (NTU)	Free Cl (mg/L)	DO (mg/L)	BOD_5 (mg/L)	NO_3 (mg/L)	NO_2 (mg/L)	PO_4 (mg/L)	COD (mg/L)	FC (CFU/100ml)	EHEC (CFU/100ml)
pH	1													
TDS (mg/L)	.135	1												
EC (mS/cm)	.124	1.000**	1											
Temp (oC)	.406	.130	.138	1										
Turbidity (NTU)	-.256	.549	.539	-.527	1									
Free Cl (mg/L)	-.432	-.011	-.020	-.940*	.751	1								
DO (mg/L)	-.301	.179	.173	-.935*	.576	.854	1							
BOD5 (mg/L)	.635	.196	.202	.907*	-.629	-.957*	-.755	1						
NO3 (mg/L)	-.100	.165	.174	.802	-.131	-.603	-.822	.489	1					
NO2 (mg/L)	-.701	.054	.066	.294	.126	-.117	-.377	-.085	.777	1				
PO4 (mg/L)	-.386	.647	.645	-.089	.844	.374	.154	-.333	.394	.572	1			
COD (mg/L)	-.222	-.272	-.281	-.966**	.359	.858	.882*	-.811	-.902*	-.487	-.140	1		
FC (CFU/100ml)	.870	.485	.481	.480	-.234	-.546	-.224	.761	-.033	-.584	-.255	-.365	1	
EHEC (CFU/100ml)	-.174	.732	.729	.080	.791	.219	.021	-.138	.469	.492	.972**	-.284	-.046	1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Key: STD- Stutterheim discharge point; TDS- Total dissolved solid; EC- Electrical conductivity; Temp- Temperature; Free Cl- Free chlorine; DO- Dissolved oxygen; BOD- Biological oxygen demand; NO_3- Nitrate; NO_2- Nitrite; PO_4- Phosphate; COD- Chemical oxygen demand; FC- Faecal coliforms; EHEC- Enterohemorrhagic *E. coli*.

Table 4.7: The correlation half matrix between the measured physicochemical and microbiological qualities for the KWWTW final effluent tank (KHF).

Parameters	pH	TDS (mg/L)	EC (mS/cm)	Temp (^oC)	Turbidity (NTU)	Free Cl (mg/L)	DO (mg/L)	BOD_5 (mg/L)	NO_3 (mg/L)	NO_2 (mg/L)	PO_4 (mg/L)	COD (mg/L)	FC (CFU/100ml)	EHEC (CFU/100ml)
pH	1													
TDS (mg/L)	-.439	1												
EC (mS/cm)	-.445	1.000**	1											
Temp (oC)	.390	-.165	-.171	1										
Turbidity (NTU)	-.665	.772	.770	.014	1									
Free Cl (mg/L)	.192	-.815	-.819	.265	-.282	1								
DO (mg/L)	.056	-.909*	-.907*	-.123	-.575	.817	1							
BOD_5 (mg/L)	.314	-.147	-.143	.498	-.465	-.279	-.096	1						
NO_3 (mg/L)	.605	-.638	-.638	-.386	-.864	.260	.536	.006	1					
NO_2 (mg/L)	.695	-.659	-.664	-.089	-.588	.605	.519	-.330	.798	1				
PO_4 (mg/L)	.241	.680	.675	.488	.422	-.586	-.912*	.255	-.498	-.350	1			
COD (mg/L)	-.196	.614	.616	-.795	.200	-.699	-.474	-.358	.168	-.079	.137	1		
FC (CFU/100ml)	.005	.723	.716	.434	.721	-.372	-.837	-.131	-.680	-.326	.895*	.097	1	
EHEC (CFU/100ml)	-.163	.500	.493	.374	.814	.046	-.497	-.483	-.687	-.152	.563	-.083	.867	1

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

Key: KHF- Keiskammahoek final effluent; TDS- Total dissolved solid; EC- Electrical conductivity; Temp- Temperature; Free Cl- Free chlorine; DO- Dissolved oxygen; BOD- Biological oxygen demand; NO_3- Nitrate; NO_2- Nitrite; PO_4- Phosphate; COD- Chemical oxygen demand; FC- Faecal coliforms; EHEC- Enterohemorrhagic *E. coli*.

Table 4.8: The correlation half matrix between the measured physicochemical and microbiological qualities for the KWWTW discharge point (KHD).

Parameters	pH	TDS (mg/L)	EC (mS/cm)	Temp (°C)	Turbidity (NTU)	Free Cl (mg/L)	DO (mg/L)	BOD_5 (mg/L)	NO_3 (mg/L)	NO_2 (mg/L)	PO_4 (mg/L)	COD (mg/L)	FC (CFU/100ml)	EHEC (CFU/100ml)
pH	1													
TDS (mg/L)	-.638	1												
EC (mS/cm)	-.651	1.000**	1											
Temp (oC)	-.038	-.260	-.269	1										
Turbidity (NTU)	-.737	.728	.726	.085	1									
Free Cl (mg/L)	.475	-.894*	-.899*	.567	-.388	1								
DO (mg/L)	-.062	-.217	-.205	-.641	.024	.086	1							
BOD_5 (mg/L)	-.678	.773	.786	-.683	.528	-.855	.374	1						
NO_3 (mg/L)	.983**	-.699	-.711	.028	-.836	.506	-.127	-.725	1					
NO_2 (mg/L)	.933*	-.720	-.734	.035	-.562	.662	.132	-.714	.882*	1				
PO_4 (mg/L)	-.388	.795	.784	.289	.577	-.572	-.737	.240	-.413	-.505	1			
COD (mg/L)	-.443	.673	.673	-.483	.795	-.524	.433	.716	-.597	-.288	.268	1		
FC (CFU/100ml)	-.514	.844	.834	.216	.829	-.536	-.478	.362	-.590	-.505	.926*	.565	1	
EHEC (CFU/100ml)	-.717	.575	.576	.024	.966**	-.249	.252	.514	-.823	-.476	.348	.822	.661	1

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

Key: KHD- Keiskammahoek discharge point; TDS- Total dissolved solid; EC- Electrical conductivity; Temp- Temperature; Free Cl- Free chlorine; DO- Dissolved oxygen; BOD- Biological oxygen demand; NO_3- Nitrate; NO_2- Nitrite; PO_4- Phosphate; COD- Chemical oxygen demand; FC- Faecal coliforms; EHEC- Enterohemorrhagic *E. coli*.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Gastrointestinal-related diseases such as cholera and diarrhea remain a leading cause of morbidity and mortality in the developing world most especially in young children and immunocompromised individuals (Casburn-Jones and Farthing, 2004; Quadri, *et al.* 2005). Diarrheal outbreaks have severally been linked to the consumption of enteric bacteria contaminated water (Dauda, 2010). Many rural dwellers in South Africa depend on surface and underground water sources for their daily activities. Many of the sources are often impacted by untreated or poorly treated wastewater effluents from many of the under-performing wastewater treatment works (Palaniappan *et al.*, 2010). Pollution, as a result of poor wastewater and sewage treatment infrastructure, has a direct impact on human health and the environment (Mema, 2009). In order to forestall the outbreak of waterborne diseases, it is imperative that these various wastewater treatment plants are routinely monitored in order to evaluate their performances, and efficiency in line with their compliance with the set guidelines for discharged final effluents.

This study assessed the working efficiency of two wastewater treatment plants in South Africa over a five month period by investigating the physicochemical and microbiological qualities of their discharged final effluents. The study shows that some of the physicochemical parameters were within the DWAF and WHO set guidelines for discharged effluents.

The observed pH range (6.7 - 7.6) for the duration of the study fell within the DWAF guidelines of 5.5 – 9.5 (DWAF, 2004) for discharged effluent. Generally, the obtained values are also within the WHO ranges of 6.5 to 8.5 for water meant for full contact recreation

(Igbinosa and Okoh, 2009). Shifts in surface water pH towards acidity or alkalinity from the recommended limit generally have adverse effects on sensitive fish and other aquatic life (UNIDO, 2000). The neutral to alkaline pH range obtained in this study is similar to that reported elsewhere (Obi *et al.*, 2004).

The DO measurement obtained at the effluent tank and discharge point of SWWTW ranged between 4.88 and 8.48 mg/L. Although there is no set limit for DO in the revised South African Government Gazette of October 1999, a previous government Gazette of 1984 stipulated discharged effluent shall be at least 75% saturated with oxygen (Government Gazette 1984; DWAF, 2004). The WHO however, has a set limit of DO greater or equal to 5 mg/L in order to sustain aquatic life and a DO limit of 6 mg/L for drinking water (Rao 2005; WHO, 2006). The range of measurements obtained at SWWTW therefore fall within the WHO acceptable limits.

The observed measurements at KWWTW were, however, different. The DO at the final effluent tank ranged between 2.20 and 2.64 mg/L which was well below the WHO acceptable limit for sustenance of aquatic life (Rao, 2005). The DO content of water is an important water quality characteristic for protecting fish and other aquatic life. Low DO levels can induce fish kills and reduce reproduction rates in aquatic biota (Carmago and Alonso, 2006). Low DO in water or effluent can also encourage microbial reduction of nitrate to nitrite and sulphate to sulphite (WHO, 2006).

Unlike the low DO at the final effluent tank, the observed DO at the discharge point ranged from 5.01 to 5.94 mg/L. The sharp increase in the DO between the effluent tank and the discharge point was due to the flow and mixing of the effluent as it runs from the effluent tank to the discharge point. The low DO at KWWTW final effluent tank could be attributed to the presence of biodegradable organic material (Igbinosa and Okoh 2009).

Oxygen depletion in water is measured as BOD (DWAF, 1996a). The BOD measurements at the SWWTW mostly fell within the EU recommended limit of 3-6 mg/L (Chapman, 1996). There is no set guideline for BOD in DWAF discharged effluent guide (DWAF, 2004; Momba *et al.,* 2006). The low BOD at the site shows the efficiency of the secondary treatment processes in converting organic content in the effluents to sludge (UNEP, 2000). Although the BOD range at the discharge point of KWWTW fell within the EU guidelines, the observed BOD values at the final effluent tank generally fell short of the recommended guideline. This high BOD content at the effluent tank was probably due to high organic content in the treated effluent. This could have been caused by the fact that the mechanical aerator which is small compared to the volume of wastewater treated at this plant.

The turbidity of the discharged effluent at SWWTW ranged between 2.64 and 9.80 NTU while it ranged between 13.74 and 58 NTU at KWWTW. While there is no set guideline by DWAF for turbidity level in effluents, the turbidity measurements at SWWTW generally complied with the WHO guideline of less than 5 NTU (<5 NTU) for drinking water except in November 2012 where the turbidity values were 9.8 NTU and 8.82 NTU at the final effluent tank and the discharged point respectively. In contrast to what was observed at the SWWTW, the turbidity levels at KWWTW were excessively high above the WHO guideline. Although no health-based guideline value for turbidity has been proposed; ideally, however, turbidity should be below 0.1 NTU for effective disinfection. Turbidity is also an important process control parameter and can indicate problems with treatment processes particularly sedimentation. High levels of turbidity in effluents can overwhelm treatment processes, allowing faecal bacteria and other enteric pathogen escape disinfection processes (WHO, 2008). While high turbidity negatively affects the aesthetic value of recreational receiving water bodies, it may also potentially cause the formation of carcinogenic by-products

(trihalomethanes) with chlorine during the disinfection process (Odjadjare and Okoh 2009, Mazibuko, 2012).

The temperature regimes observed in this study generally ranged between 15^{o}C and 24^{o}C at both plants. These temperature regimes comply with the special limit of 25^{o}C or general limit of 35^{o}C set by DWAF for discharged effluents (Government Gazette, 1984). Higher temperature in discharged effluents can offset the homeostatic balance of receiving water bodies (Odjadjare, 2010).

Electrical conductivity is a measure of the ability of water to pass an electric current. While DWAF recommended a general EC guideline limit of 7000 µS/cm above intake to a maximum of 15000 µS/cm for discharged effluents, the EC measurement in this study ranged between 168 µS/cm and 266 µS/cm. All the observed EC values did not exceed the set guidelines limit throughout the sampling period.

Total dissolved solids (TDS) is a measure of the amount of all soluble substances most of which carry electrical charges while COD determination measures the amount of oxygen required for chemical decomposition of organic and inorganic contaminants, dissolved or suspended in water (DWAF, 1996a; Salem *et al.,* 2011). The WHO and DWAF set guideline values for TDS in discharged effluents are 2000 mg/L and 450 mg/L respectively (DWAF, 1996b; Akan *et al.,*2008). The measured TDS at both treatment works in this study ranged between 129 mg/L and 170 mg/L. This observed range of TDS is within the set guideline by both the WHO and DWAF. The COD measurement in the study ranged between 46 mg/L and 482 mg/L at both treatment works. Most COD readings obtained during the sampling period were above the set guideline of 75 mg/L after the removal of algae (DWAF, 2004). Excessively high COD in discharged effluents will exert high oxygen demand in receiving water bodies with the attendant problems such as a rise in pH and insufficient oxygen for fish and other aquatic life in such receiving water bodies (Salequzzaman *et al.*, 2008).

An important strong positive correlation exists between TDS and EC at all the sampling point throughout the sampling period. Tables 4.5, 4.6, 4.7 and 4.8 show that the amount of change in the TDS of effluents also result in the same amount of change in the EC of the effluents. EC is recommended as an important factor for reclaimed water quality in different fields such as agriculture and industrial reuse (US EPA, 2004b). Also, wastewater from different sources has been shown to have high concentrations of ions, which increased the conductivity of the effluent samples, requiring additional treatment to maintain the parameters within established guidelines (Kim, 2007). However, limited work has been conducted to determine the correlation between TDS and EC in industrial reclaimed wastewater. Every industry discharges different kinds of effluents with different concentrations of salts and ions, so TDS/EC ratios are usually different across industries.

Nutrient concentrations in the discharged effluents were determined by measuring the concentrations of nitrate (NO_3), nitrite (NO_2) and phosphate (PO_4). All readings obtained for the nutrient components in the discharged effluents at both treatment works fell within the set maximum guideline level of 15 mg/L (for both NO_3 and NO_2) and 10 mg/L (for PO_4) throughout the sampling period. It implies that the treatment plants are efficient in terms of nutrient removal, as excessive nutrient loads in discharged effluents causes eutrophication in receiving water bodies (DWAF 1996a).

The free chlorine concentration measures the amount of chlorine that is available to react for disinfection purposes. The set guideline for the amount of permissible free chlorine in discharged effluent in South Africa is 0.25 mg/L. This value is meant to protect the health of persons that may come into contact with such water and also safeguard the integrity of receiving water bodies. High levels of inconsistencies in the free chlorine regimes were observed in the final effluent of both wastewater treatment works in this study, with the worst case scenarios being observed at the KWWTW. Free chlorine concentrations at the SWWTW

final effluent tank generally ranged between 0.13 and 32 mg/L over the entire sampling period, while it ranged between 0.14 and 0.41 mg/L at the discharge point.

At the KWWTW final effluent tank, the free chlorine concentration varied widely with the least value of 0.15 mg/L in September 2012 and the highest value of 0.61 mg/L in January 2013. The situation was also similar at the discharge point with the least value of 0.14 mg/L in September 2012 and the highest value of 0.65 mg/L in January 2013. A major implication of low free chlorine concentration in discharged effluent is encouragement of survival of microorganisms including pathogens. This poses a health risk for persons that come in contact with such effluents, as well as the immediate communities that depend on water from the receiving water bodies where such effluents are discharged. High free chlorine concentration on the other hand (as was observed in October 2012 and January 2013), poses a major threat to aquatic life and may also react with organics in receiving water bodies to form trihalomethanes (e.g. chloroform, dichloro-bromomethane, bromoform among others) which are possible human carcinogens (Mills *et al.,* 1998). High levels of chlorine can also impart a brackish salty taste to receiving water bodies which can result in nausea and electrolyte problems in receiving water bodies meant for domestic use (DWAF, 1996a).

One of the main functions of wastewater treatment plants is to remove pathogens and other hazardous components from wastewater, in order to protect public health and conserve freshwater sources that often serve as receiving bodies for discharged effluents. Although the negative impact of discharged effluents on surface water has received attention in recent years, the production of effluent of high microbiological quality remains a major challenge (Dungeni *et al.*, 2010). High levels of faecal coliforms and enterohemorrhagic *E. coli* counts over the sampling period suggest the inadequacy of the understudied wastewater treatment plants in producing effluents of high microbiological quality. This might be suggestive of a possible threat to the environment and public health.

Generally, the faecal coliform counts obtained at Stutterheim final effluent tank (STF) and discharge point (STD) fell within the DWAF guideline of not more than 10^3 CFU/100ml of faecal coliforms in discharged effluents. A striking exception was however observed in the month of October 2012, where a very high count of 2.7×10^4 CFU/100 ml was recorded at the final effluent tank. This could have been a result of low chlorine dosage. The concentration of free chlorine in the final effluent in that month was 0.13 mg/L, a concentration far below the recommended 0.25 mg/L for discharged effluent. Also, the FC count at the discharge point (1.2×10^3 CFU/100ml) exceeded the DWAF guideline of 10^3 CFU/100ml.

The range of FC counts at KHF was between 5.5×10^1 CFU/100ml (in December 2012) and 8.5×10^3 CFU/100ml (in November 2012). At this sampling point, the FC counts were also within the set guideline except in the months of October and November 2012 where the counts were 1.5×10^3 CFU/100 ml and 8.5×10^3 CFU/100 ml respectively. Although the free chlorine concentrations in the effluents for these months were fairly high: 0.49 mg/L (October) and 0.21 mg/L (November), they failed to eliminate the FC bacteria. Studies in the past have reported cases of bacterial survival in treated water at chlorine concentration of up to 1 mg/L (Olivieri, *et al,* 1985; LeChevallier, *et al.,* 1987). A count of zero FC count was recorded at KHD in the month of January 2013. This could be attributed to high free chlorine concentration (0.61 mg/L) in the final effluent tank in that month. Although the high chlorine concentrations sufficiently wiped out the FC bacteria, it however poses a different threat to the environment (Fatoki *et al.*, 2001).

Enterohemorrhagic *E. coli* (EHEC) is the most virulent serotype of all pathogenic *E. coli,* and has risen in the past two decades as an important public health problem (OIE Terrestrial Manual, 2008). Presumptive EHEC were recovered from the two understudied wastewater treatment plants in high numbers. Although no count of EHEC was obtained from

the sample collected at SWWTW in September, EHEC was however recovered for the rest of the sampling months at this site. For the four months in which EHEC was recovered from the samples collected from both the final effluent tank (STF) and discharge point (STD), the counts generally ranged between 1.6×10^2 CFU/100 ml and 4.7×10^3 CFU/100 ml.

At the KWWTW, EHEC was also recovered from all samples except the discharge point sample of January 2013 where no count was obtained. The 0 CFU/100 ml of sample analysed for FC in the same month. For the months that EHEC was recovered, counts generally ranged between 38 CFU/100 ml at the discharge point in December 2012 and 9.3×10^3 CFU/100 ml at the discharge point in November 2012.

The correlation analysis of data obtained from the physicochemical and microbiological analysis at STF show a fairly strong inverse relationship between FC counts and free chlorine concentration. There was also a fairly strong inverse relationship between FC counts and DO. Weak inverse correlations were, however, observed between EHEC counts and free chlorine and also between EHEC counts and DO. The case was also similar between FC counts and free chlorine concentration at the discharge point. However, DO showed positive correlation with enterohemorrhagic *E. coli* counts.

5.2 Conclusion and recommendation

The two understudied wastewater treatment plants showed high levels of inconsistency in the physicochemical and microbiology qualities of their discharged effluents. Though, microbiological parameters are usually the most important in determining the safety of water; including discharged treated effluents, it is equally important to assess the

different physical and chemical properties in order to understand the true nature and pollution level of different water samples.

From the findings of this study, some of the physicochemical parameters assessed were within the stipulated guidelines by DWAF and the WHO for discharged final effluents, while others did not conform to set guidelines. High turbidity and inconsistent chlorine dosage observed throughout the sampling period at KWWTW is unacceptable and efforts should be made to solve this problem to ensure the production of effluent that meets required guideline values.

The results of the microbiological analysis revealed the inadequacy of the treatment plants to effectively remove FC (which could be suggestive of the presence of pathogenic microorganisms including enteric viruses) from the treated effluents. Of major concern is the recovery of the highly virulent enterohemorrhagic *E. coli* pathotype from the effluents throughout the sampling period. Considering the public health implications of this strain, utmost priority should be given to regular monitoring of these treatment works to ascertain their efficiency at producing effluents that meet regulatory guidelines in terms of both physicochemical and microbiology qualities. A review of the disinfection (chlorination) methods used at these treatment works is also recommended to ensure that all pathogenic microorganisms are effectively inactivated before the treated effluents are discharged back into the environment.

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